Preclinical assessment of a novel anti-TNFα Vorabody™ as an oral therapy for Crohn's Disease

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Introduction

- VHsquared Ltd is a biotechnology company based in Cambridge, UK, using proprietary technology to engineer domain antibodies (Vorabodies) for oral delivery in the treatment of inflammatory bowel diseases. V565, the lead Vorabody product, is a 115 amino acid, 12.6 kDa single domain antibody that has potent TNFα-neutralising activity and engineered resistance to intestinal proteases.

- Crohn's disease (CD) is an incurable life-long disease that is difficult to control with conventional therapies. Selective neutralisation of TNFα has already been established as an effective therapeutic strategy for CD.

- Anti-TNFα antibodies such as infliximab, adalimumab and certolizumab - are currently being used clinically for the treatment of CD. However, they must be injected or intravenously infused which is inconvenient and painful for the patient.

- V565 is administered in a convenient oral formulation to enhance delivery to inflamed mucosal tissues - the site of overproduction of TNFα in CD. This is expected to limit systemic exposure and potential side-effects (such as systemic immunosuppression and infections) seen with conventionally administered anti-TNFα antibodies.

Background

- Potent TNFα-neutralising domain antibodies were isolated from a phage display library prepared from the blood cells of a llama that had been hyper-immunised with soluble human TNFα.
- Domain antibody leads with some intrinsic resistance to intestinal proteases were selected.
- V565 was developed through the engineering of these domain antibodies to enhance resistance to small intestinal and faecal proteases while retaining the TNFα-neutralising potency against both soluble and membrane forms of human TNFα.
- V565 does not cross-react with rodent TNFα and no suitable non-human primate model of IBD exists for preclinical efficacy testing. Instead, the TNFα-neutralising activity of V565 was investigated in *ex vivo* cultures of inflamed CD colonic tissue using the assay system described by Vossenkämper et al (2014).
- This model tested the inhibitory effects of the V565 Vorabody on the raised levels of signalling phosphoproteins and spontaneous production of cytokines that exist in CD under pathophysiological conditions.

Results

V565 neutralises soluble and transmembrane TNFα with comparable efficacy to adalimumab

- V565 effectively inhibits the biological activities of both soluble and membrane-associated forms of human TNFα with similar potency to the clinical TNFα-neutralising mAb adalimumab (Fig. 1).

