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A NOVEL ERK1/2 INHIBITOR HAS POTENT ACTIVITY IN KRAS-MUTANT NON-SMALL CELL LUNG CANCER MODELS

Aurelie Courtin, Luke Bevan, Jessica Brothwood, Tom Heightman, Birikiti Kidane, Justyna Kucia-Tran, John Lyons, Sandra Muench, Alpesh Shah, Lukas Stanczuk, Neil Thompson, Nicola Wallis, Nicola Wilsher, Joanne Munck

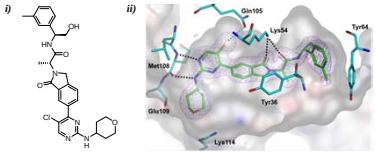
Astex Pharmaceuticals, 436 Cambridge Science Park, Milton Road, Cambridge, CB4 0QA, United Kingdom

INTRODUCTION

- KRAS mutations occur in 25-30% of non-small cell lung cancer (NSCLC) patients. Although, NSCLC patients harboring EGFR or ALK mutations can benefit from personalized therapies, there are currently no approved targeted therapies for KRAS-mutant NSCLC tumors.
- The constitutive activation of the MAPK pathway in these tumors provided the rationale for targeting MEK1/2 (MEK). However, when tested clinically, MEK inhibitors had limited activity[1].
- As ERK1/2 (ERK) is the primary downstream effector of the MAPK pathway, it is hypothesized that ERK inhibitors may prove to be less susceptible to oncogenic bypass than MEK inhibitors and therefore have the potential to overcome the limitations of MEK inhibitors in KRAS-mutant NSCLC.
- Using fragment-based drug discovery we have developed a novel, potent and selective ERK inhibitor, which inhibits *in vitro* ERK catalytic activity with a low nM IC_{50} value and has strong anti-proliferative effects in KRAS-mutant NSCLC cell lines.
- In addition to inhibiting ERK catalytic activity, the compound also inhibits the phosphorylation of ERK by MEK and confers a decrease in cellular pERK levels in KRAS-mutant NSCLC cell lines (*in vitro* and in *in vivo* pharmacodynamic [PD] studies).
- Once daily oral dosing of the lead compound (50 mg/kg) conferred significant anti-tumor activity in a range of KRAS-mutant NSCLC *in vivo* models.
- This work demonstrates the *in vitro* and *in vivo* activity of a novel, highly potent, selective ERK inhibitor in models of KRAS-mutant NSCLC and supports the further optimisation of this series of compounds for clinical development.

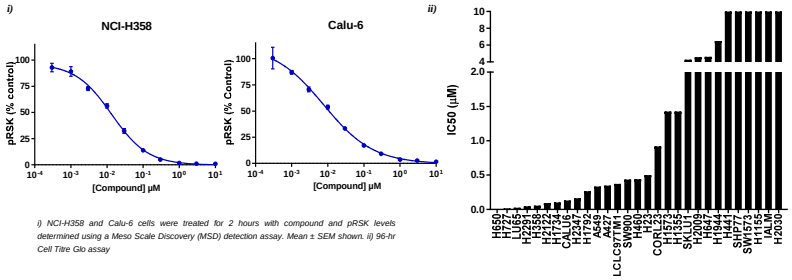
1. A novel potent and selective ERK inhibitor

- The lead compound inhibits ERK catalytic activity with an IC_{50} of 3 nM (as determined in an ERK TRF Kinase assay).
- It binds to the active site of ERK2 (where the adenine of ATP binds) and then expands in an elongated shape, exploiting a pocket which is created by an unusual movement of the P-Loop Tyr36 residue.



i) Structure of lead compound ii) Crystal structure of lead compound bound to human ERK2

2. Inhibition of ERK catalytic activity and the proliferation of KRAS-mutant NSCLC cells

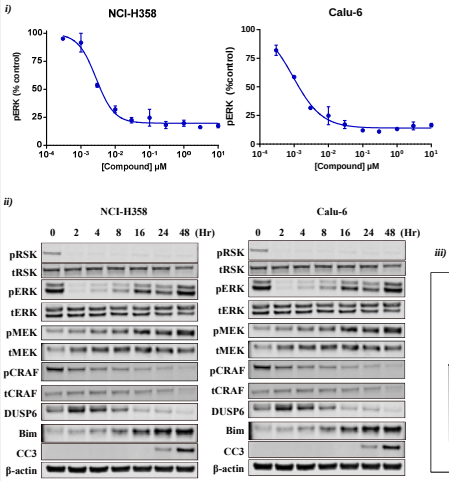


i) NCI-H358 and Calu-6 cells were treated for 2 hours with compound and pRSK levels determined using a Meso Scale Discovery (MSD) detection assay. Mean ± SEM shown. ii) 96-hr Cell Titre Glo assay

- The lead compound potently inhibited ERK catalytic activity in NCI-H358 and Calu-6 (KRAS-mutant NSCLC) cells, with an IC_{50} value of 11 nM and 15 nM, respectively.
- The lead compound potently inhibited the proliferation of a panel of KRAS-mutant NSCLC cell lines. 55% of the KRAS-mutant NSCLC cell lines exhibited antiproliferative IC_{50} values ranging from 1 nM to 500 nM.

