Narrow spectrum kinase inhibitors (NSKIs) potently inhibit inflammatory cytokines in both *in vitro* and *in vivo* inflammatory eye models

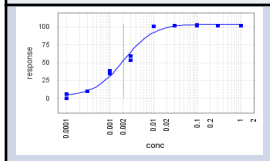
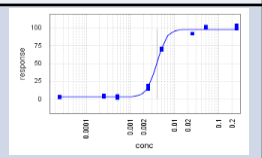
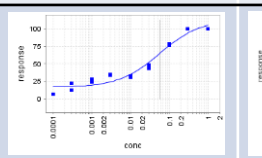
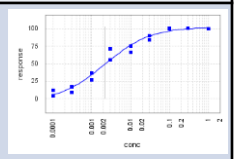
Claire Walshe[1], Martyn Foster[1], Isabel Crane[2], Gayathri Devarajan[2], Yemisi Solanke[1], Sameer Sirohi[1], Matthew Fyfe[1], Steve Webber[1]

[1] Topivert Pharma Limited, Imperial College Incubator, Level 1 Bessemer Building, London SW7 2AZ, UK
[2] The Institute of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen AB25 2ZD, UK

- NSKIs are a novel class of small molecule which selectively target key kinases involved in inflammatory signalling cascades
- NSKIs are very potent and efficacious inhibitors of inflammatory cytokine responses across a broad range of inflammatory cell types, spanning both the adaptive and innate immune responses
- In stimulated primary human retinal endothelial and epithelial cells nanomolar concentrations of NSKI (TOP1106) were more potent than corticosteroids (CS) in reducing inflammatory cytokine production
- Topical and intravitreal administration of TOP1106, in an endotoxin induced inflammatory eye model, were more potent than CS in reducing inflammatory cytokines in the anterior and posterior tissues of the eye, as well as inflammatory cell infiltrate into the aqueous humor
- These data demonstrate the potential utility of NSKIs for the treatment of inflammatory eye disorders

TOP1106 Is a Potent Narrow Spectrum Kinase Inhibitor With Broad Anti-inflammatory Activity

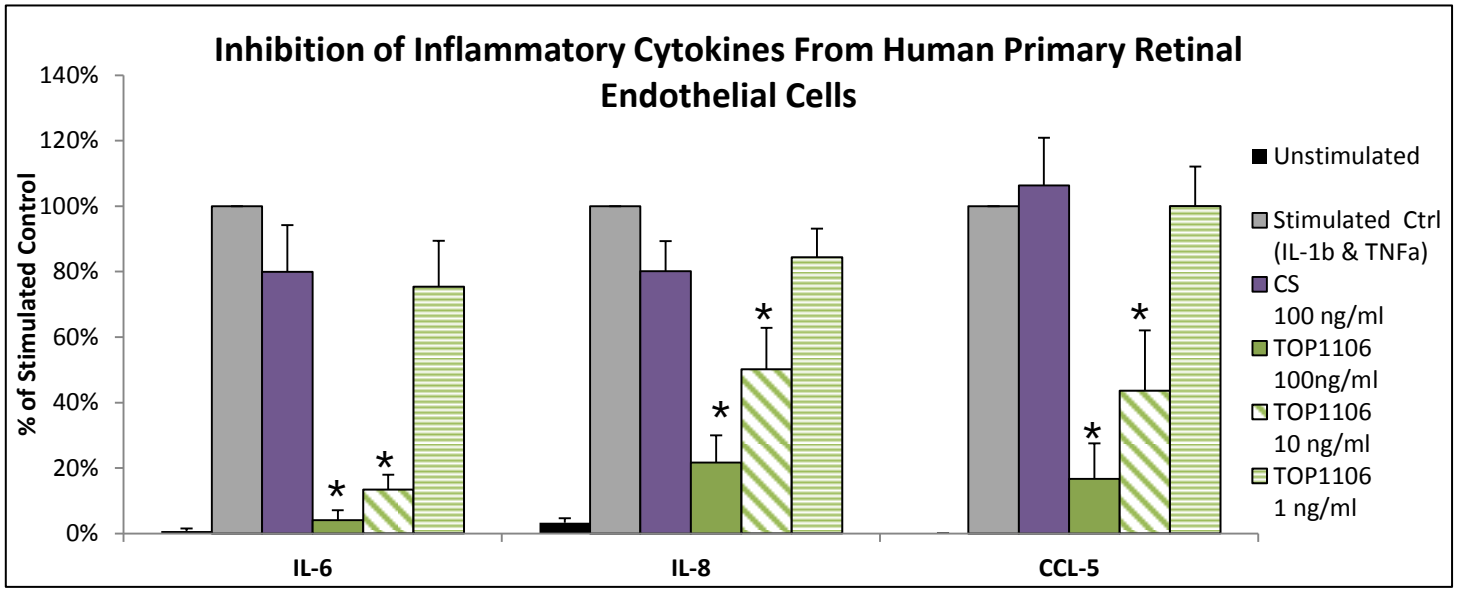
Kinase profile	P38 alpha (IC50 nM)	Src (IC50 nM)	Syk (IC50 nM)
TOP1106	65	10	17

