The narrow spectrum kinase inhibitor (NSKI) TOP1288 Demonstrates Potent Anti-Inflammatory Effects in a T cell Adoptive Transfer Colitis Model through a Topical Mode of Action

Yemisi Solanke, Martyn R Foster, Sameer Sirohi, Adele Rowley, Matthew CT Fyfe, Steve Webber, and Claire A Walshe
Topivert Pharma Ltd., London, UK;

Introduction

- Intracellular kinase activation plays a key role in inflammation and kinase inhibitors have been proposed as potential therapies in chronic inflammatory disorders such as ulcerative colitis
- Unwanted systemic side effects are often associated with current therapies for inflammatory bowel disease (IBD), particularly corticosteroids and immunomodulators
- A range of narrow spectrum kinase inhibitors (NSKI) have been developed to achieve topical, non-systemic, effects in the gastrointestinal (GI) tract when administered orally
- The activity of TOP1288, an NSKI in Phase 2 clinical development, has been compared to the immunomodulator, cyclosporine A (CsA), and budesonide in *in vitro* inflammatory assays and an adoptive transfer colitis model.

Methods

- Cellular assays were performed after 2hr pre-incubation with test compound or vehicle. Peripheral blood monocytes (PBMCs) were stimulated with either lipopolysaccharide (LPS) or anti-CD3/CD28 for 24 hours and 48 hours respectively. Inflammatory cytokine release was measured by ELISA. Data shown is mean of at least three independent experiments
- Pharmacokinetics of TOP1288, after oral administration, was assessed in naïve C57BL/6 mice. A single dose of 5mg/kg was administered by oral gavage in Corn oil, Transcutol, Maisine and Cremophor ELP (32.5:20:12.5:35)
- CD45RB^{high} CD4^{+} T cells were injected into SCID mice and disease allowed to develop for 14 days before treatment with either TOP1288 (0.03 – 3 mg/kg, P.O., BID in vehicle (as above) or cyclosporine A (50 mg/kg P.O., Q.D. in neoral/CMC) for a further 28 days. Efficacy was measured by histopathology score of the proximal colon. IL-8 and IFNγ levels in homogenised colonic tissue were also determined.

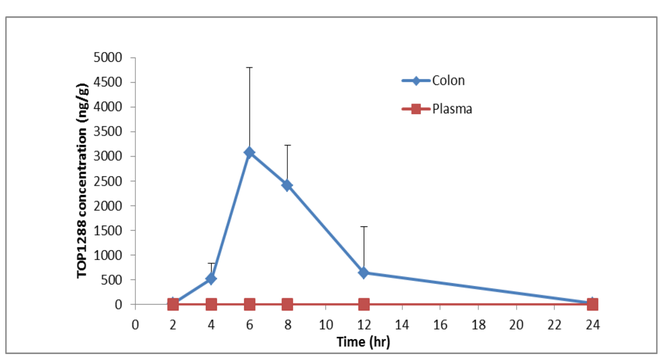
Results

- TOP1288 potently inhibits pro-inflammatory cytokine release in innate and adaptive immunity *in vitro* assays. In contrast, budesonide and CsA are only active in select assays (Table 1).

	Innate Response (LPS stimulated PBMCs)		Adaptive Response (α-CD3/CD28 stimulated PBMCs)	
	IL-8 release (IC_{50}, nM)	TNFα release (IC_{50}, nM)	IFNγ release (IC_{50}, nM)	IL-2 release (IC_{50}, nM)
TOP1288	1.6	0.6	6.8	77.2
Budesonide	>2322	6.2	>2322	7.9
CsA	>831	>831	51.4	5.9

Table 1: *In vitro* activity of TOP1288 and comparator compounds in assays related to innate and adaptive immunity

- TOP1288 achieves good colonic exposure with minimal systemic exposure (levels below detection limit of 1ng/ml) after oral administration in C57BL/6 mice (Figure 1)



	C_{max} (ng/ml)	AUC (hr*ng/ml)
Colon	3083	19841
Plasma	NC#	NC#

#Not calculated;levels below detection limit

Figure 1. Oral PK profile of TOP1288. Single administration of TOP1288 (5 mg/kg by oral gavage). Compound levels in plasma and colon tissue were measured over 24 hours.

Results

- TOP1288 achieves minimal systemic exposure after oral administration in the adoptive transfer model (Table 2). Exposure does not accumulate between days 22 and 39 and is consistent with exposure in naïve mice.

	Dose	Day 22 Mean ± SE ng/ml	Day 39 Mean ± SE ng/ml
TOP1288	3mg/kg	0.92±0.19	1.09±0.25
CsA	75 mg/kg	1739±338	1306±303

Table 2. Snap-shot plasma PK from adoptive transfer animals. Plasma taken on Days 22 and 39, 30 minutes after the first daily dose.

- TOP1288 is well tolerated and demonstrates anti-inflammatory effects comparable to CsA with marked effects on histological endpoints (Figure 2 & 3) and inflammatory cytokine release (Figure 3).

