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Oral delivery of the anti-tumor necrosis factor \(\alpha\) domain antibody, V565, results in high intestinal and fecal concentrations with minimal systemic exposure in cynomolgus monkeys

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**ABSTRACT**

**Objective:** V565 is a novel oral anti-tumor necrosis factor (TNF)-\(\alpha\) domain antibody being developed for topical treatment of inflammatory bowel disease (IBD) patients. Protein engineering rendered the molecule resistant to intestinal proteases. Here we investigate the formulation of V565 required to provide gastro-protection and enable optimal delivery to the lower intestinal tract in monkeys.

**Methods:** Enteric-coated V565 mini-tablets were prepared and dissolution characteristics tested \textit{in vitro}. Oral dosing of monkeys with enteric-coated mini-tablets containing V565 and methylene blue dye enabled \textit{in vivo} localization of mini-tablet dissolution. V565 distribution in luminal contents and feces was measured by enzyme-linked immunosorbent assay (ELISA). To mimic transit across the damaged intestinal epithelium seen in IBD patients an intravenous (i.v.) bolus of V565 was given to monkeys and pharmacokinetic parameters of V565 measured in serum and urine by ELISA.

**Results:** Enteric-coated mini-tablets resisted dissolution in 0.1 M HCl, before dissolving in a sustained release fashion at neutral pH. In orally dosed monkeys methylene blue intestinal staining indicated the jejunum and ileum as sites for mini-tablet dissolution. Measurements of V565 in monkey feces confirmed V565 survival through the intestinal tract. Systemic exposure after oral dosing was very low consistent with limited V565 mucosal penetration in healthy monkeys. The rapid clearance of V565 after i.v. dosing was consistent with renal excretion as the primary route for elimination of any V565 reaching the circulation.

**Conclusions:** These results suggest that mini-tablets with a 24% Eudragit enteric coating are suitable for targeted release of orally delivered V565 in the intestine for topical treatment of IBD.

**Introduction**

Crohn’s disease (CD) and ulcerative colitis (UC) are serious and lifelong chronic inflammatory bowel diseases (IBDs). The two forms of IBDs differ in the location and distribution of intestinal inflammation; CD is a segmental, transmural disorder, which can affect any part of the gastrointestinal (GI) tract but most commonly occurs in the ileum, cecum, and ascending colon, while UC is characterized by continuous inflammation of the colon [1]. Selective neutralization of membrane and soluble tumor necrosis factor (TNF)-\(\alpha\) by antibodies is an effective and transformative treatment for IBD. Four anti-TNF\(\alpha\) antibodies: infliximab, adalimumab, certolizumab pegol, and golimumab are currently used clinically for the treatment of IBD [2–4]. These monoclonal antibodies (mAbs) must be administered parenterally requiring either a hospital visit or multiple injections that are inconvenient for both patients and medical practitioners, and can be painful. There are also major safety concerns with these anti-TNF\(\alpha\) antibodies including infusion reactions, injection site reactions, and increased risk of infections and malignancy associated with long-term systemic suppression of the immune system [5–7]. An orally administered anti-TNF\(\alpha\) domain antibody, which is active at the intestinal inflammatory sites in IBD patients and which is cleared rapidly from the bloodstream would therefore have major benefits including ease of administration and reduced systemic immunosuppression. However, oral delivery of antibodies represents a significant challenge due to the exposure to proteases present in intestinal fluids during transit through the GI system both in man and in animal species to be used for preclinical development.

Variable domain antibodies retain the potency and specificity of conventional antibodies, and have unique properties including their small size (12–15 kDa), that make them a useful starting point for developing an oral therapy [8]. This scaffold can be engineered to produce domain antibodies that are resistant to intestinal proteases and are suitable for oral delivery (Vorabodies). V565 is a novel anti-TNF\(\alpha\) Vorobody with potency against soluble and membrane TNF\(\alpha\) equivalent to adalimumab [9]. Engineering of V565 was undertaken during the lead optimization process to enhance resistance of the domain antibody to inactivation by intestinal proteases, while retaining the TNF\(\alpha\)-neutralizing activity and potency against membrane and soluble TNF\(\alpha\). In \textit{in vitro} studies established that V565 retained activity after incubations with small intestinal proteases and in human ileal and fecal supernatants.
over extended time periods that are both stringent and highly relevant for simulating oral transit through the GI tract in man. However, under acidic conditions, the resistance of V565 to pepsin digestion was poor over long periods of time, demonstrating the need for formulation to protect V565 during passage through the stomach [9].