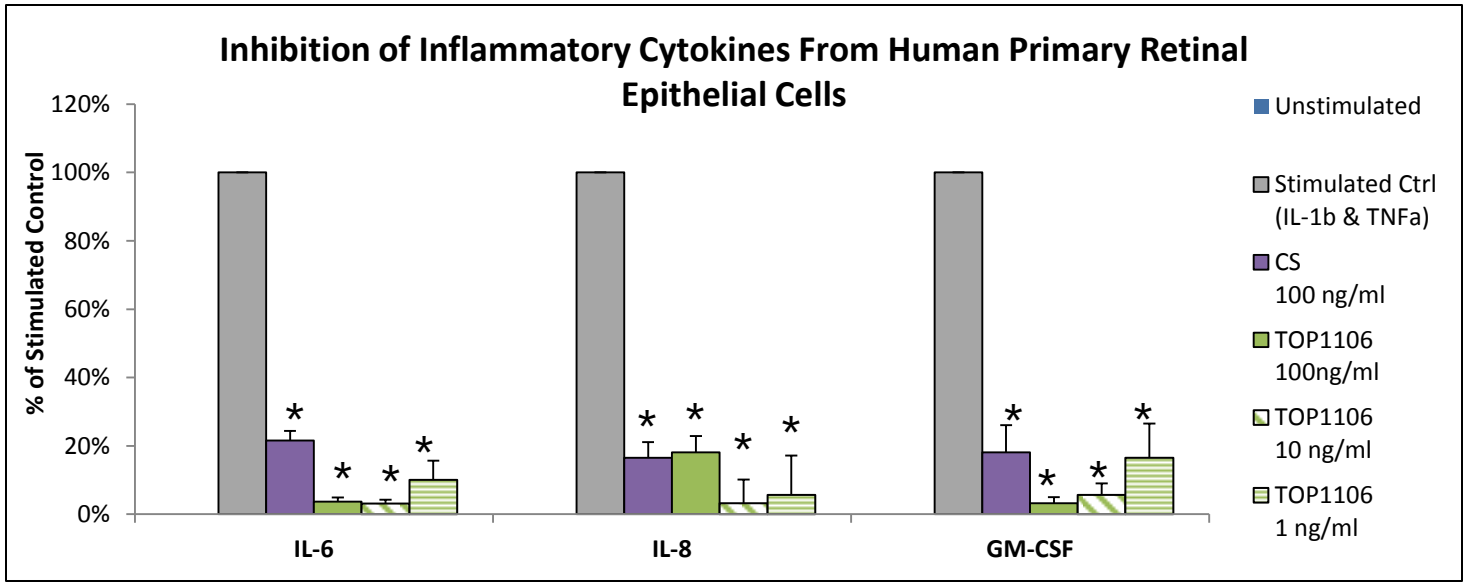
Phenotypic profile	Innate response		Adaptive response	
	Monocytes (IC50 nM)		T cells (IC50 nM)	
	PBMC (LPS stimulated IL-8 release)	Primary Macrophage (LPS stimulated TNFα release)	PBMC (α-CD3/CD28 stimulated IL-2 release)	PBMC (α-CD3/CD28 stimulated IFNγ release)
				
