Introduction

Dry Eye Disease (DED) is a chronic, multifactorial disease that affects up to 30% of the over-50s population globally.

DED is characterised by pain, visual disturbances, tear film instability and, in severe cases, blindness. Ocular surface inflammation has a key role in DED progression and Current DED treatment options are limited: Topical Cyclosporine and Lifitegrast are the only FDA-approved therapeutics.

This study investigated the potential of TOP1362, a Narrow Spectrum Kinase Inhibitor (NSKI), as a novel treatment for DED.

Methods

Inhibitory kinase activity

NSKI TOP1362 was assayed in competition binding assays of p38-α, Lck and spleen tyrosine kinase (Syk), leading to determination of dissociation constants (KD) (KINOMEscan™, USA). Inhibition of p38-α and Src substrate phosphorylation was also assessed in a fluorescence resonance energy transfer (FRET)-based kinase assay (Z-lyte, Invitrogen, UK).

Eyepim Sample Collection and qPCR analysis

Human conjunctival epithelial cells (HCEs) were harvested from DED patients (n=9) and healthy controls (n=8) by impression cytology, using the Eyepim® device (Fig 1, Opio Technologies, France). No DED subjects were using preservative-containing eyedrops (Table 1). RNA was extracted and real-time polymerase chain reaction (qPCR) was performed to quantify differences in p38-α, Src and Syk expression.

Purpose

Clinical & Research

Results

Table 2. Inhibition of p38-α, Src family kinases and Syk by TOP1362. Compound potently inhibited all target kinases tested.

<table>
<thead>
<tr>
<th>Kinase</th>
<th>IC50 (nM)</th>
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<tbody>
<tr>
<td>p38-α</td>
<td>37</td>
</tr>
<tr>
<td>Src Family</td>
<td>3.7 (Lck)</td>
</tr>
<tr>
<td>Syk</td>
<td>18</td>
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</table>

Fig 2. Gene expression comparison of (A) p38-α, (B) Syk and (C) Src in subjects with DED versus healthy controls. Target gene expression was standardised to the housekeeping gene GAPDH. Key: ****p<0.0001.

Fig 3A. Graph of p38-α gene expression in hyperosmolar–challenged Chang cells. B) Table showing stimuli, time points and significance levels. Target gene expression was standardised to GAPDH. Assays were carried out in triplicate. Key: ****p<0.0001.

Fig 4. Effects of Dose-response treatment (TOP1362) on p38-α gene expression in 100mM NaCl–challenged Chang cells. All treatments are compared with vehicle (0.9% DMSO). Target gene expression was standardised to GAPDH. Key: ns, not significant. **p<0.005, ****p<0.0001.

Discussion

TOP1362 is a potent inhibitor of p38-α, Src family kinases (Src and Lck) and Syk kinases (Table 2).

In DED subjects, p38-α, Syk and Src were significantly upregulated in HCEs versus healthy controls (p<0.0001, Fig 2). p38-α expression in Chang cells was significantly increased by a hyperosmolar (NaCl) challenge (Fig 3).

Hyperosmolar-induced p38-α expression in Chang cells was dose-dependently inhibited by TOP1362 (Fig 4).

Conclusions

Further PD/PK profiling of TOP1362 in vivo models is required to determine its potential for future clinical studies.

TOP1630, another compound from this NSKI series, is currently being assessed in DED patients in a Phase 2 clinical trial (NCT03088605).

References


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