[1]Centre for Immunology and Infectious Disease, Blizard Institute, Barts and the London School of Medicine and Dentistry, London, UK; [2]First Department of Medicine, Fondazione IRCCS Policlinico S. Matteo, University of Pavia, Pavia, Italy; [3]Department of Experimental Immunobiology, Division of Transplantation Immunology and Mucosal Biology, King's College London, London, UK; [4]TopiVert Pharma Ltd, London, UK; [5]RespiVert Ltd, London, UK

Down-modulation of Intestinal Production of Pro-inflammatory Cytokines in Experimental Colitis and in Inflammatory Bowel Disease by a Narrow Spectrum Kinase Inhibitor

Paolo Biancheri,[1,2] Renata Curciarello,[1] Nick Powell,[3] Jonathan W. Lo,[3] Claire A. Walshe,[4] Steve Webber,[4] Kazuhiro Ito,[5] Paolo Giuffrida,[1,2] Antonio Di Sabatino,[2] Gino R. Corazza,[2] Graham Lord,[3] Thomas T. MacDonald[1]

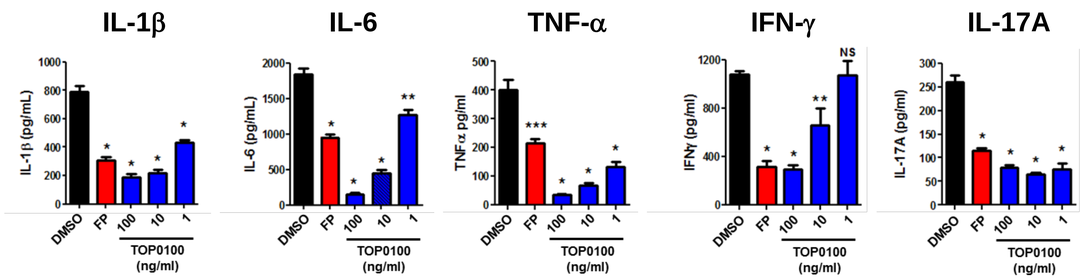
Background and aims

- Increased mucosal levels of pro-inflammatory cytokines such as interleukin (IL)-1β, IL-6, IL-8 and tumor necrosis factor (TNF)-α play an important role in sustaining gut inflammation in the two main forms of inflammatory bowel disease (IBD), Crohn's disease (CD) and ulcerative colitis (UC)
- Since protein kinases such as mitogen-activated protein kinases (MAPK) are crucial regulators of pro-inflammatory cytokine expression, we evaluated the *ex vivo* and *in vitro* effects of a narrow spectrum kinase inhibitor (TOP0100) in mouse colitis and on the inflamed mucosa of IBD patients

Methods

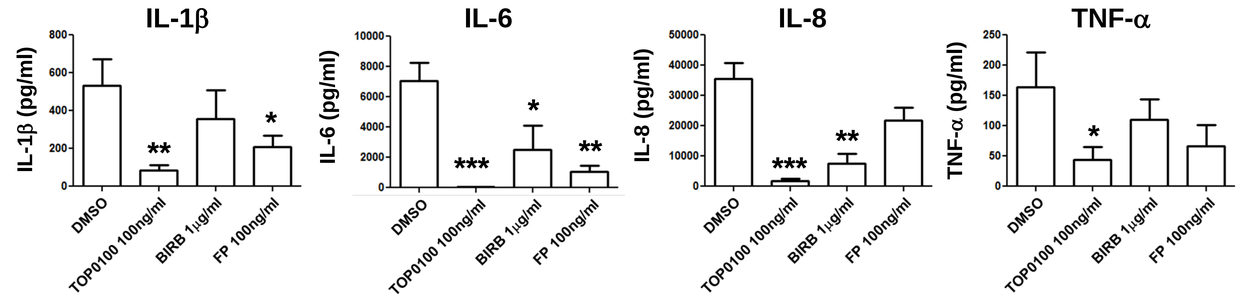
- Colitis was induced in Rag2-/- mice by means of naïve T cell transfer, and colonic explants were cultured *ex vivo* for 24 hours in the absence or presence of TOP0100 at 1-100ng/ml or fluticasone propionate (FP) (100ng/ml)
- Biopsies from inflamed intestinal mucosa of 19 IBD patients (9 CD and 10 UC) were either cultured *ex vivo* for 24 hours in the absence or presence of TOP0100 (100ng/ml) or the known MAPK inhibitor BIRB796 (1μg/ml) or FP (100ng/ml), or were used for lamina propria mononuclear cell (LPMC) isolation
- Anti-CD3/anti-CD28-stimulated IBD LPMCs were cultured for 48 hours with or without increasing concentrations (1-100ng/ml) of TOP0100 or BIRB796
- The production of IL-1β, IL-6, IL-8, TNF-α, interferon (IFN)-γ and IL-17A was evaluated in mouse and human and culture supernatants by ELISA

Results – Experimental colitis – Ex vivo



- TOP1081 inhibited in a dose-response manner IL-1β, IL-6, TNF-α, IFN-γ and IL-17A production by explants of inflamed mouse colon. The effect of TOP1081 was superior or comparable to FP

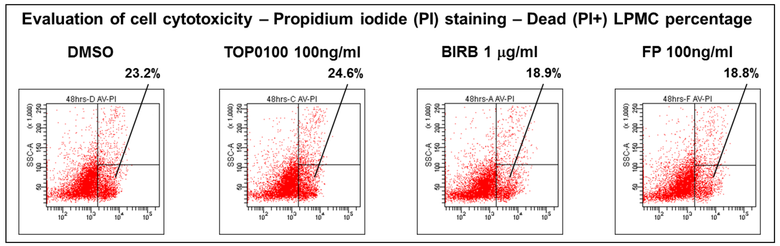
Results – IBD – Ex vivo

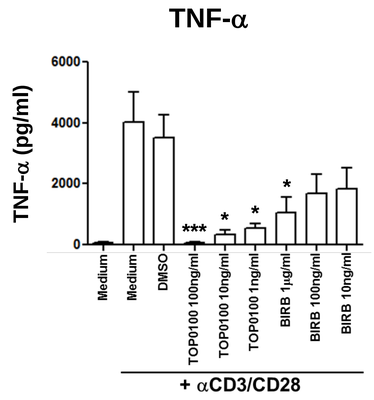


- TOP0100 significantly inhibited IL-1β, IL-6, IL-8 and TNF-α production by inflamed IBD mucosal explants cultured *ex vivo*. BIRB796 inhibited IL-6 and IL-8 release by IBD biopsies, whereas FP only inhibited IL-1β and IL-6 production

Results – IBD – In vitro

- TNF-α production by anti-CD3/anti-CD28stimulated IBD LPMCs was reduced in a dose-response manner by both TOP0100 and BIRB796, however TOP0100 was markedly more potent than BIRB796
- TOP0100 did not affect LPMC viability





Conclusions

- The narrow spectrum kinase inhibitor TOP0100 showed consistent down-regulatory effects on mouse *ex vivo* and human *ex vivo* and *in vitro* models of intestinal inflammation
- The efficacy profile compared to BIRB796 and FP and the effects on both innate and T cell-derived pro-inflammatory cytokines suggest that narrow spectrum kinase inhibitors are a potentially useful class of drugs for IBD treatment

p.biancheri@qmul.ac.uk