Effects of TOP1210, a narrow spectrum kinase inhibitor, and selective kinase inhibitors on the intestinal pro-inflammatory immune response in ulcerative colitis

Martyn R Foster², Paolo Biancheri¹, Matthew CT Fyle, Thomas T MacDonald ¹, Adele Rowley¹, Sameer Sirohi, Yemisi Solanke, Steve Webber¹, Eleanor Wood⁵ and Claire A Walseh.²
²Topivert Pharma Ltd., London, UK; ¹Centre for Immunobiology, Barts and the London School of Medicine and Dentistry, London, UK; ⁵Academic Department of Medical and Surgical Gastroenterology, Homerton University Hospital, 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.
• Selective kinase inhibitors, however, have proved disappointing, particularly in the treatment of rheumatoid arthritis and inflammatory bowel disease (IBD).
• Multi-kinase inhibition has been investigated as a strategy to improve efficacy.
• The activity of a narrow spectrum kinase inhibitor (NSKI), TOP1210, has been compared to selective kinase inhibitors (BIRB-796, dasatinib and BAY-61-3606) in a range of innate and adaptive inflammatory cell assays and in inflamed biopsies from ulcerative colitis (UC) patients.

Methods

• Inhibitory effects on recombinant p38, Src and Syk kinases was assessed in an ATP dependent ZLYTE™ based assay.
• Cellular assays were performed after 2hr preincubation with compound or vehicle. Peripheral blood monocytes (PBMCs) were stimulated with either lipopolysaccharide (LPS) or anti-CD3/CD28, monocyte derived macrophages with LPS, HT29 epithelial cells with IL1β and myofibroblasts isolated from inflamed UC mucosa with TNFα.
• Biopsies from inflamed ulcerative colitis patients were incubated with compound (24hr) and spontaneous cytokine release measured.
• A range of inflammatory cytokines in cellular and biopsy supernatants were measured by ELISA. Data is expressed as means ±s.e.m.of at least 3 determinations.

Results

• TOP1210 is a potent and efficacious inhibitor of innate and adaptive immune responses. Generally, compared to TOP1210, the selective kinase inhibitors have weak potency and efficacy.

Table 2: Effect of TOP1210 and selective kinase inhibitors on a range of innate, adaptive and epithelial cellular response assays.

<table>
<thead>
<tr>
<th>Kinase Inhibitor</th>
<th>IL-6 (IC50 ng ml⁻¹)</th>
<th>IL-8 (IC50 ng ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAY-61-3606</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>Dasatinib</td>
<td>315</td>
<td>630</td>
</tr>
<tr>
<td>BIRB-796</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
</tr>
</tbody>
</table>

![Figure 2](image2.png)  
Figure 2. Effect of TOP1210, BIRB796 (BIRB), dasatinib (DAS), or BAY-61-3606 (BAY) on the release of IL-6 and IL-8 by UC myofibroblasts.

Table 3. Inhibitory effects of the NSKI TOP1210 and the selective kinase inhibitors on pro-inflammatory cytokine release by Ulcerative Colitis myofibroblasts.

• TOP1210 is more potent than selective kinase inhibitors in reducing cytokine release from TNFα-stimulated UC mucosal myofibroblasts.

• TOP1210 potently inhibits all kinases tested, whilst BIRB-796, dasatinib and BAY-61-3606 show selective profiles.

Table 1: Inhibitory effects of selective kinase inhibitors and the NSKI TOP1210 on kinase activity in a biochemical Z-lyte based assay.

<table>
<thead>
<tr>
<th>Kinase Inhibitor</th>
<th>p38α IC50 (µM)</th>
<th>Src IC50 (µM)</th>
<th>Syk IC50 (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOP1210</td>
<td>65</td>
<td>10</td>
<td>17</td>
</tr>
<tr>
<td>BIRB-796</td>
<td>&gt;1895</td>
<td>&gt;1895</td>
<td>&gt;1895</td>
</tr>
<tr>
<td>Dasatinib</td>
<td>378</td>
<td>6</td>
<td>&gt;2049</td>
</tr>
<tr>
<td>BAY-61-3606</td>
<td>&gt;2562</td>
<td>&gt;2562</td>
<td>136</td>
</tr>
</tbody>
</table>

![Figure 3](image3.png)  
Figure 3. TOP1210 inhibitory effects on pro-inflammatory cytokine release from UC biopsies.

Conclusions

• Multi-kinase inhibition with NSKIs like TOP1210 leads to a efficacious and broad inhibitory profile in UC tissues and across a range of cell types including epithelial cells, innate and adaptive immune cells. Thus, NSKIs provide significant advantages over existing selective kinase approaches, and potentially offer much improved therapeutic benefit in IBD.