The rationale for treatment of IBD with an orally administered Vorabody is that very high levels will be released into the gut lumen, but will only permeate inflamed mucosal tissue at sites where the epithelium is ulcerated or where tight junctions have been disrupted [9–11]. Previous studies in mice with dextran sulphate (DSS) colitis demonstrated that V565, dosed orally with milk and bicarbonate to protect the Vorabody from stomach acid and pepsin, was able to penetrate inflamed intestinal mucosal tissues [9]. Due to their small size, the clearance of variable domain antibodies from the circulation is reported to be rapid, with elimination via renal filtration and excretion into the urine [12]. Consequently, we should not anticipate quantifiable serum levels after oral dosing. In patients with CD, we hypothesize that any V565 reaching the circulation from the inflamed lamina propria will be cleared rapidly by this mechanism, limiting bloodstream exposure, and potential systemic immunosuppression.

To provide a convenient dosing strategy that will protect V565 from degradation in the stomach, we describe in this article the formulation of purified freeze-dried V565 into 3 mm mini-tablets coated with a polymer sub-coat and a gastro-protective Eudragit enteric coat, and the study of the gastric resistance and intestinal dissolution characteristics of the mini-tablets following oral administration to monkeys. To facilitate this, methylene blue dye was incorporated into the mini-tablets to stain the intestinal tract on dissolution. This was used to reveal the location of V565 release from the mini-tablets (Figure 1). There is no primate model of IBD and the monkeys used in this study had no intestinal epithelial damage. Therefore, to confirm the rapid clearance of any Vorabody that would enter the circulation in IBD patients with epithelial lesions, an additional experiment to investigate the serum pharmacokinetics (PK) and confirm the rapid clearance and route of excretion of V565 from the circulation was conducted in monkeys using intravenous (i.v.) administration of V565.

Using these strategies, the aims of the study were to demonstrate in vivo that an enteric-coated mini-tablet formulation of V565 could survive passage through the stomach following oral administration and to show that active V565 is released directly to the required site of action in the ileum, cecum, colon, and rectum of the GI tract, with minimal absorption into the systemic circulation. A further aim of the study was to confirm that any V565 reaching the circulation was rapidly cleared via the kidneys and excreted in urine. The study was conducted in cynomolgus monkeys as V565 cross-reacts with primate, but not rodent, TNFα and therefore cynomolgus monkeys are the most appropriate species for toxicology studies. Also, as a primate species, data generated in cynomolgus monkeys are more likely to predict the pharmacokinetics of V565 in man than a rodent model.

**Materials and methods**

**Drug substance (V565) production**

V565 is a 115 amino acid 12.6 kDa single domain antibody with potent TNFα-neutralizing activity, engineered for resistance to intestinal proteases [9]. Using a proprietary fermentation process, V565 was secreted from *Saccharomyces cerevisiae* into the fermentation media and then separated from cells using microfiltration. V565 was purified using CaptoS (GE Healthcare, Amersham, UK) ion exchange chromatography followed by ultrafiltration. Purified V565 was then either freeze-dried to produce the drug substance (DS) for downstream manufacture of the oral drug product (DP) or further purified for i.v. administration.

**DP production**

**Formulation for IV Administration**

Purified V565 was endotoxin reduced using serial anion exchange chromatography and 5 μm filters. The material was further processed through a bioburden reduction filter (0.2 μm) and supplied as a sterile solution in 10 mM sodium acetate (pH 5, 26.7 mg/ml) and refrigerated. The solution was diluted 1:9 with vehicle (0.9% saline) to a V565 concentration of 2.67 mg/ml on the day of dosing.

**Formulation for oral administration**

V565 DS (56% per tablet core) was formulated with Pearlitol 200 (Roquette), Ac-Di-Sol (FMC), Avicel 102 (FMC), and magnesium stearate (Ligamed SA-2-V) and compressed into 3 mm mini-tablets.
These were then coated with a seal coat of Methocel E3 (5% polymer weight gain) and two thicknesses of Eudragit L100 (Evonik) enteric polymer according to the manufacturer’s recommendations. Methylene blue (Sigma, Gillingham, UK, M4159) (4% per core tablet) was included in the formulation as a marker to stain gut tissues, marking the site of dissolution. The mini-tablets were filled into Size 0 hydroxypropyl methylcellulose capsules (Capsugel) as described in Table 1. Capsules were stored in a cool, dry, and dark place at 2–8°C in a well-sealed container.