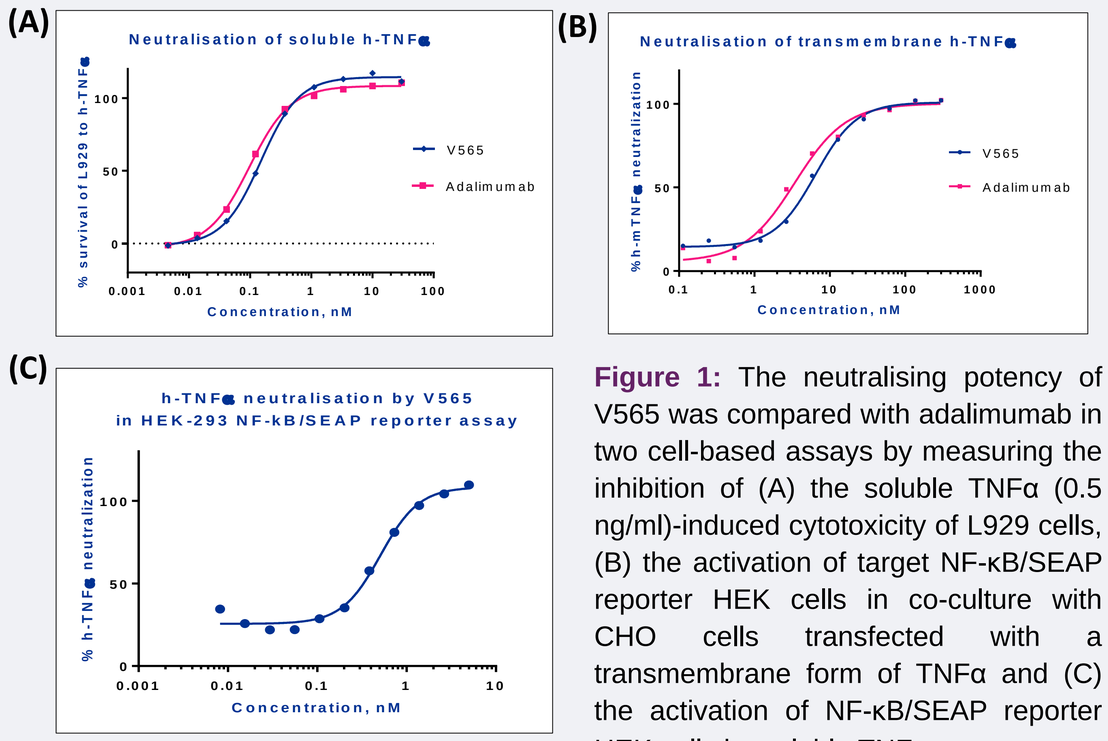
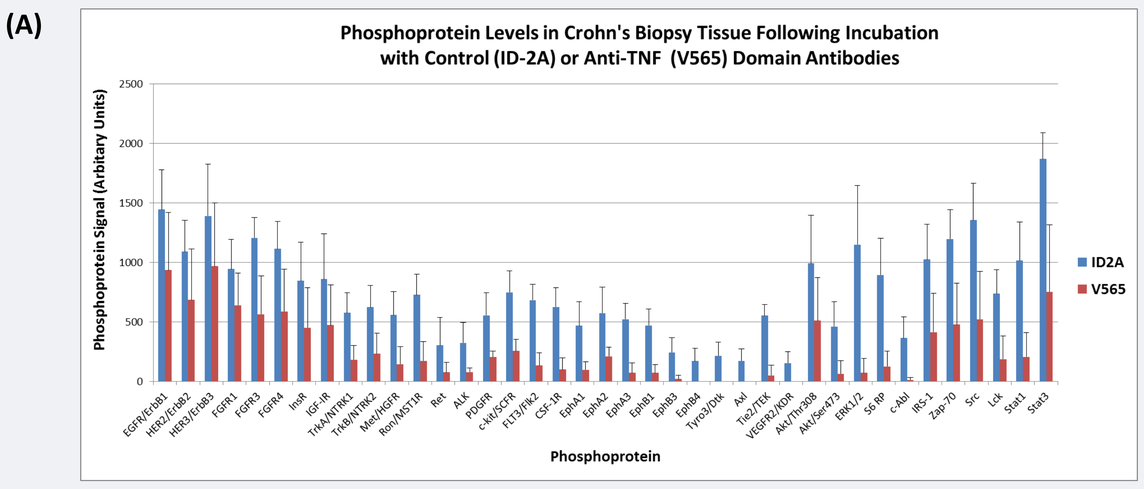


Figure 1: The neutralising potency of V565 was compared with adalimumab in two cell-based assays by measuring the inhibition of (A) the soluble TNFα (0.5 ng/ml)-induced cytotoxicity of L929 cells, (B) the activation of target NF-κB/SEAP reporter HEK cells in co-culture with CHO cells transfected with a transmembrane form of TNFα and (C) the activation of NF-κB/SEAP reporter HEK cells by soluble TNFα.

Analysis of phosphoproteins in active Crohn's biopsy tissue following culture with V565 or control for 24 hours

- V565 inhibited the phosphorylation of most of the proteins included on the array (Figure 2).
- V565 neutralisation of TNFα in *ex vivo* cultures of inflamed CD colonic tissue inhibited receptor signalling pathways in cell types that are important for the regulation of inflammation and disease pathology.