3. Modulation of pERK levels and MAPK signalling in KRAS-mutant NSCLC cell lines

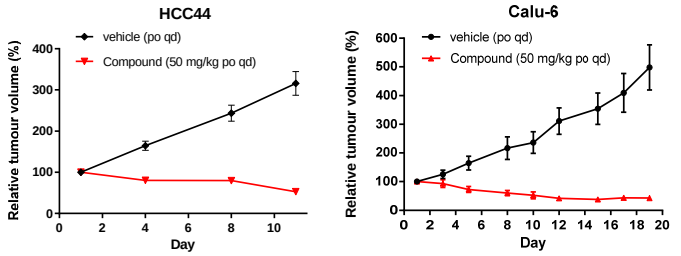
- We have previously shown that the lead compound prevents the phosphorylation of ERK by MEK, but does not inhibit MEK activity.
- Here we confirm that the lead compound confers a decrease in the phosphorylation of ERK itself in both NCI-H358 and Calu-6 cell lines.



i) Cells were treated for 2 hours with compound and pERK levels determined by ELISA ii) NCI-H358 and Calu-6 cells were treated for up to 24 hours with 900 nM and 650 nM, compound, respectively (equivalent of 5 x proliferation assay EC_{50} value). Cell lysates were analysed by western blotting. iii) ERK directly phosphorylates and inhibits wild-type RAF. In KRAS-mutant cell lines, ERK inhibitors therefore confer an increase in RAF activity and increase in phosphorylation of MEK and ERK.

- The lead compound inhibited ERK catalytic activity in both NCI-H358 and Calu-6 cell lines. This was indicated by a decrease in phosphorylation of the ERK substrates RSK and CRAF, a decrease in the levels of DUSP6 (which is positively regulated by ERK) and an increase in the levels of BIM1 (a pro-apoptotic protein, which is negatively regulated by ERK).
- An increase in the apoptosis was observed in response to the lead compound, as indicated by an increase in cleaved caspase 3 (CC3) .
- An increase in pMEK was observed in both cell lines. This is consistent with previous observations that the inhibition of ERK-dependent negative feedback confers an increase in MAPK signalling in KRAS-mutant cells[2].
- The lead compound conferred a decrease in the phosphorylation of ERK in both cell lines. pERK levels were restored by 16 hours. The restoration of pERK levels at later time-points is likely to be due to the inhibition of ERK-dependent negative feedback described above.

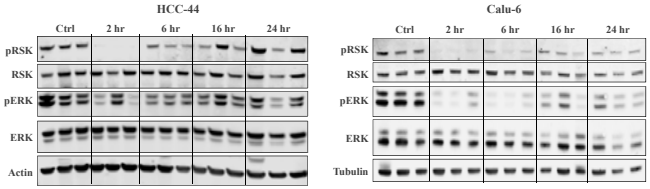
4. Anti-tumour activity in KRAS-mutant NSCLC tumor xenografts



The lead compound was administered orally at 50 mg/kg once daily (qd) to BALB/c nude mice bearing either sub-cutaneous HCC44 or Calu-6 tumor xenografts. Each data point represents mean ± SEM. n = 8 mice per treatment group. Compound was well tolerated.

- Once daily oral administration of 50 mg/kg lead compound conferred significant anti-tumor activity ($p < 0.0001$) in the HCC44 KRAS-mutant NSCLC xenograft models and significant tumour regression ($p < 0.0001$) in the Calu-6 KRAS-mutant NSCLC xenograft model.

5. Inhibition of ERK catalytic activity and the phosphorylation of ERK in KRAS-mutant NSCLC xenograft tumor tissue



A single dose of 50 mg/kg lead compound was administered orally to BALB/c mice bearing subcutaneous Calu-6 tumor xenografts. Animals were sacrificed and tumors removed at indicated time-points. Tumor lysates were analysed by western blotting.

- A single dose of 50 mg/kg inhibited ERK catalytic activity in both HCC44 and Calu-6 tumor xenografts (indicated by the decrease in phosphorylation of the ERK substrate RSK).
- Furthermore, the compound conferred a decrease in the phosphorylation of ERK itself.
- The effects on ERK catalytic activity and ERK phosphorylation were sustained for up to 16 hours in the HCC44 model and upto 24 hours in the Calu-6 model.

SUMMARY AND CONCLUSIONS

- The direct targeting of ERK is an attractive therapeutic approach for the treatment of KRAS-mutant NSCLC.
- Using fragment-based drug discovery we have developed a novel, potent and selective ERK inhibitor, which in addition to inhibiting ERK catalytic activity also inhibits the phosphorylation of ERK by MEK.
- The compound potently inhibits ERK signalling and the proliferation of KRAS-mutant NSCLC cell lines.
- *In vivo*, the compound confers significant anti-tumor activity in several KRAS-mutant NSCLC xenograft models.
- These data support the further optimisation of this compound series for future clinical development.

References: 1. Jänne et al., (2017) *JAMA*. 317(18),1844-1853 5 2. Hatzivassiliou et al., 2013 Nature12; 501 (7466): 232-6

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