School of Health and Life Sciences

Narrow Spectrum Kinase Inhibitor (NSKI) Targets Are Up-Regulated in Conjunctival Cells from Dry Eye Patients Compared to Healthy Controls

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Introduction

- Dry Eye Disease (DED) is a chronic, multifactorial disease that affects up to 30% of the over-50s population globally[1]
- DED is characterised by pain, visual disturbances, tear film instability and, in severe cases, blindness[2,3]
- Ocular surface inflammation has a key role in DED progression[4-6]
- Current DED treatment options are limited: Topical Cyclosporine and Lifitegrast are the only FDA-approved therapeutics

Purpose

- 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 assessed 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[7] (Z-lyte, Invitrogen, UK).

Eyeprim 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 Eyeprim® device (Fig 1, Opia 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, Syk and Src expression.

Fig 1. Eyeprim® device utilised to harvest HCE cells from subjects with DED.

Gender	Age	OSDI	NITBUT	Schirmer (mm)	Eye sampled	DED (D) or Normal (N)
F	31	31.8	5	4	R	D
F	22	22.7	5	8	R	D
F	44	20.8	7	0	R	D
F	53	20.8	4	0	R	D
M	43	16.7	7.33	4	R	D
F	42	14.6	6	2	R	D
F	27	15	4	5	R	D
M	50	27.1	6.33	8	R	D
F	86	13.9	5	8	R	D
F	23	4.2	14	27	R	N
F	24	6.25	13.67	10	R	N
M	20	0	12.33	13	R	N
F	32	4.5	12	35	R	N
M	57	0	11	20	R	N
F	57	6.8	11	13	R	N
F	42	8.3	10	25	L&R	N
M	37	0	11	16	R	N

Table 1. Clinical data of study subjects. Definitions of normal: **OSDI**= <10, **NITBUT**= >10 seconds, **Schirmer**= >10mm of wetting (5 mins). **Key**: **R**-Right eye, **L**-Left eye.

Dose response of TOP1362 on Hyperosmolar-challenged Chang conjunctival cell line

The effects of **TOP1362** (0.01 – 1 μg/ml) on p38-α expression in hyperosmolar-challenged Chang cells (100mM NaCl, 6 hrs) were assessed. Target genes were standardised to Glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Assays were carried out in triplicate.

Results

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

	p38-α	Src Family	Syk
Competition Binding (Kd, nM)	37	3.7 (Lck)	18
Substrate Phosphorylation (IC_{50}, nM)	137	32 (Src)	N.D.

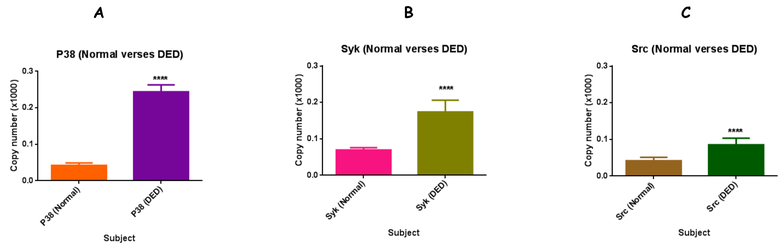
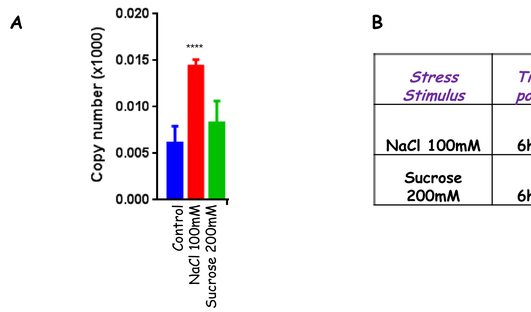


Fig 2. Gene expression comparison of (A) **p38-α**, (B) **Syk** and (C) **Src** in subjects with DED versus healthy controls. Target gene expression was standardised to the housekeeping gene GAPDH. **Key**: ****p<0.0001.



B

Stress Stimulus	*Time point*	*Number of replicates*	*P values*
NaCl 100mM	6hrs	3	<0.0001
Sucrose 200mM	6hrs	3	0.107

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.

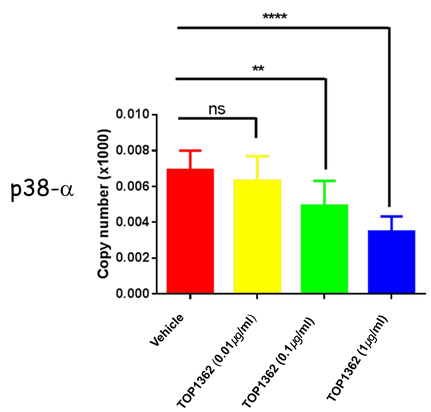


Fig 4. Effects of Dose-response treatment (TOP1362) on p38-α gene expression in 100mM NaCl-challenged Chang cells. All treatments are compared with vehicle (0.5% DMSO). Target gene expression was standardised to GAPDH. **Key**: ns, not significant, **p=0.005, ****p<0.0001.

Results

- **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

- **TOP1362** potently inhibits **p38-α**, **Src** family kinases (Src and Lck) and **Syk**, which are key kinases involved in inflammatory signalling cascades
- We have shown that these kinases are **significantly up-regulated in DED** patients (versus healthy controls)
- A Chang cell model of tear hyperosmolarity confirmed increased p38-α expression, which was inhibited by **TOP1362**
- This demonstrates the potential of NSKIs as novel DED treatments

Future Work

- Further PD/PK profiling of **TOP1362** in *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)

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