Oral delivery of a novel domain antibody (Vorabody™) for the treatment of Crohn's Disease

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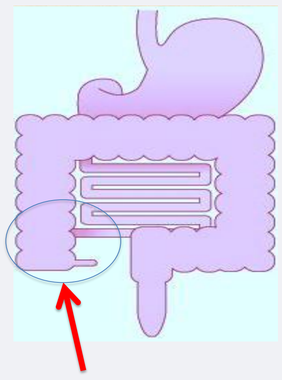
Introduction

Crohn's disease (CD) is a serious and lifelong chronic inflammatory bowel disease (IBD) which can affect any part of the gastrointestinal tract (GIT) but most commonly occurs in the lower part of the ileum, caecum and ascending colon.



Main region for targeted release of Vorabody™ in CD to ensure coverage over the full lower GI tract

Selective neutralisation of TNFα by antibodies is established as an effective and transformative treatment for CD. Three anti-TNFα antibodies: infliximab, adalimumab and certolizumab are currently used clinically for the treatment of CD.

These monoclonal antibodies (mAbs) must be administered parenterally requiring either a hospital visit which is inconvenient for the patient, or multiple injections. This mode of administration can be painful and can also result in unwanted systemic side effects associated with long term systemic suppression of TNFα. An oral antibody delivered locally to the site of inflammation in IBD could therefore have major benefits: ease of administration, preservation of efficacy, and the potential to minimise unwanted systemic side effects.

To date, other than protein replacement products, very few oral peptides have been commercialised, which reflects the significant challenge of oral biologic delivery.

Background

VHsquared Ltd. is a biotechnology company based in Cambridge, UK using proprietary technology to engineer domain antibodies for intestinal protease stability while retaining high potency against the target. This process generates proteins suitable for oral delivery for the treatment of immuno-inflammatory GI diseases. V565, the lead compound of the VHsquared Vorabody™ platform technology, is a 115 a.a., 12.6 kDa single domain antibody with potent TNFα-neutralising activity and resistance to inactivation by intestinal proteases.

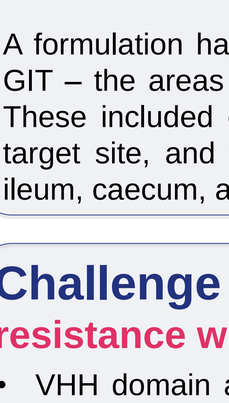
A formulation has been designed to deliver active V565 to the ileum and colon of the GIT – the areas most commonly affected by CD – by overcoming several challenges. These included ensuring that the Vorabody™ remains stable, releases at the correct target site, and that an adequate amount of drug is available where required in the ileum, caecum, and distal parts of the GIT.

Challenge 1: Stabilising V565 to increase GI protease resistance while retaining anti-TNFα activity

- VHH domain antibodies with potent TNFα-neutralising activity were isolated from a llama VHH phage display library.
- V565 was engineered by altering its amino acid sequence to better resist proteolytic inactivation. Variants with improved stability that retained potency to both soluble and membrane forms of TNFα were modified further resulting in the fully optimised Vorabody™ V565.

Challenge 2: Producing a formulation to minimise the risk of dose dumping and the effect of food

- V565 is produced by a proprietary *S. cerevisae* fermentation process which yields multiple grams per litre. V565 is easily purified from the yeast supernatant, freeze or spray dried, and formulated into 3 mm diameter mini-tablets which are encapsulated for ease of adherence to oral therapy.
- The mini-tablet formulation has sustained release characteristics such that the V565 is released over an extended period in the GIT. The selection of a multi-particulate dosage form of this type and size has a number of advantages:



- 3 mm mini-tablets are relatively easy to make using existing equipment
- Dosage can be altered easily by simply varying the number of mini-tablets in the capsule
- Their small size enables the mini-tablets to exit the pylorus intact
- The mini-tablets will distribute over the GIT maximising the chance of exposing the diseased mucosa to the drug
- The mini-tablet technology, whilst widely used in small molecule drug formulation, can only be applied to a biologic because of the robustness of the Vorabody™

Challenge 3: Protecting V565 from acid and proteases in stomach and upper GIT

- All proteins are destabilised in acid leaving them vulnerable to digestion from various proteases prevalent in the stomach and upper GIT.
- V565 mini-tablets are coated with an enteric Eudragit® L100 polymer, remaining intact until the coating begins to dissolve after exit from the stomach once a higher pH is reached (Tables 1 and 2).

Table 1: pH tolerance of Eudragit® polymers (Evonik literature).