TOP1106	2.5	8.3	19.2	3.0
Budesonide	> 2300	1.0	> 2300	>2323

100% max. inhibition in all cell types

Kinase inhibition was measured using Z-LYTE Assay technology (Life Technologies™) which measures the inhibition of substrate phosphorylation. Human monocyte assays involved stimulation with 1-10 ng/ml LPS, in the presence of compound, for 24hrs before assaying the supernatant. Human T cell assays involved stimulation with α-CD3/CD28 , in the presence of compound, for 3 days before assay of the supernatant. Data shown are mean values of at least three independent experiments.

TOP1106 is a Potent Inhibitor of Pro-inflammatory Cytokine Release From Primary Retinal Cells

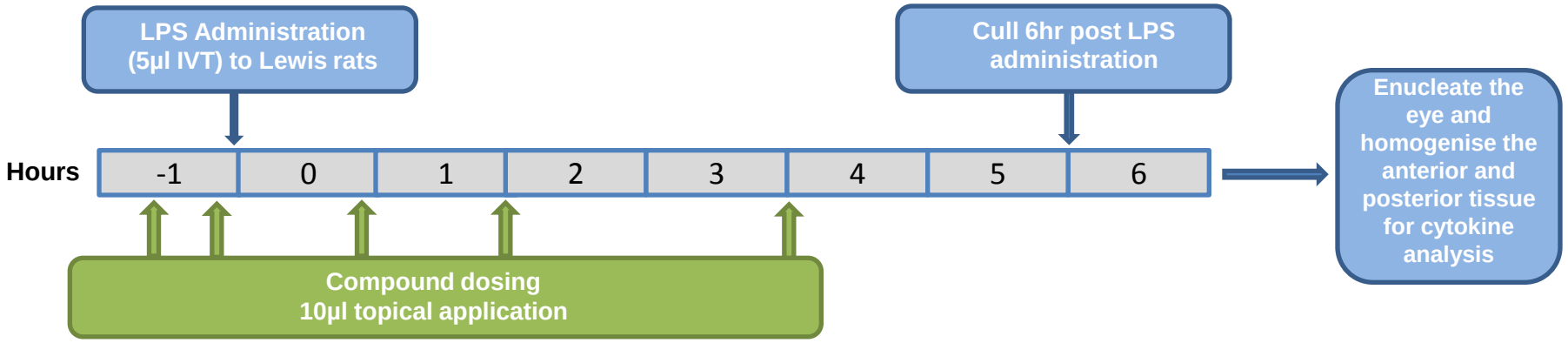


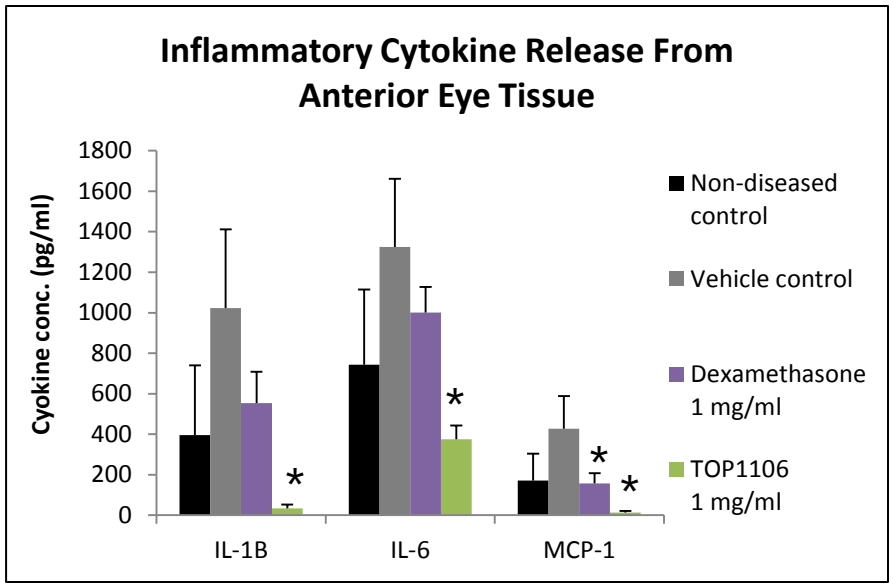


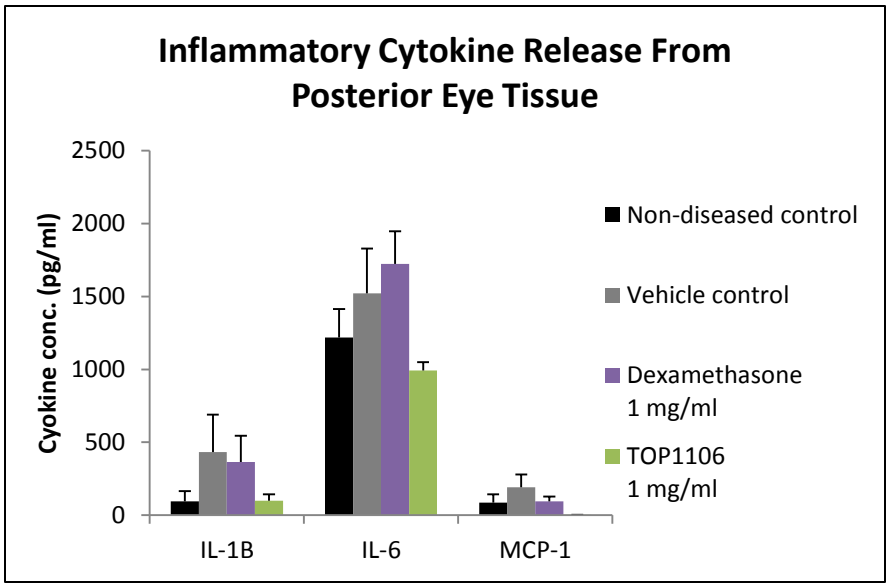
- TOP1106 is comparable, and in some cases superior, to corticosteroid (CS, fluticasone propionate) in retinal endothelial and epithelial cells
- Data shown is mean and standard error from at least three independent donors