Dynamic dissolution test of mini-tablets
Separately, mini-tablets to the same formulation, but containing no added methylene blue (11 mini-tablets with 127.9 mg drug per capsule) were tested using a dynamic dissolution method [13] to simulate fasting intraluminal pH gradients with a USP II apparatus set with a paddle rotation speed of 50 rpm. The pH gradients were simulated using a dynamic pH controller for hydrogen carbonate buffers, pHysio-grad (Physiolution, GmbH). This device provides continuous pH adjustment of the buffer medium by microcomputer controlling both acidification by CO2 influx and alkalization by degassing with mixtures of CO2 and N2. The mini-tablets were emptied from the capsule for the purposes of this test. Following an initial 2 h period in 0.1 M HCl, the dissolution medium was replaced with modified Hanks’ bicarbonate buffer with modification of the pH with time using the pHysio-grad controller to simulate the pH changes that are predicted to occur during passage through the GI tract [13,14]. Overall, the test simulates a median pH profile of residence in the stomach, passage through the small bowel as well as modeling the pH profile of the colonic transit [14]. Samples over time were analyzed by UV absorbance at 279 nm to determine the concentration of V565 in solution.

Enzyme-linked immunosorbent assays
Adalimumab competition enzyme-linked immunosorbent assays (ELISAs) were used to detect V565 in serum, urine, and feces in the non-Good Laboratory Practice (GLP) pharmacokinetic studies.

V565 ELISAs. Briefly, ELISA plates coated with 175 ng/ml human TNFα were blocked with 1% bovine serum albumin (BSA) in phosphate buffered saline (PBS). Urine, fecal, and GI tract samples were centrifuged to remove all solids. For urine analyses, standards and samples were diluted in 1% BSA, 0.5% human AB + serum (Sigma H6914), and 0.1% urea in PBS. For fecal and GI tract sample analyses, supernatants and standards were diluted in 1% BSA, 1% human AB + serum, 0.6 M NaCl, 0.05% Tween20, and protease inhibitors (Sigma S8820, 2x conc.) in PBS. For serum analyses, standards and samples were diluted in 1% BSA, 0.5% human AB + serum, 0.6 M NaCl, and 0.05% Tween20 in PBS. Biotinylated adalimumab (LGC) was mixed with all standards and samples to give a final concentration of 2 nM before adding the mixtures to the plates. Bound biotinylated adalimumab was detected using ExtrAvidin-horseradish peroxidase (Sigma E2886) and visualized using TMB Microwell Substrate (KPL 50–76–00) before stopping with 0.5 M HzSO4 and reading at 450 nm.

Animal experiments
Animal experiments were conducted at Envigo (Huntingdon, UK). Cynomolgus monkeys were used, as V565 is pharmacologically active in this species, but not in other species typically used for preclinical safety assessment studies. Furthermore, non-human primates, including cynomolgus monkeys have been used for the preclinical evaluation of other anti-TNFα antibodies that are now used clinically for the treatment of IBD. The in-life experimental procedures undertaken during the course of these studies were subject to the provisions of the United Kingdom Animals (Scientific Procedures) Act 1986 Amendment Regulations 2012. The number of animals used was the minimum that was consistent with scientific integrity and regulatory acceptability, consideration having been given to the welfare of individual animals in terms of the number and extent of procedures to be carried out. Animals used in the study were female, aged ≥2 years, and weighing 2.5–3.0 kg. The health status and welfare of the animals was evaluated in accordance with the accepted animal husbandry procedures. The experimental procedures used in these studies and justification for the use of non-human primates were reviewed and approved by the institutional ethical review committee at Envigo.

Intravenous administration of V565 to cynomolgus monkeys
Dose administration and sample collection. Three cynomolgus monkeys were given an i.v. injection (slow bolus) of V565 (formula above) 1 h before feeding. The dose of 2.5 mg/kg was achieved by individual dose volumes calculated from the most recently recorded bodyweight. Blood samples were taken pre-dose and at 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, and 36 h post-dose. The blood samples were allowed to clot at room temperature, centrifuged at 2000g for 10 min to separate the serum, and the resultant serum samples were snap frozen on dry ice and stored at −70°C until analysis. Urine samples were collected separately into containers cooled by solid carbon dioxide, from each animal pre-dose, and 0–6, 6–12, 12–24, and 24–36 h post-dose. The resultant plasma and urine samples were analyzed for V565 levels by ELISA.