Eudragit® Polymer grade	pH at which soluble
L100-55	5.5
L100	6.0
S100	7.0

Table 2: pH and transit time in different human GIT compartments (Sjogren et al, 2014)

GI tract region	pH (fasted state)	Time to transit (hours)
Stomach	1.2-2.0	0.25-2.0
Duodenum	6.0-	0.5-0.7
Jejunum	6.6	1.5-2.0
Ileum	7.5	
Colon	6.4-7.0	13-68

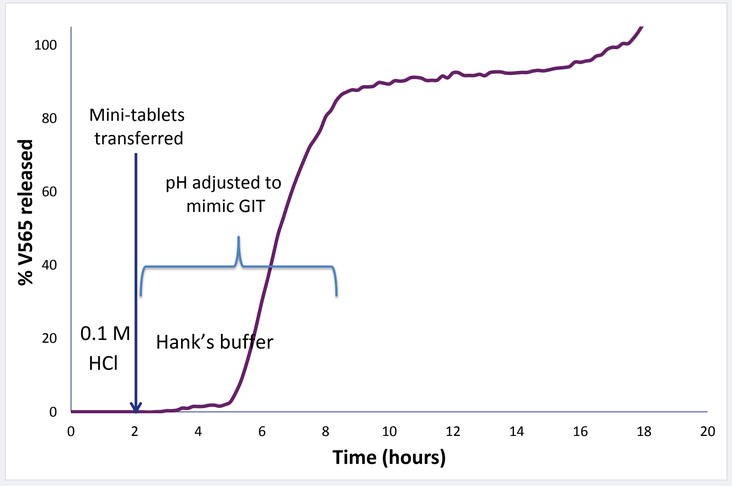
2-4 hours

- The pharmacopoeial requirement for delayed release (e.g. enteric coated) tablets is to release <10% of their content during 2 h in acid.
- V565 mini-tablet coating formulation has exceeded this requirement to ensure complete passage of the dose through the stomach, releasing no drug during 6 hours in 0.1 M HCl.
- This demonstrates tailoring small-molecule technology for biologic formulation.

Challenge 4: Optimising the Eudragit® coating thickness to target the desired regions of the GIT

1. *In vitro* dynamic dissolution testing

- Release of V565 from enteric coated mini-tablets was tested in a dynamic dissolution system using a USP 2 (paddles) bath (Garbacz et al, 2014). After a 2 hour period in 0.1M HCl the mini-tablets were transferred into a modified Hank's buffer medium (bicarbonate based) which more closely matches physiological fluid.



- Results showed no release of V565 in acid, with dissolution commencing after ≈2-3 hours.
- The start of release correlates to ≈ pH 6.0 (Fig. 1).

Figure 1: Dynamic dissolution. The dynamic dissolution system uses a combination of CO_2 and N_2 bubbled through the medium to either lower or raise the pH to mimic the pH profile that the dosage form would experience as it transitions the GIT.

2. Testing in non-human primates

- V565 toxicology tests were conducted in cynomolgus monkeys, since V565 only cross-reacts with primate TNFα. To select the optimum coating for the toxicology studies, pharmacokinetic studies were conducted to test the dissolution characteristics of mini-tablets *in vivo.*
- Animals were dosed orally with capsules containing mini-tablets with either a thinner (Set 1) or thicker (Set 2) Eudragit® coating. GIT contents were analysed after 4h.
- V565 release in the stomachs of Set 1 animals indicated inadequate gastric protection. V565 detection further down the GIT of Set 2 animals confirmed better bio-distribution with the thicker coating, progressed into toxicology and clinical studies.
- Detection of very high concentrations of active V565 in the ileum, caecum and colon in Set 2 animals, and in the faeces of orally-dosed animals, demonstrates that active V565 survives in the intestinal tract of primates, when optimally formulated.

3. Testing in humans

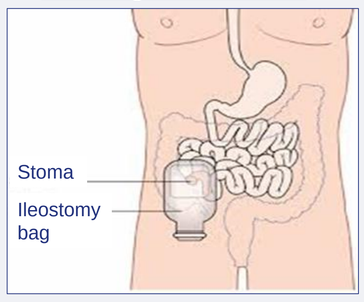


Figure 2: Ileostomy. A stoma in the ileum of a patient gives unique access to mid-GIT contents in humans.

- As part of a Phase 1 clinical study, 4 otherwise healthy individuals with ileostomy bags (Fig. 2) were given a single dose of V565 and ileostomy bag contents were analysed hourly.
- Peak V565 detection in the ileostomy bags occurred at 2-4 h, and V565 was detected up to 16 h in some patients reflecting the delayed release formulation.
- In addition, high concentrations of V565 were detected in the faeces of CD patients without an ileostomy, indicating survival through the entire patient intestinal tract.

Conclusions

- A protease-resistant, potent and selective domain antibody (Vorabody™) against TNFα has been successfully formulated for oral delivery.
- This formulation provides a delayed, sustained release of active antibody in the intestinal tract of humans, including those with Crohn's disease.
- This formulation is unprecedented for the delivery of a biologic, and the oral delivery of high concentrations of V565 anti-TNFα Vorabody™ directly to the site of disease has the potential to revolutionise IBD treatment.
- V565 is currently in a Phase 2 clinical study in North America and Europe.

References:

- Garbacz, G., et al. (2014). "A dynamic system for the simulation of fasting luminal pH-gradients using hydrogen carbonate buffers for dissolution testing of ionisable compounds." *Eur J Pharm Sci* **51**: 224-231.
- Sjogren, E., et al. (2014). "In vivo methods for drug absorption - comparative physiologies, model selection, correlations with in vitro methods (IVIVC), and applications for formulation/API/excipient characterization including food effects." *Eur J Pharm Sci* **57**: 99-151 and references therein.