Development of methods to investigate effects of narrow spectrum kinase inhibitors on cellular phosphorylation of kinase substrates

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Introduction

• Protein kinases play a critical role in intracellular signalling by catalysing the transfer of a phosphate group to substrate proteins, leading to activation of the inflammatory signalling cascade.

• Selective kinase inhibitors have been used as therapies in the treatment of chronic inflammatory diseases, such as rheumatoid arthritis and inflammatory bowel disease (IBD), but with limited success.

• In order to improve efficacy, targeted multi-kinase inhibitors have been developed, as exemplified by narrow-spectrum kinase inhibitor (NSKI) TOP1288, which target key kinases of the inflammatory signalling cascade; mitogen-activated protein (MAP), Src-family and Syk kinases.

• TOP1288 is proposed as a potential therapy for IBD and in order to investigate activity in inflammatory cells isolated from colon biopsies, flow cytometric methods have been developed to demonstrate inhibition of protein kinase in a clinical setting.

Methods

• Phospho-flow methods were developed in vitro in both a human Jurkat T cell line and primary cells derived from human whole blood (PBMC) or colon biopsies (LPMC).

• Activation was assessed using phospho-site specific antibodies against auto-phosphorylation epitopes of two key kinases (P38α and Lck) in the pathology of IBD, and subsequent inhibition with NSKI.

• Phosphorylation induced by stimulation with hydrogen peroxide (H2O2) and subsequent TOP1288-mediated inhibition was assessed by intracellular flow cytometry (in morphologically intact lymphocytes).

• In all cases, cells were pre-incubated with vehicle or compound for 2hrs prior to stimulation.

Results

Peripheral Blood Mononuclear Cells (PBMC)

• TOP1288 maintained similar potency and efficacy when assessed in human PBMCs isolated from healthy donors, compared to the T cell line (Figure 2).

• TOP1288 (3 µg/ml) achieved complete inhibition of the stimulated phospho-signal against both targets (Figure 2).

![Figure 2](image)

Lamina Propria Mononuclear Cells (LPMC)

• In LPMCs, phospho-Lck stimulation was achieved in response to H2O2. However, only minimal induction of phospho-P38α was evident (Figure 3A).

• TOP1288 potently inhibits phospho-Lck induction with an IC50 of 90ng/ml (Figure 3B), achieving maximal efficacy similar to that observed in PBMCs (Figure 3C).

![Figure 3](image)

Conclusions

• The methods developed allow semi-quantitative measurement of target engagement in individual cells of primary origin. We have been able to demonstrate that the NSKI, TOP1288, can potently inhibit activation of kinase targets in a range of different cells. Therefore, these methods have potential for use in clinical studies for biomarker measurements in pathologically relevant cells of IBD.