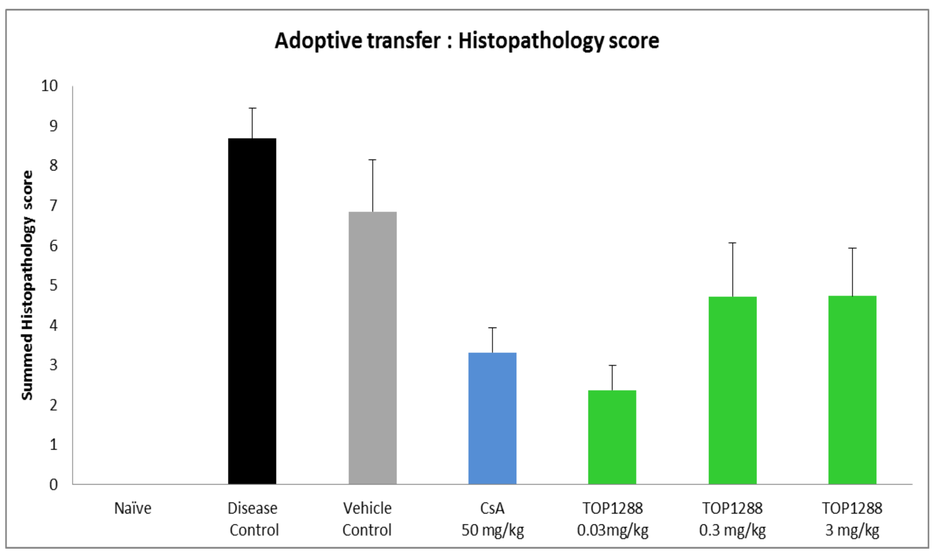


Figure 2: Effect of TOP1288 and CsA on disease pathology in an adoptive transfer model of colitis.

- TOP1288 effectively reduces IFNγ (Figure 3A) and IL-8 (Figure 3B). levels in the colon of animals with effects comparable to CsA. Inhibition of IL-8 release correlates with neutrophil infiltration (Figure 3C)

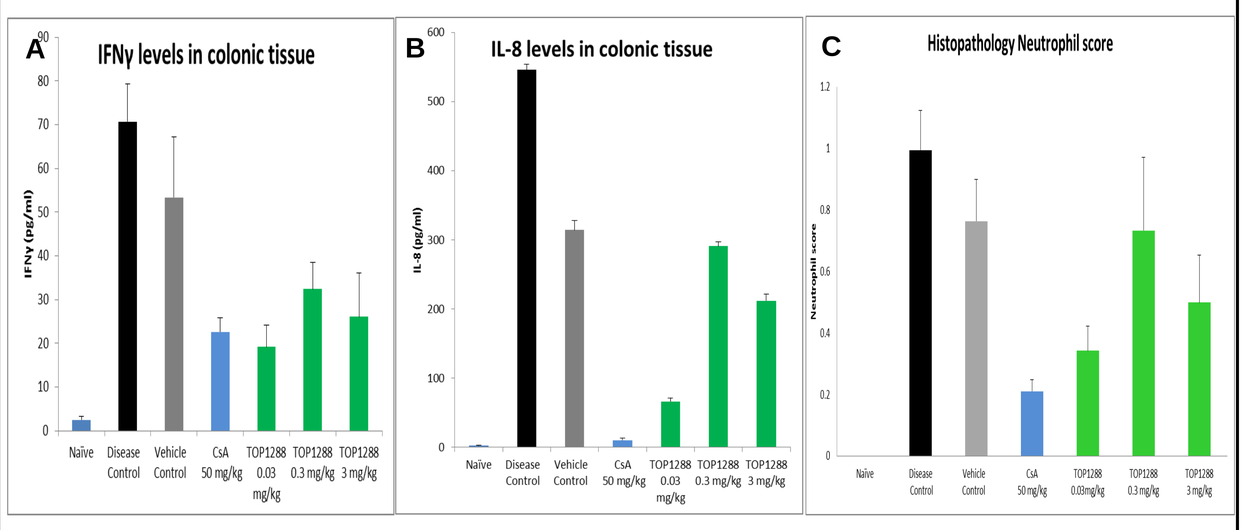


Figure 3: (A) IFNγ and **(B)** IL-8 levels in colon homogenates from adoptive transfer animals and matching **(C)** histopathology neutrophil score

- Although TOP1288 achieves comparable efficacy to CsA, there is a marked difference in systemic exposure, TOP1288 plasma concentrations >1000x fold lower than CsA, suggesting that TOP1288 acts through a topical mode of action.

Conclusions

- Unwanted systemic side effects are associated with some of the mainstay treatment options for IBD patients. A series of NSKIs has been developed to achieve topical, non systemic, effects in the GI tract. TOP1288 is a potent inhibitor in innate and adaptive in vitro cellular assays. Here, TOP1288 achieves anti-inflammatory efficacy comparable to CsA in a preclinical model of colitis at, approximately, 15x fold lower doses. In addition, TOP1288 has very low systemic exposure suggesting effects are mediated through a topical mode of action. TOP1288 is in Phase 2 clinical development and potentially offers improved safety and therapeutic benefit in IBD.