TOP1210 NSKI Demonstrates Superior Activity and Improved Safety Profile Potential Compared to Corticosteroid and Immunomodulators in Preclinical Models of Colitis

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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 inflammatory bowel disease (IBD)
- Unwanted systemic side effects are often associated with current therapies for IBD, particularly corticosteroids and immunomodulators
- 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 TOP1210, an exemplar NSKI, has been compared to a corticosteroid (budesonide) and the immunomodulator, cyclosporine A (CsA), in *in vitro* inflammatory assays and the adoptive transfer colitis model

Methods

- Cellular assays were performed after 2hr pre-incubation with test compound or vehicle. Human peripheral blood monocytes (PBMCs) were stimulated with either lipopolysaccharide (LPS), anti-CD3/CD28 or anti-CD3+ IL-2 for 24 hours and 48 hours respectively. Inflammatory cytokine release was measured by R&D ELISA. Data shown is mean of at least three independent experiments
- Pharmacokinetics of TOP1210, after oral administration, was assessed in naïve C57BL/6 mice. Five administrations of 5mg/kg was administered by oral gavage in peanut oil
- CD45RB^{high} CD4^{+} T cells were injected into SCID mice and disease allowed to develop for 14 days before treatment with either TOP1210 (0.04 – 5 mg/kg, P.O., BID in peanut oil), Budesonide (1 mg/kg, P.O., BID in peanut oil) or cyclosporine A (75 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 levels of homogenised colonic tissue were also assessed

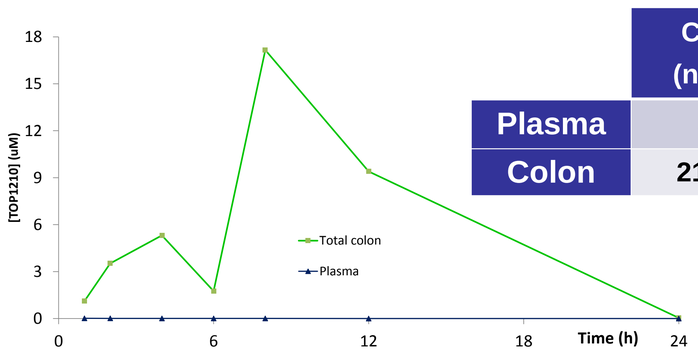
Results

- TOP1210 potently inhibits pro-inflammatory cytokine release in innate and adaptive immunity *in vitro* assays. In contrast, budesonide is only active in select assays (Table 1).

	Innate Response (LPS stimulated PBMCs)		Adaptive Response PBMCS	
	IL-8 release (IC_{50}, nM)	TNFα release (IC_{50}, nM)	α-CD3/CD28 stim IFNγ release (IC_{50}, nM)	α-CD3 + IL-2 stim IFNγ release (IC_{50}, nM)
TOP1210	2.0	0.7	2.5	0.3
Budesonide	>2322	6.2	>2322	1.2
CSA	>831.5	>831.5	59.2	3.3

Table 1: *In vitro* activity of TOP1210 and comparator compounds in assays related to innate and adaptive immunity

- Good colonic exposure with minimal systemic exposure is achieved after oral administration of TOP1210 in C57BL/6 mice (Figure 1)



	Cmax (ng/ml)	AUC (hr*ng/ml)
Plasma	3.6	15.8
Colon	2170.7	16896.7

Figure 1. Oral PK profile of TOP1210. Five administrations of TOP1210 (5 mg/kg by oral gavage). Compound levels in plasma and colon tissue were measured over 24 hours.

Results

- Dose dependent inhibition of disease pathology is achieve with TOP1210 (Figure 2). Efficacy of TOP1210 is superior to the highest tolerated dose (based on body weight loss) of budesonide (1 mg/kg) and comparable to CsA (75 mg/kg).