Oral administration of V565 to cynomolgus monkeys
Oral dosing study schedule. Animals were given a single dose of V565 for each experiment, with at least 1 week washout in between. Each study had a defined objective (Table 2): to investigate transit of formulated V565 through the entire GI tract, to investigate the systemic exposure of orally administered V565, and, in the final study where animals were culled 4 h post-dose, to measure V565 concentrations and to determine sites of

<table>
<thead>
<tr>
<th>Composition of formulated V565 in capsules.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manufacturing parameter</td>
</tr>
<tr>
<td>V565 per mini-tablet</td>
</tr>
<tr>
<td>Total mini-tablets per capsule</td>
</tr>
<tr>
<td>Total V565 per capsule</td>
</tr>
<tr>
<td>Eudragit L100 mini-tablet coating thickness (coating suspension weight gain)</td>
</tr>
<tr>
<td>Corresponding L100 polymer weight gain</td>
</tr>
<tr>
<td>Methylene blue per capsule</td>
</tr>
</tbody>
</table>
Animals

Dose administration and sample collection. Animals (n = 3) were given one capsule of V565 1 h before feeding. The animals were housed together except for the period just before dosing until approximately 2 h later when they were housed individually. In the final study, the animals were individually housed.

Blood samples. In Study 2, venous blood samples (0.5 ml) were taken from each animal at the following times in relation to dosing: pre-dose, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, and 24 h. The blood samples were processed as described above. A terminal blood sample (4 h post-dose) was taken from each animal in Study 3. Blood samples were processed as described above. A terminal blood sample (4 h post-dose) was taken from each animal in Study 3.

Fecal samples. Feces were collected at specified intervals throughout each study, and were frozen and stored at –80 °C until slurry preparation. The thawed feces were homogenized on ice with 4 ml/g feces of ice-cold sample buffer A (0.1% BSA, 0.6 M NaCl, 0.05% Tween 20, 10 mM EDTA, and 1X SigmaFAST protease inhibitor cocktail S8830 in PBS). Samples were mixed using an ultra-turrax to achieve a uniform slurry. If additional buffer was required to make a free-flowing slurry, the volume was noted. Small volumes were centrifuged to produce supernatants free of solid material before analysis.

GI samples. In Study 3 post mortem, the GI tract was separated by ligation into the following sections: stomach, duodenum, jejunum, ileum (30 cm of the small intestine before the ileum/cecum junction), cecum, proximal colon, distal colon, and rectum. Each GI section was collected into separate pre-weighed pots kept on ice. Each section was opened by cutting along its length, the contents removed and each section flushed with a recorded volume of ice-cold buffer B (0.1% BSA, 0.05% Tween 20, and 10 mM EDTA in PBS). The mixtures were kept ice-cold until homogenization. Any non-dispersed mini-tablets were identified and removed taking care to ensure they remained intact and their position noted. Contents of each section plus buffer B were homogenized and stored at –80 °C. Samples were centrifuged as above.

Table 2. Schedules for i.v. and oral dosing studies.

<table>
<thead>
<tr>
<th>Route</th>
<th>Study</th>
<th>Objective</th>
<th>Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV</td>
<td></td>
<td>To confirm the rapid clearance and route of excretion of V565 from the circulation</td>
<td>Serum and urine V565 concentrations</td>
</tr>
<tr>
<td>Oral</td>
<td>1</td>
<td>To investigate the transit of formulated V565 through the entire GI tract after oral dosing.</td>
<td>Concentrations of V565 in feces over 24h post-dose period</td>
</tr>
<tr>
<td>Oral</td>
<td>2</td>
<td>To investigate systemic exposure following oral administration of formulated V565</td>
<td>Serum V565 concentrations</td>
</tr>
<tr>
<td>Oral</td>
<td>3</td>
<td>To investigate localization of mini-tablet dissolution 4h after oral dosing of formulated V565</td>
<td>Methylene blue staining of GI tissue and luminal V565 concentrations. Serum V565 concentrations</td>
</tr>
</tbody>
</table>

