**Gastrointestinal Stability and Tissue Penetration of V565: a Novel Orally Administered Anti-TNFα Vorabody™**


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### Introduction

- VHsquared Ltd is a biotechnology company based in Cambridge, UK, using proprietary technology to engineer llama single domain antibodies for oral delivery (Vorabodies) for the treatment of inflammatory diseases of the intestine.
- V565, the lead Vorabody product, potently neutralises TNFα and has been engineered to be resistant to intestinal and inflammatory proteases.
- Crohn’s disease (CD) is an incurable life-long inflammatory disease of the intestine, although selective neutralisation of TNFα has already been established as an effective therapeutic strategy.
- Conventional anti-TNFα antibodies must be injected or intravenously infused, which is inconvenient and painful for the patient and results in systemic suppression of TNFα.
- V565 is administered in a convenient oral formulation to enhance delivery direct to the disease site where there is overproduction of TNFα, while limiting systemic exposure and potential associated side-effects.

### Development

- VH domain antibodies with potent TNFα-neutralising activity were isolated from a llama VH phage display library.
- V565 was engineered by altering its amino acid sequence to better resist proteolytic inactivation. Variants with improved stability that retained potency were modified further resulting in the fully optimised Vorabody V565.
- PK studies were performed in mice to assess the stability of V565 in transit through the GI tract and to determine luminal concentrations in different regions of the intestines following oral administration of the Vorabody.
- The penetration of V565 into inflamed regions of the intestinal tract was investigated in mice with DSS-induced colitis using immunocytochemistry.

### Results

**In vitro stability of V565 in Intestinal & Faecal Extracts**

- Vorabodies are formulated for release at the site of inflamed mucosal tissues. In this case, the ileum and colon.
- V565 strongly resisted degradation in different regions of the GI tract: (i) mouse small intestine (ii) the human ileum and (iii) pooled human faeces (Fig. 1).

**Resistance of V565 to disease-associated MMPs**

- Matrix metalloproteinases (MMPs) are increased in the inflamed mucosa of patients with CD and can digest native human IgG and therapeutic agents that contain a human IgG scaffold (Biancheri et al, 2015).
- V565 was highly resistant to digestion by MMPs, compared to the IgG-based anti-TNFα therapy Enbrel (Fig. 2).

**In vivo GI stability and distribution of V565 in the mouse**

**Local Delivery to the Intestinal Tract following Oral Administration**

- Oral dosing of mice with V565 resulted in rapid transit of the Vorabody through the GI tract and by 3h V565 in the stomach and SI was below measurable levels.
- Meanwhile, high concentrations were present in both the caecum and colon.
- V565 levels in faeces varied between animals, but a peak concentration was detected at 3h post-dosing in all mice (Fig. 3B).
- Overall, V565 was shown to transit through the mouse GI tract delivering high concentrations in the lower GI tract and faeces up to 7h post-dosing.

### Figure 3: α-TNFα Neutralising Activity Remaining in Mouse Small Intestinal Fluid, Human Ileal Fluid and Faecal Supernatants

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Remaining Activity (%)</th>
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<tbody>
<tr>
<td>2h</td>
<td>Mouse SI fluid</td>
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<tr>
<td>10h</td>
<td>Human Faecal Supernatant</td>
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**Figure 4: DSS colitis in mice**

- Based on immunostaining there was very little penetration of V565 into normal colonic mucosal tissue, but immuno-staining for V565 in colonic mucosal tissue was markedly increased in mice with DSS colitis.
- V565 can be seen throughout the sub-mucosa as early as 1h post-dosing (Fig. 5).
- V565 staining in the colon tissue was maximal at 3-5h, corresponding to the peak concentrations of V565 in the lumen of the colon.
- V565 does not bind to murine TNFα and tissue staining decreased in parallel with luminal concentrations of the VH1, consistent with murine intestinal transit time.

### Conclusions

- V565 is highly resistant to proteases found in the lumen and mucosa of the gut.
- Recovery of high concentrations of V565 in the colons of normal and colitis mice following oral administration demonstrates the feasibility of delivering active Vorabody to the site of inflammation.
- Results showing that V565 can penetrate into inflamed colonic mucosal tissue from the gut lumen provides in vivo confirmation that orally delivered V565 can reach and potentially neutralise TNFα activity in sub-mucosal tissues.

### References