Narrow spectrum kinase inhibitors (NSKIs) are superior to corticosteroid in both *in vitro* and *in vivo* inflammatory eye models (# 2687757)

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Introduction

- Inflammatory eye diseases such as non-infectious posterior uveitis and dry eye syndrome can be debilitating and in severe cases lead to visual impairment
- Corticosteroids (CS) are one of the primary treatment options for these patients however long-term CS use is limited by ocular side effects of cataract and iatrogenic glaucoma
- NSKIs have been developed to target p38-α, Src and Syk, key kinases involved in signalling cascades of innate and adaptive immunity
- Here, NSKI TOP1106 is compared to CS in primary retinal epithelial cells and an acute *in vivo* model of eye inflammation

Methods

- Inhibitory effects on recombinant p38-α, Src and Syk kinases were assessed in an ATP dependent ZLYTE™ assay
- Cellular assays were performed after 2hr pre-incubation with compound or vehicle. Peripheral blood monocytes (PBMCs) and primary human moncyte derived macrophages (HMDM) were stimulated with lipopolysaccharide (LPS). $CD4^+$ isolated lymphocytes were stimulated with anti-CD3/CD28. Primary human retinal pigmented epithelial (RPE) cells were stimulated with TNFα/IL-1β. Inflammatory cytokine release was measured by R&D ELISA
- In vivo efficacy was assessed in an endotoxin induced inflammatory model (EIU) where LPS was administered IVT into the eye of Lewis rats. Compound or PBS control was administered as a eye drop at -1hr, 0hr, 1hr, 2hr & 4hr relative to LPS exposure. Inflammatory cytokine levels in eye tissue homogenates were assessed 6 hours post LPS stimulation

In Vitro Results

- TOP1106 is a potent inhibitor of p38-α, Src and Syk kinases, key kinases involved in inflammatory signalling cascades (Table 1)

Table 1: Inhibition of substrate phosphorylation by TOP1106

	p38-α	Src	Syk
TOP1106 (IC_{50}, nM)	61	10	17

- TOP1106 potently inhibits pro-inflammatory cytokine release from cells of the innate (LPS stimulated PBMCs and primary macrophages) and the adaptive (α-CD3/CD28 stimulated $CD4^+$ T lymphocytes) immune response (Figure 1)

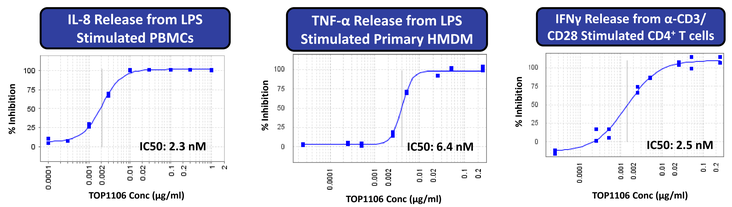

Figure 1: TOP1106 inhibition of inflammatory cytokine release from PBMCs, primary human macrophages and $CD4^+$ T lymphocytes. IC_{50} values quoted are geometric means from n≥3 experiments

- TOP1106 achieves marked inhibition of pro-inflammatory cytokines from primary RPE cells and is superior to CS (Figure 2)

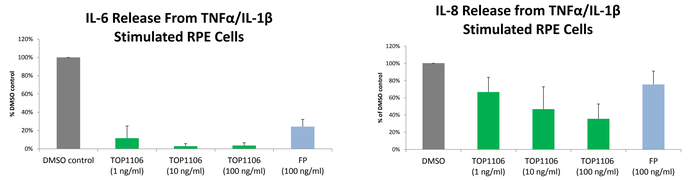

Figure 2: Inhibition of inflammatory cytokine release from primary RPE human Mean values quoted are from n≥3 experiments. (FP=fluticasone propionate)

In Vivo Results

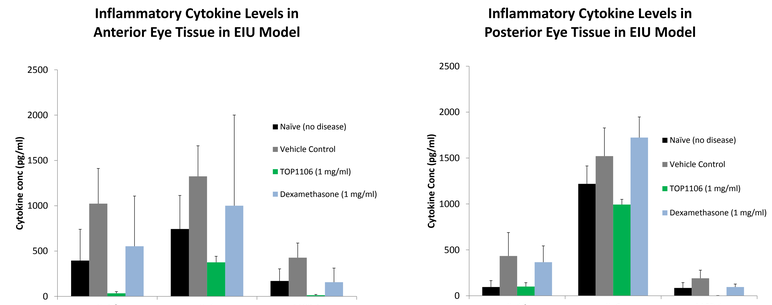

Figure 3: Inflammatory cytokine levels in anterior and posterior eye tissue 6 hours post LPS challenge. Data shown is mean of all animals in the group (naïve n=4, all other treatment groups n=8)

- Inflammatory cytokines IL-1β, IL-6 and MCP-1 are increased post LPS challenge in both anterior and posterior eye tissue in EIU model. TOP1106 achieves marked inhibition of all three cytokines in both anterior and posterior tissue.
- TOP1106 is superior to dexamethasone in the EIU model

Conclusions

- TOP1106 is a potent NSKI, which inhibits key kinases involved in inflammatory signalling cascades, leading to broad anti-inflammatory effects across cells of both the innate and adaptive immune response as well as primary RPE cells
- In the EIU model, following topical administration, TOP1106 effectively inhibits inflammatory cytokine release in both anterior and posterior eye tissue and is superior to corticosteroid
- This data highlights the potential of NSKIs as a novel treatment option for inflammatory eye disease

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