Table 3. Pharmacokinetics following i.v. bolus administration at 2.5 mg/kg to cynomolgus monkeys (n = 3).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (ng/ml)</td>
<td>42529 ± 6791</td>
</tr>
<tr>
<td>AUC 0-24h (ng.h/ml)</td>
<td>25448 ± 3883</td>
</tr>
<tr>
<td>AUC 0-4h (ng.h/ml)</td>
<td>25505 ± 3916</td>
</tr>
<tr>
<td>Clp (ml/min/kg)</td>
<td>1.65 ± 0.23</td>
</tr>
<tr>
<td>Terminal half-life (h)</td>
<td>4.77 ± 0.97</td>
</tr>
<tr>
<td>Elimination half-life (h)</td>
<td>0.83 ± 0.07</td>
</tr>
<tr>
<td>Vss (l/kg)</td>
<td>0.16 ± 0.04</td>
</tr>
</tbody>
</table>

Data collection and analysis

V565 in fecal and GI tract supernatants was measured using the adalimumab competition ELISA. Dilution factors were calculated using sample weight, assuming the material had a specific gravity of 1 and that V565 was freely diffusible throughout. The data presented here are the V565 concentrations in feces and GI tract contents before addition of buffer, calculated from the V565 concentrations in the slurry supernatants, and the dilution factors used in their preparation.

Data availability

All data generated or analyzed during this study are included in this published article or are available from the corresponding author on reasonable request.

Health and safety

All mandatory laboratory health and safety procedures have been complied with in the course of conducting the experimental work reported in this article.

Results

V565 is cleared rapidly from the bloodstream into the urine following i.v. administration to cynomolgus monkeys

Purified V565 was administered to three cynomolgus monkeys by slow i.v. bolus at 2.5 mg/kg. Serum and urine samples were taken at intervals over 36 h and analyzed by ELISA. Pharmacokinetic parameters were calculated using non-compartmental analysis. Mean data are presented in Table 3. The elimination half-life was 0.83 h, which is consistent with that of other single domain antibodies [12,15]. The volume of distribution (Vss) was relatively small, and the plasma clearance was close to the physiological value for glomerular filtration rate in cynomolgus monkeys (2.2 ml/min/kg) [16]. The urinary recovery was variable, ranging between 18.4% and 89.5%. This is likely due to the technical difficulties of conducting a renal excretion balance study without radiolabelled material. Nonetheless, the 89.5% recovered from one animal strongly indicates that renal clearance is the primary elimination route for V565 from the bloodstream.

V565 mini-tablets coated with Eudragit-L100 were tested in vitro dissolution systems and demonstrated resistance to dissolution in acid (pH 1.0) and sustained release of V565 above pH 6

Oral delivery of V565 for the treatment of IBD presents a series of formulation challenges including protection against pepsin degradation...
during passage through the stomach, combined with targeted release of the Vorabody proximal to the site of intestinal disease activity (Figure 1). Acid-resistant Eudragit polymer was chosen for the coating of compressed V565 mini-tablet cores to provide gastric protection. Eudragit-L100 is designed to dissolve at pH 2.7, after exit of mini-tablets from the stomach via the pyloric sphincter. In this case, a coating timed for dissolution within the lower small intestine would allow V565 mini-tablets to transit through the duodenum and upper jejunum but begin dissolution thereafter (Table 4) delivering high concentrations of V565 to the ileum, cecum, colon, and rectum; the most common disease sites in IBD.

To investigate the survival of mini-tablets in an acid environment, and their subsequent dissolution at higher pH, mini-tablets were first incubated in 0.1 M HCl (pH 1.0), for 2 h, then subjected to a dynamic dissolution test in which the pH was increased to 6, after acid exposure, and modulated over time to reflect passage through the intestinal tract (Figure 2). No V565 was released during the 2 h acid incubation. Moreover, V565 release was only detected after a further 3 h, at which point the pH had reached 6.7. V565 solubilization continued gradually for approximately another 3 h during which time the pH varied between 6.5 and 7.5, until it was complete.

Oral dosing of V565 mini-tablets coated with Eudragit-L100 to cynomolgus monkeys demonstrated survival of the mini-tablets in the stomach and dissolution of the mini-tablets in the small intestine leading to V565 distribution throughout the intestine. To investigate the dissolution profile of mini-tablets in an acid environment, and their subsequent dissolution at higher pH, mini-tablets were first incubated in 0.1 M HCl (pH 1.0), for 2 h, then subjected to a dynamic dissolution test in which the pH was increased to 6, after acid exposure, and modulated over time to reflect passage through the intestinal tract (Figure 2). No V565 was released during the 2 h acid incubation. Moreover, V565 release was only detected after a further 3 h, at which point the pH had reached ≈7.7. V565 solubilization continued gradually for approximately another 3 h during which time the pH varied between 6.5 and 7.5, until it was complete.

