Narrow spectrum kinase inhibitors (NSKI) target kinases involved in both innate and adaptive immune responses leading to potent anti-inflammatory effects in experimental models of inflammatory bowel disease

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1. Introduction

- Pathogenesis of inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn’s disease (CD), is driven by increased levels of pro-inflammatory cytokines in the mucosal and sub-mucosal layers of the colonic wall.
- Key mitogen-activated protein (MAP) kinases involved in the inflammatory cascade include p38α and Src family (c-Src) kinases.
- The efficacy of NSKI TOP1210 as a MAP kinases inhibitor has been demonstrated in vitro assays, in vivo models of colitis and in explants from IBD patients.

2. Methodology

**In vitro**
- TOP1210-driven inhibition of recombinant p38α & Src was assessed in a cell-free, ATP-dependent, FRET-based biochemical assay.
- Interleukin (IL)-8 & interferon (IFN)γ release from LPS or anti-CD3/anti-CD28-stimulated human peripheral blood mononuclear cells (PBMC) was evaluated by ELISA.
- Inhibition of phospho-p38α (p-p38α) & phospho-Lck (pLck) in H2O2-stimulated human PBMCs was analysed by flow cytometry.

**In vivo**
- Naïve T cell transfer was used to induce colitis in Rag2-/- mice, with or without orally administered TOP1210 (5 mg/kg), twice daily for 28 days. Efficacy was assessed based on histopathology scores and cytokine measurements from colon homogenates.

Patient Explants
- Biopsies and Lamina Propria mononuclear cells (LPMC) isolated from IBD patients were cultured ex vivo in the presence or absence of TOP1210 and assessed for pro-inflammatory cytokine release by ELISA.

3. Results – in vitro

- Fig 1 - TOP1210 exhibited sub 100nM IC50 against recombinant p38α and Src family (c-Src) kinases.
- Fig 2 - In both macrophages and T cells, TOP1210 inhibition of inflammatory cytokines was more potent than corticosteroid Budesonide.
- Fig 3 - Inhibition of pP38α and pLck in stimulated human PBMCs was linked to a reduction in IL-8 and IFNγ (Fig 1) from stimulated human macrophages and T cell.

4. Results – in vivo – mouse T cell colitis model

- Fig 3 - Orally administered TOP1210 (5 mg/kg) achieved 42% inhibition in the summed histopathology scores, close to the greatest achievable inhibition for the adoptive transfer model (approx. 50%).
- Fig 4 - TOP1210 was also superior in efficacy to the maximum tolerated dose of Budesonide (1 mg/kg).
- Fig 5 - TOP1210 (5 mg/kg) exhibited a reduction in IL-8 and IFNγ from the colon homogenates to levels equal or greater than Budesonide (1mg/kg).

5. Results – Patient explants

- Fig 5 - TOP1210 is a potent inhibitor of IL-8 release from UC patient explants cultured ex vivo.
- Fig 6 - In LPMCs isolated from IBD patient biopsies, TOP1210 demonstrated more potent inhibition of IFNγ release compared to Budesonide.

6. Conclusions

- Through in vitro assays, in vivo models of colitis, and explants from IBD patients, we demonstrate that NSKI TOP1210 can modulate protein kinases (p38α & Lck) in cells of both the innate and adaptive immune system leading to inhibition of inflammatory cytokine release.
- TOP1210's efficacy, relative to Budesonide, suggests NSKIs may provide an important alternative to corticosteroids in the treatment of IBD.

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