The pharmacological profile of TOP1288, a narrow spectrum kinase inhibitor (NSKI) in clinical development as an anti-inflammatory treatment for ulcerative 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 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 and a series of narrow spectrum kinase inhibitors developed, including TOP1288 which is now in Phase 2 clinical development, which target p38, Src and Syk kinases.
- The activity of TOP1288, has been compared to selective kinase inhibitors (BIRB-796, dasatinib and BAY-61-3606 targeting p38, Src and Syk respectively) in a range of innate and adaptive inflammatory cell assays and in inflamed biopsies from ulcerative colitis (UC) patients.

Results

- TOP1288 is a potent and efficacious inhibitor of innate and adaptive immune responses. Generally, compared to TOP1288, the selective kinase inhibitors have weak potency and efficacy.

Table 2: Effect of TOP1288 and selective kinase inhibitors on a range of innate, adaptive and epithelial cellular response assays (NA=not active).

<table>
<thead>
<tr>
<th>Kinase</th>
<th>PBMCs</th>
<th>Primary macrophages</th>
<th>Adaptive response: T cells</th>
<th>Epithelium</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-8 release</td>
<td>IL-8 release</td>
<td>TNF-α release</td>
<td>IL-2 release</td>
<td>IFN-γ release</td>
</tr>
<tr>
<td>BIRB-796</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Dasatinib</td>
<td>NA</td>
<td>NA</td>
<td>52</td>
<td>2.1</td>
</tr>
<tr>
<td>BAY-61-3606</td>
<td>607</td>
<td>NA</td>
<td>291</td>
<td>247</td>
</tr>
</tbody>
</table>

- TOP1288 is a potent and efficacious inhibitor in down-regulating pro-inflammatory cytokine release from Ulcerative Colitis biopsies with effects comparable to or better than those of prednisolone.

Figure 2. TOP1288 inhibitory effects on pro-inflammatory cytokine release from UC biopsies.

- TOP1288 is a potent inhibitor of cytokine release from TNFα-stimulated UC mucosal myofibroblasts.

Figure 3. Effect of TOP1288 or prednisolone on the release of IL-6 and IL-8 by TNFα stimulated UC myofibroblasts (n=2)

Conclusions

- Multi-kinase inhibition with NSKIs like TOP1288 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. TOP1288 which is in clinical development, may provide significant advantages over existing selective kinase approaches, and potentially offer much improved therapeutic benefit in IBD.

Methods

- Inhibitory effects on recombinant p38, Src and Syk kinases were assessed in an ATP dependent ZLYTE™ 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. or geometric means of at least 3 determinations.

Results

- TOP1288 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 TOP1288 on kinase activity in a biochemical Z-lyte assay (NA = not active).

<table>
<thead>
<tr>
<th>Kinase</th>
<th>TOP1288</th>
<th>BIRB-796</th>
<th>Dasatinib</th>
<th>BAY-61-3606</th>
</tr>
</thead>
<tbody>
<tr>
<td>p38α</td>
<td>116</td>
<td>24</td>
<td>659</td>
<td>NA</td>
</tr>
<tr>
<td>Src</td>
<td>13</td>
<td>NA</td>
<td>NA</td>
<td>136</td>
</tr>
<tr>
<td>Syk</td>
<td>378</td>
<td>6</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Figure 1. TOP1288 inhibits IL-8 release by LPS stimulated PBMCs (Figure 1A) and macrophages (Figure 1B), and also LPS stimulated TNFα release by macrophages (Figure 1C), with greater potency and efficacy than any of the selective kinases tested.