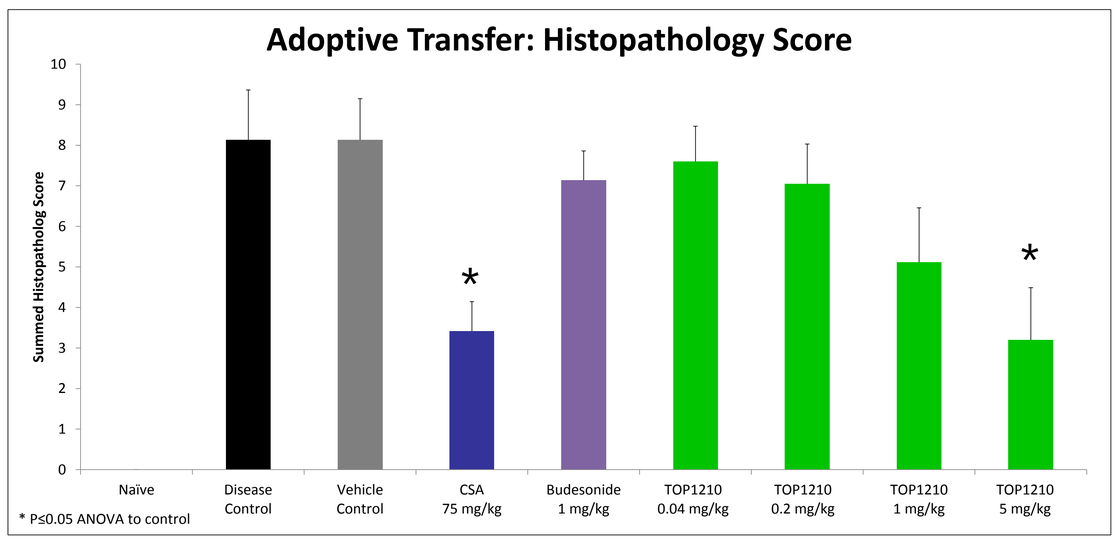


Figure 2: Effect of TOP1210 and clinical comparators on disease pathology in an adoptive transfer model of colitis.

- TOP1210 reduces IL-8 levels in the colon of treated animals (Figure 3A) and demonstrates improved tolerability over budesonide and CsA (Figure 3B).

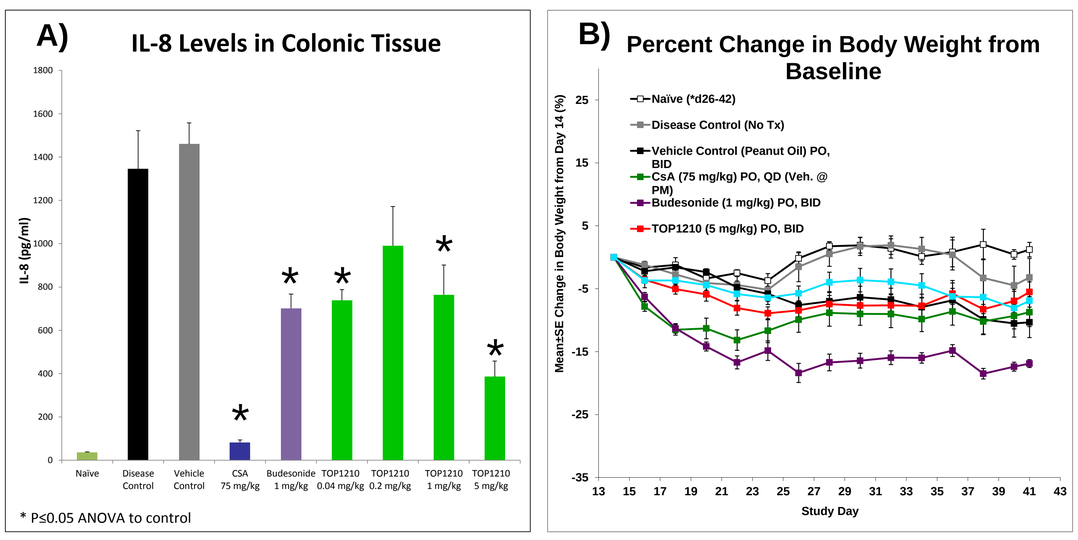


Figure 3: (A) IL-8 levels in colon homogenates from adoptive transfer animals **(B)** Body weight changes during in-life phase of the study

- TOP1210 has minimal systemic exposure after oral administration in the adoptive transfer model (Table 2). Exposure does not accumulate between days 22 and 38 and is consistent with exposure in naïve mice. Although TOP1210 achieves comparable efficacy to CsA, there is a marked difference in systemic exposure suggesting that TOP1210 is acting through a topical mode of action

	Dose	Day 22 Mean ± SE ng/ml	Day 38 Mean ± SE ng/ml
TOP1210	5 mg/kg	8.7 ± 2.2	7.2 ± 2.3
	1 mg/kg	3.6 ± 0.7	4.4 ± 1.3
CsA	75 mg/kg	5293 ± 638	1514 ± 113

Table 2. Snap-shot plasma PK from adoptive transfer animals. Plasma taken on Days 22 and 38, 30 minutes after the first daily dose.

Conclusions

- Unwanted systemic side effects are associated with some of the mainstay treatment options for IBD patients. NSKIs have been developed to achieve topical, non systemic, effects in the GI tract. Here, TOP1210 achieves superior efficacy over budesonide in a panel of *in vitro* assays and a preclinical model of colitis. In addition, TOP1210 (5 mg/kg) demonstrates an improved tolerability profile over Budesonide (1 mg/kg) and CsA (75 mg/kg).