Oral dosing of V565 mini-tablets coated with Eudragit-L100 to cynomolgus monkeys demonstrated survival of the mini-tablets in the stomach and dissolution of the mini-tablets in the small intestine leading to V565 distribution throughout the intestine. To investigate the dissolution profile of mini-tablets in vivo, methylene blue was added to the formulation as a marker. Since methylene blue binds to gut tissue and contents, its release acts as a spatial marker for mini-tablet dissolution, staining gut walls in dissolution areas and beyond. This approach allowed us to determine the locations of initial mini-tablet dissolution.

Three, co-housed animals were each given a single oral dose of formulated V565. All feces were collected at 4 h intervals and pooled for the purpose of determining V565 transit time and fecal concentrations of V565. No intact mini-tablets were found. Fecal concentrations of V565 up to 5 μM were observed (Figure 3), suggesting that the formulation enabled successful passage of active V565 through the entire GI tract.

Table 4. Average pHs of and estimated transit times to different compartments of the human and cynomolgus monkey GI tracts.

<table>
<thead>
<tr>
<th>GI Region</th>
<th>pH (fasted)</th>
<th>Transit time (min)</th>
<th>pH (fasted)</th>
<th>Transit time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach</td>
<td>2.7 ± 0.8a</td>
<td>30 (range 15–32)a</td>
<td>1.2–4.3d</td>
<td>153 ± 87d</td>
</tr>
<tr>
<td>Small Intestine (proximal)</td>
<td>6.0 ± 0.2a</td>
<td>247 (range 210–352)a</td>
<td>1.9–2.2d</td>
<td>174 (range 132–252)c</td>
</tr>
<tr>
<td>Small Intestine (distal)</td>
<td>7.7 ± 0.2a</td>
<td>241 ± 39b</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Small Intestine (average)</td>
<td>7.0 ± 0.2a</td>
<td>1503 ± 470a</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Colon</td>
<td>6.5 ± 0.3a</td>
<td>1591 ± 978b</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Koziolek et al. [15].
Maurer et al. [17].
Ikegami et al. [18].
Chen et al. [19].

Table 5. V565 Concentrations in the sera of monkeys 4h after oral dosing with a single capsule of formulated V565 (nd: not detected).

<table>
<thead>
<tr>
<th>Animal</th>
<th>Mean [V565], nM</th>
<th>Standard deviation, nM</th>
</tr>
</thead>
<tbody>
<tr>
<td>M234</td>
<td>nd</td>
<td></td>
</tr>
<tr>
<td>M236</td>
<td>4.45</td>
<td>0.47</td>
</tr>
<tr>
<td>M238</td>
<td>10.89</td>
<td>1.13</td>
</tr>
</tbody>
</table>

Systemic V565 exposure after oral dosing to monkeys was very low

After a wash-out period of 1 week the same animals were redosed as before and serum samples were collected to measure
concentrations of V565. The concentrations of V565 measured in sera were below the limit of detection (2.5 nM), precluding analyses of pharmacokinetic parameters such as AUC and oral bioavailability. In a further study of systemic exposure, serum samples taken at the time of cull in Study 3 were analyzed for V565. Despite very high V565 concentrations in the intestines of these monkeys (see below), serum V565 was only found at very low levels in two monkeys (Table 5). These results were not unexpected as V565 will poorly traverse the intact epithelium of normal animals in which there are no intestinal inflammatory lesions.

Methylene blue staining revealed mini-tablet dissolution occurred in the distal jejunum-proximal ileum

Following a further wash-out period of 1 week, the animals were re-dosed as before and sacrificed 4 h after dosing, when mini-tablets would be expected to have exited the stomach and begun dissolution. Feces were collected from post-dose to time of cull. At 4 h, a terminal blood sample was taken from each animal, and the GI tract was separated by ligation into sections. During removal of the GI luminal contents, it was observed that there were some mini-tablets intact and others that were partially dissolved (Table 6). Some intact mini-tablets were still present in the stomach, indicating that release from the stomach was not synchronous. Although intact or partially dissolved mini-tablets were found in the jejunum of some monkeys, no intact mini-tablets were found in the ileum or beyond. The higher numbers of intact mini-tablets present in the upper GI of M234 may suggest a slower exit of mini-tablets through the pyloric sphincter relative to the other animals in the study.