Spot ID	Phosphoprotein	Possible Functions
5	FGFR3	Epithelial cell regulation
6	FGFR4	Epithelial cell regulation
9	TrkA	Mast cell / enteric glia regulation
10	TrkB	Upregulated in colon cancer
12	Ron	Macrophage stimulating receptor
16	c-kit	Haematopoiesis & mast cell development
17	FLT3	Haematopoisis & immune cell regulation
18	M-CSFR	Macrophage activation
19	EphA1	Epithelial cell regulation / crypt architecture
20	EphA2	Epithelial cell regulation / crypt architecture
21	EphA3	Epithelial cell regulation / crypt architecture
22	EphB1	Epithelial cell regulation / crypt architecture
24	EphB4	Epithelial cell regulation / crypt architecture
25	Tyro3/Dtk	Macrophage activation
27	Tie2/TEK	Macrophage activation / Angiogenesis
28	VEGFR2	Angiogenesis
31	ERK1/2	Cell activation
32	S6 ribos. Protein	Cell activation
33	c-Abl	Immune cell regulation
36	Src	Immune cell regulation
37	Lck	T cell activation
38	Stat1	Cytokine signalling
39	Stat3	Cytokine signalling

Figure 2: Biopsies from four patients with active CD were incubated for 24h with an irrelevant control (ID2A; 250nM) or anti-TNF (V565; 250nM) VHH and tissue lysates were analysed on phosphoprotein antibody arrays (PathScan RTK Signalling Antibody Arrays; Cell Signaling Technology) with ECL detection. (A) Mean pixel values (n=4 biopsies) of the different phosphoproteins for each treatment; (B) Phosphorylation levels detected on the arrays for the control ID2A and V565-treated biopsies from one patient. White boxes = Spot IDs; (C) Protein identities.

V565 inhibits production of pro-inflammatory cytokines in cultures of active Crohn's disease biopsy tissue

- V565 also inhibited the spontaneous production of pro-inflammatory cytokines IL-1β, IL-17, IL-6, IL-8 and TNFα in *ex vivo* cultures of colonic biopsy tissue from patients with active CD (Fig. 3).

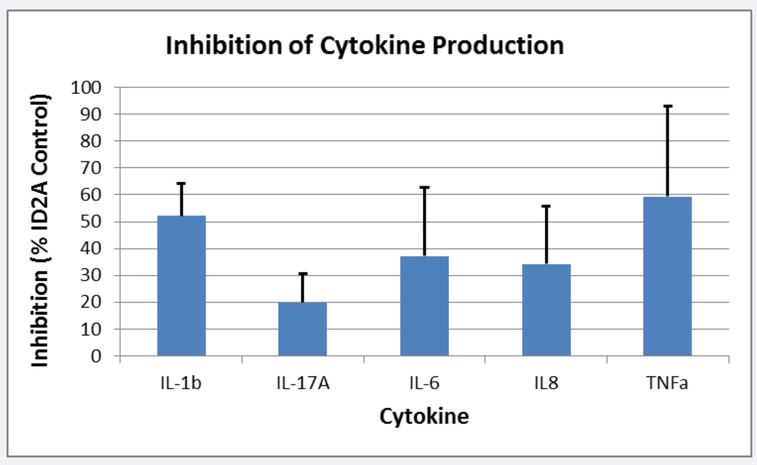


Figure 3: Culture supernatants were analysed for levels of IL-1β, IL-17A, IL-6, IL-8 and TNFα using multiplexed cytokine assay kits (R&D Systems MagPix). Cytokine data for the V565-treated biopsies were normalised to corresponding biopsy controls (n=4 CD patients). % inhibition values are given as mean +/- SD.

Conclusions

- In a model that closely reflects CD, Vorabody V565 suppressed the phosphorylation of multiple receptor tyrosine kinases and cytoplasmic signalling proteins and inhibited the release of inflammatory cytokines.
- The pattern of tissue phosphoproteins inhibited by V565 is almost identical to that achieved in a previous study with a clinically relevant concentration of infliximab (10 µg/ml) (data not shown).
- The inhibition of tissue biomarkers of inflammation in this model provides confidence that oral V565 will be effective in CD patients where the mucosal epithelial barrier is compromised.
- Analysis of phosphoprotein levels or cytokine production in colonic biopsies taken following oral treatment with V565 could be used to provide early markers of pharmacodynamic activity.

References

Vossenkämper et al 2014: A CD3-specific antibody reduces cytokine production and alters phosphoprotein profiles in intestinal tissues from patients with inflammatory bowel disease. Gastroenterology 147:172-83.