- P<0.05 compared to stimulated control by one-way ANOVA

Topical TOP1106 is Superior To Dexamethasone in an Acute Model of Uveitis



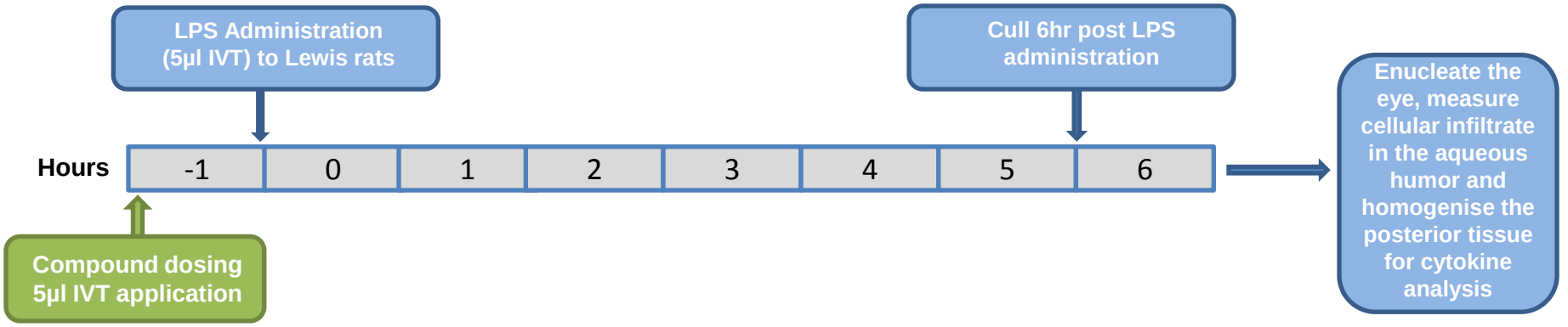


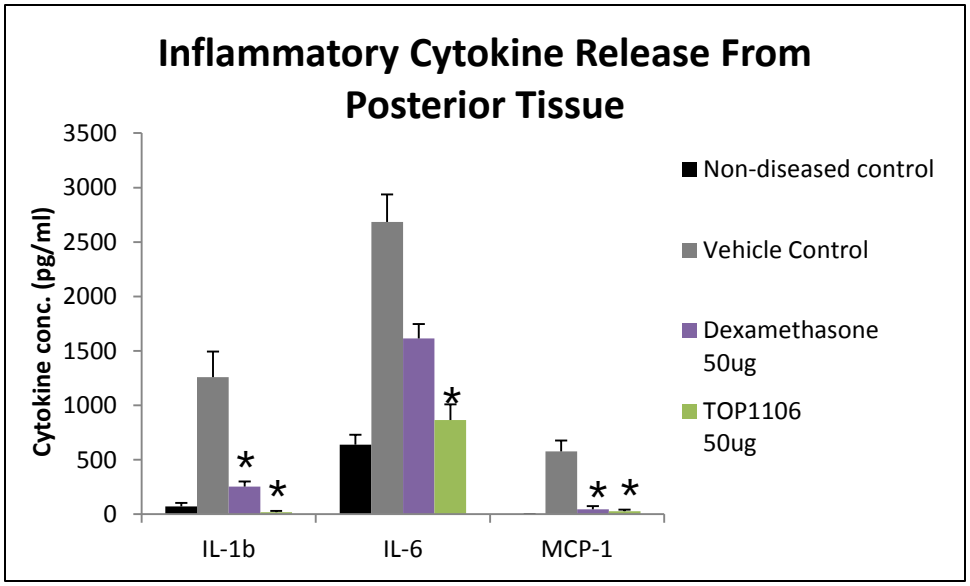


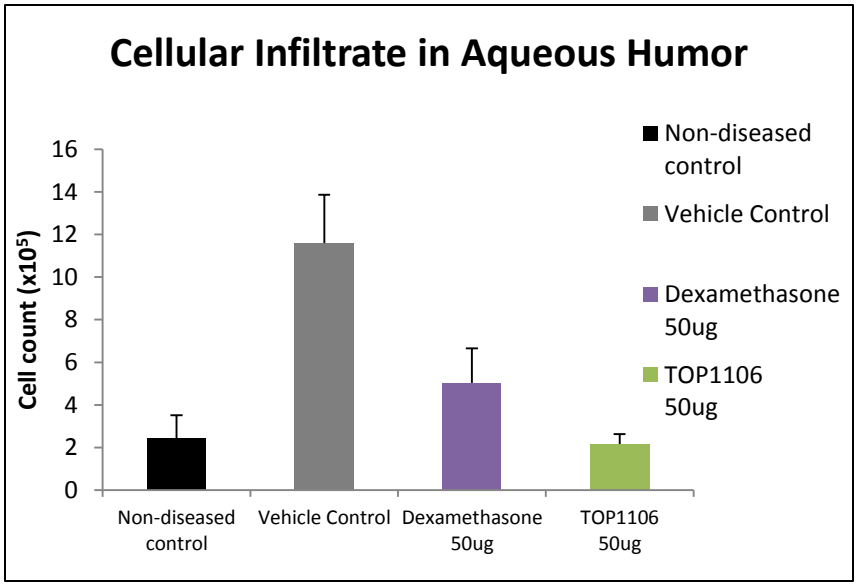
* P<0.05 compared to vehicle control by one-way ANOVA

Topical application of TOP1106 leads to superior inhibition of inflammatory cytokines in both anterior and posterior eye tissue compared to dexamethasone

Intravitreal Administration of TOP1106 is Highly Efficacious In EIU Model of Uveitis







* P<0.05 compared to vehicle control by one-way ANOVA

IVT administration of TOP1106 leads to superior inhibition of inflammatory cell infiltrate into the aqueous humor compared to dexamethasone. This reduction is most likely mediated through the inhibition of chemotactic cytokines expression in inflamed ocular tissues.