When the monkey intestines were examined, intense blue coloration was observed indicating the sites of mini-tablet dissolution (Figure 4(a,b)). In monkey M238 gut shown in Figure 4, mini-tablet dissolution was confined to the distal jejunum and proximal ileum. Staining occurred in the same gut area in the other two monkeys (M234, M236), although it was also observed in the cecum (Table 7).

Table 6. Undissolved or partially dissolved mini-tablets present in GI compartments 4 h post-dose.

<table>
<thead>
<tr>
<th>Monkey</th>
<th>Stomach</th>
<th>Duodenum</th>
<th>Jejunum</th>
<th>Stomach</th>
<th>Duodenum</th>
<th>Jejunum</th>
<th>Ileum</th>
</tr>
</thead>
<tbody>
<tr>
<td>M234</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>M236</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>M238</td>
<td>0</td>
<td>0</td>
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To determine the gastric protection efficiency of the mini-tablet enteric coating and V565 exposure in the different gut compartments after mini-tablet dissolution, monkeys were culled at 4 h post-ingestion and the luminal contents of each compartment removed for V565 analyses. This cull time was chosen because small amounts of fecal V565 were detected in the 4–8 h post-ingestion samples (Figure 3). V565 concentrations within all the monkey GI tract samples were analyzed by ELISA (Figure 5). Two of the three monkeys contained no detectable V565 in the stomach, while the V565 concentration in the third monkey (M234) was probably due to high residence time as discussed above and was relatively low compared with that found in the other intestinal compartments. All monkeys had V565 in their upper colons, demonstrating V565 survival into the lower GIT, whilst only monkey M234 contained detectable V565 in the lower colon, probably reflecting a slightly faster transit in this animal than in the other two. Very high V565 levels were found in the jejunum and/or ileum; the primary areas of mini-tablet dissolution. Moreover, in agreement with the methylene blue staining (Table 7), cecal V565 concentrations in M234 and M236 were >100 μM, while M238 cecal V565 was 34.7 μM. V565 was found in the duodenum of only one monkey (M234), where it's level was <1 μM.

Discussion

The clinical use of anti-TNFα antibodies has demonstrated their effectiveness in treating CD and UC patients, but systemic exposure to these agents is accompanied by increased infection risk and increased risk of malignancy [5–7]. An alternative approach for treatment of IBD using oral anti-TNFα delivery results in topical exposure of the diseased tissue in the intestine and greatly mitigates risks associated with systemic exposure. In this study, we describe the development of an oral anti-TNFα Vorabody, V565, which has been engineered to resist degradation by intestinal and inflammatory proteases and survive at high concentrations within the intestinal tract of cynomolgus monkeys.

While the intended delivery route of V565 is oral, it was necessary to confirm its topical, rather than systemic, distribution. In a mouse model of IBD, DSS colitis, V565 delivered by oral gavage was demonstrated to transit across the inflamed intestinal epithelia into the lamina propria, where TNFα is produced [9]. Excess V565 is cleared via lymphatic drainage or entry into inflamed blood vessels in the lamina propria and is subsequently cleared from the circulation via renal filtration. Since no IBD model is

Figure 4. Methylene blue staining in the cynomolgus monkey GI tract following oral dosing with V565 mini-tablets containing methylene blue. (a) Example of methylene blue staining of gut tissue in one animal (M238), dashed box indicates section magnified in (b).
available in monkeys, and transit from the intestinal lumen across an intact intestinal epithelium will occur at a very low level, the clearance of V565 from the bloodstream was simulated by administering V565 via i.v. injection. Rapid clearance of V565 from the circulation was observed and V565 levels detected in the urine of intravenously dosed animals demonstrated that renal clearance of V565 from the bloodstream also occurs in monkeys. Taken together, these results build confidence that oral dosing of V565 to IBD patients should confine its anti-TNFα activity to the inflamed regions of the intestine, and rapidly clear upon access to the systemic circulation, minimizing systemic side-effects.

Although we were able to enhance the resistance of V565 to intestinal and fecal proteases, early work indicated there was insufficient stability to pepsin in an acidic environment, such as in the stomach. For this reason, lypo-phosphatidyl V565 was formulated in mini-tablets along with excipients to facilitate tablet disintegration under appropriate conditions, and these were covered with an enteric coat for protection during gastric transit. Observations during dynamic dissolution revealed that in vitro, V565 forms a gel on initial exposure to the aqueous buffer, from which V565 is slowly released into solution. mAbs at high concentration have also previously been reported to form gels [20]. The gel formation facilitated sustained release of V565 over several hours when dissolved at a physiological pH (Figure 2). This property should be beneficial in vivo as it will facilitate transit further down the small intestine before V565 release, consequently resulting in higher concentrations over a greater area in the lower intestinal tract.

