Receptor-interacting protein 1 kinase inhibition therapeutically ameliorates experimental T cell-dependent colitis in mice

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Receptor-interacting protein 1 (RIP1) has emerged as a key protein for transducing signals induced by several immune receptors, including tumour necrosis factor receptor 1 (TNFR 1). When these receptors are engaged, RIP1 kinase activity can drive cell death (apoptosis and necroptosis) and proinflammatory cytokine production which has been implicated in the pathogenesis of multiple inflammatory diseases1,2,3. To date, although RIP1 kinase has been implicated in driving inflammation in multiple tissues, the data points to a key role of RIP1 in the intestines.

Inflammatory bowel disease (IBD) is a multifactorial disorder characterised by chronic intestinal inflammation and comprises two distinct diseases: ulcerative colitis (UC) and Crohn’s disease (CD). Human mutations in the RIP1 pathway, as shown in patients carrying a deficiency in NF-κB essential modulator (NEMO) or the linear ubiquitin chain assembly complex (LUBAC), result in TNF-dependent intestinal inflammation4,5. Similar to the human data, NEMOEC-KO mice display Paneth cell apoptosis and microbiota-driven chronic inflammation in the colon6. Additionally, it has been shown that SHARPIN-deficient cpdm mice, that have a defect in LUBAC, develop TNF-dependent multiorgan inflammation6. Moreover, RIP1 might be a key driver of Paneth cell death as suggested by increased necroptosis in human Paneth cells in the terminal ileum of patients with CD7. It has also recently been shown that necroptosis is strongly associated with intestinal inflammation in children with IBD and that it contributes to strengthening the inflammatory process8. Together, these data suggest that the inhibition of RIP1 kinase activity represents an attractive therapeutic target for TNF-driven IBD in an integrated system.

At GlaxoSmithKline (GSK) we have developed small molecule inhibitors which potently bind to RIP1 with exquisite kinase selectivity and excellent activity in protecting from hypothermia in a model of TNF-induced sterile shock9. Furthermore, these molecules have also been shown to reduce the spontaneous production of cytokines from human UC and CD explants10. However, the use of animal studies is also critical in addressing mechanistic and translational questions related to IBD.

A recent study showed that necrostatin-1 (Nec-1) reduced intestinal inflammation in the chemically-induced DSS model of colitis11. Nec-1 is a tool used to explore the function of RIP1 kinase activity. However, its utility is limited due to the moderate potency, off-target activity against indoleamine-2,3-dioxygenase (IDO), and poor pharmacokinetic properties. GSK547 is a potent and highly-selective inhibitor of RIP1 kinase activity with suitable pharmacokinetic properties for dosing in chronic mouse models. In this study, we have tested for the first time the anti-inflammatory potency of GSK547 in the CD4+CD45RBhigh T cell transfer mouse model of colitis. Among all models of colitis, the T cell transfer model is significantly more relevant to human disease to investigate the immunological mechanisms responsible for the induction and perpetuation of chronic intestinal disease. This model has proved the most reliable in predicting the efficacy of therapies that have successfully progressed into the clinic (i.e. anti-IL-12/23 p40 monoclonal antibody therapy)12. Moreover, when compared with other animal models, the CD4+CD45RBhigh T cells transfer model

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Fig. 1 (See legend on next page.)
shows the most resemblance to human IBD in terms of colon gene expression changes.

In this study, chronic colitis was achieved by transferring CD4+CD45RBhigh T cells into immunodeficient female SCID mice (Supplementary Materials and Methods). After confirming the development of pathology using endoscopy (Supplementary Fig. 1), animals were treated therapeutically with GSK547 (50 mg/kg) or vehicle (0.5% hydroxypropyl methylcellulose in water) twice a day per os starting at 3 weeks after cell transfer (n = 10/13 mice per group). This dose was selected to maintain coverage of IC90 for 12 h based on the integration of known pharmacokinetic profiles and relevant in vitro potency (Supplementary Fig. 2). The severity of colitis was measured at termination (day 35) (Fig. 1a). The most common in-life indicator for this autoimmune colitis is body weight loss. Our data show that treatment with GSK547 significantly prevented body weight loss when compared with the vehicle treated group (Fig. 1b).

Post-mortem pathological observations showed that the treatment significantly ameliorated experimental T cell-dependent colitis in mice. GSK547-treated mice displayed decreased colon density (ratio weight/length), macroscopic signs of inflammation (disease activity index—scores of oedema, diarrhoea, presence of blood in the stools) and colon thickness (Fig. 1c–e). In clinical IBD, endoscopic and histopathologic indices are routinely used as outcome measures in controlled clinical trials and are essential for both fundamental and translational research in mouse models. Mucosal damage, assessed both by endoscopy and histology, was significantly reduced following treatment with the RIP1 kinase inhibitor (Fig. 1f, g). It is known that T cell driven colitis is generally associated with increased expression of tissue cytokines. In this study, the reduced protein expression of IFN-γ, IL-17A, TNF-α, CXCL-1, IL-6 and IL-12/23p40 in the colon (Fig. 1h–m), suggests that Type 1 (Th1) and Type 17 (Th17) helper responses were suppressed after treatment with GSK547. High-circulating serum amyloid A and faecal calprotectin levels are widely used markers in IBD for evaluating intestinal inflammation and mucosal healing. Both of these translational biomarkers were also decreased in the GSK547-treated group compared with vehicle controls, indicating the potential for RIP1 inhibitors to decrease markers of systemic inflammation and neutrophilic infiltration in the colon (Fig. 1n, o).

Taken together, our results suggest that RIP1 kinase inhibition has anti-inflammatory potential which strongly protects against the progression of chronic colitis. The efficacy following therapeutic administration of a potent and selective RIP1 kinase inhibitor on multiple endpoints in a translatable autoimmune model of colitis provides further scientific insight to support the rationale for an existing novel target in the management of IBD. These findings specifically align to the ongoing clinical programme evaluating the effect of RIP1 kinase inhibition in IBD patients.

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Conflict of interest
The authors declare the following competing financial interest(s): all authors are current stockholders of GlaxoSmithKline. All authors, except for Scott B. Berger, are current employees of GlaxoSmithKline. Scott Berger is currently at Janssen Pharmaceuticals.

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