In the initial oral dosing study, micromolar fecal concentrations of V565 were found 8–20 h after initial dosing. This demonstrates that when dosed in an enteric-coated formulation, active V565 is able to survive for long periods in the GI tracts of primates. The extended release profile of active V565 in cynomolgus monkey feces suggests that coverage in the human intestine is likely to last for many hours. This prolonged presence of V565 in the intestinal tract is probably due to a combination of asynchronous release of mini-tablets through the pylorus, delayed release through gelling, and natural diffusion throughout the luminal gut contents. Furthermore, V565 in feces was observed at concentrations up to 4.5 μM, well above the trough serum concentration of adalimumab required for mucosal healing (54–81 nM) in IBD patients [21]. This demonstrates the feasibility of the concept of oral dosing with formulated V565, and supports the use of V565 in IBD patients.

Addition of methylene blue dye to the mini-tablet cores allowed more in-depth investigation into mini-tablet dissolution within the cynomolgus monkey GI tract. Upon removal of the GI tract from each animal, methylene blue staining was evident at the dissolution sites (Figure 4). The location of staining revealed the primary site of dissolution to be between the distal jejunum and proximal ileum, though there were occasionally other dissolution areas (Table 7). This is encouraging, since active sites in CD are most commonly found in the terminal ileum and beyond [1]. If such a dissolution profile is replicated in patients, V565 would begin its release after the acidic conditions of the stomach but before reaching key disease sites.

V565 concentrations within the intestinal lumen were very high (Figure 5), yet despite this, the concentrations of V565 measured in serum from these animals were very low or undetectable. This supports the hypothesis that very little V565 is able to access the systemic circulation from a healthy gut. These concentrations in the gut lumen are significantly higher than the fecal concentrations shown in Figure 3. Figure 5 represents a snapshot taken at 4 h where mini-tablets are likely to have only recently dissolved. However, by the much later time points shown in Figure 3, V565 is likely to be much more widely distributed across gut contents, rather than existing as a discrete bolus, leading to lower peak concentrations. Nevertheless, in IBD patients we would expect these V565 concentrations to be sufficient to neutralize membrane and soluble TNFα within the accessible inflamed lamina propria and submucosa, even if tissue V565 concentrations are only 5% of those observed in the gut lumen or feces [22].

In summary, this study demonstrates the feasibility of dosing orally with V565. A 24% Eudragit coating provides protection from the acidic conditions of the stomach, while allowing sustained release in the intestine facilitated by a potential gelling effect. This dissolution profile allows high concentrations of V565 to cover large areas of the primate intestinal tract, surviving to excretion in feces at micromolar concentrations at extended time points. This highlights the resistance of V565 to degradation in the highly proteolytic environment of the intestinal lumen, and V565’s potential to access sites of active disease in IBD patients. Furthermore, an i.v. dosing study confirmed that very high concentrations of V565 in the circulation were rapidly cleared. This study strongly supports further, clinical studies using oral dosing

| Table 7. Methylene blue staining observed in the GI tract tissue of monkeys at 4h after oral dosing. |
|---|---|---|---|---|---|
| Degree of methylene blue tissue staining | Jejunum | Distal | Proximal | Ileum | Caecum |
| Monkey | Mid | Distal | Proximal | Mid | Distal | Caecum |
| M234 | – | +++ | +++ | – | – | ++ |
| M236 | + | +++ | +++ | – | – | +++ |
| M238 | – | ++++ | ++++ | + | – | – |

Figure 5. V565 concentrations in the GI tracts of individual monkeys 4 h after dosing with a single capsule of formulated V565. Biologically active V565 concentrations in GI supernatants were measured by ELISA. Mean ± SD, n = 3 sample replicates.
of V565 contained in enteric-coated mini-tablets, with the aim of developing a safe, effective, and convenient formulation for topical anti-TNFα IBD therapy. A multinational phase II study using V565 enteric-coated mini-tablets in CD patients is ongoing.

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Disclosure statement

JSC, KJR, TMC, LM, MFC, KPR, MCD, JCW, JIH, GAW, JRR and MRW are employees of VHsquared. JSC and JRR have shares in VHsquared. The authors declare no other financial or non-financial competing interests.

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