3030

DIFFERENT PHARMACODYNAMIC PROFILES OF ERK1/2 INHIBITION CAN ELICIT COMPARABLE ANTI-TUMOR ACTIVITY

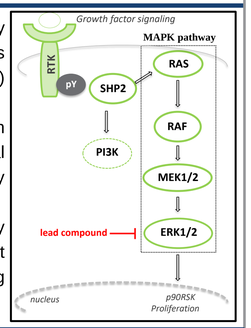
Christopher Hindley, Luke Bevan, Hannah Braithwaite, Aurélie Courtin, Lynsey Fazal, Roberta Ferraldeschi, Vanessa Martins, Sandra Muench, Nicola Wallis, Nicola Wilsher, John Lyons, Joanne Munck

chris.hindley@astx.com

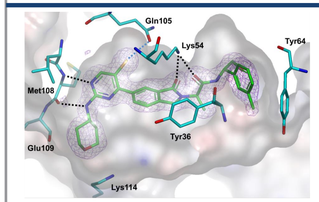
Astex Pharmaceuticals, 436 Cambridge Science Park, Milton Road, Cambridge, CB4 0QA, United Kingdom

INTRODUCTION

- The mitogen activated protein kinase (MAPK) pathway is frequently dysregulated in cancer, leading to activation of the downstream kinases ERK1/2 (ERK). Phosphorylation of ERK substrates such as p90RSK (RSK) leads to cancer cell proliferation.
- Clinical efficacy can be limited by toxicity, so it is important to establish an optimal, tolerated dose schedule which maximises efficacy. Preclinical studies investigating the duration of target engagement required for efficacy can inform on dose schedules to be tested in the clinic.
- A number of compounds under clinical development target ERK activity directly: we have recently described the development of a novel, potent and selective small molecule inhibitor of ERK, the lead compound, using fragment-based drug discovery[1].



1. A novel dual mechanism ERK inhibitor

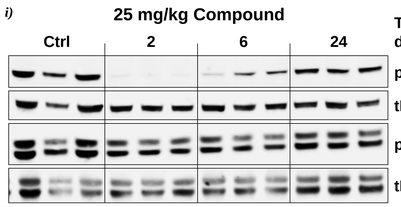


Crystal structure of lead compound bound to human ERK2

- The lead compound occupies a pocket created by an unusual movement of the P-loop Tyr36 residue, which inhibits the phosphorylation of ERK2. It therefore exhibits a dual mechanism of inhibition by inhibiting both the catalytic activity and the phosphorylation of ERK[2].
- Using this inhibitor as a tool compound, we have explored the relationship between duration of inhibition and anti-tumor activity in a colorectal BRAF^{V600E} mouse xenograft model.

2. The kinetics of ERK inhibition follow compound pharmacokinetics in vivo

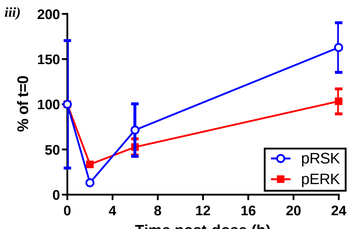
i)



ii)

	2 h	6 h	24 h
[Tumor] (μM)	3.4	1.3	0.2
[Plasma] (μM)	35.4	13.7	0.5

iii)

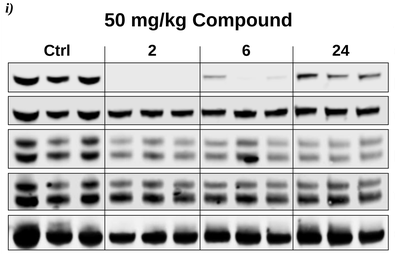


i) Western blot for total and phosphorylated RSK and ERK proteins in Colo205 xenografts grown in BALB/c Nude male mice at the indicated timepoints following a single dose of 25 mg/kg of the lead compound. ii) Concentration of lead compound in tumor and plasma at the indicated timepoints. iii) Levels of phosphorylated RSK and ERK in tumors were quantified using a Meso Scale Discovery (MSD) detection assay and ELISA detection assay, respectively.

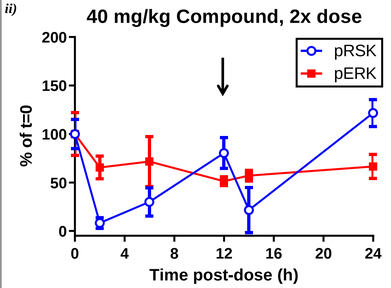
- Maximum inhibition of ERK phosphorylation and catalytic activity occurs 2 h after dosing with the lead compound.
- After 2 h the inhibition of ERK activity and phosphorylation decreases in line with the lead compound pharmacokinetics.

3. Higher doses do not result in prolonged pathway suppression

i)



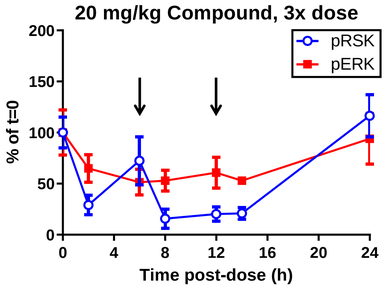
ii)



- A single 50 mg/kg dose of the lead compound does not prolong complete inhibition of ERK activity beyond 6 h.
- pRSK levels are 8% of pre-dose levels 2 h after a single 40 mg/kg dose of the lead compound but have returned to 80% by 12 h.
- An additional dose of the lead compound at 12 h results in pRSK levels of 22% at 14 h after the first dose.
- The duration of pathway suppression is not determined by dose.

i) Western blot for total and phosphorylated RSK and ERK proteins in Colo205 xenografts at the indicated timepoints following a single dose of 50 mg/kg of the lead compound. ii) Levels of phosphorylated RSK and ERK in tumors were quantified following administration of the lead compound orally at 40 mg/kg at t=0 and t=12; black arrow denotes the time of the second dose.

4. Increased frequency of dosing results in prolonged pathway suppression

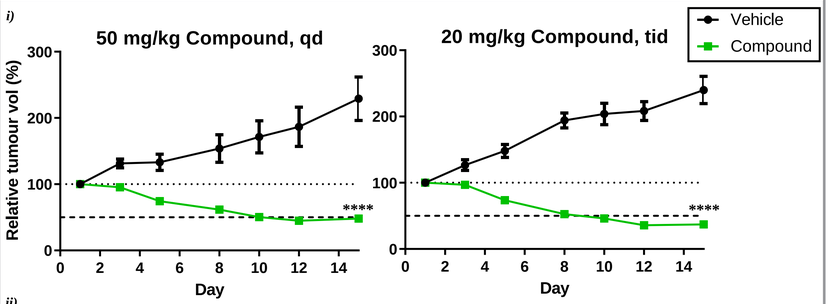


Levels of phosphorylated RSK and ERK in tumors were quantified following administration of the lead compound orally at 20 mg/kg three times (tid) within 24 hours at 0, 6 and 12 h; black arrows denote the time of the additional doses.

- A lower dose of the lead compound results in lower maximal inhibition of ERK, with pRSK levels 29% of pre-dose levels 2 h after the first 20 mg/kg dose.
- Increased frequency of dosing with 20 mg/kg of the lead compound prolongs pathway suppression – pRSK levels are 16% of pre-dose levels 8 h after the first dose and 21% at 14 h after the first dose.
- The duration of pathway suppression is determined by dosing frequency.

5. Different dosing schedules elicit comparable anti-tumor activity in vivo

i)



ii)

Dose schedule	Minimum T/C (%)	Relative tumor volume day 15 (%)	Maximum body weight loss (%)
50 mg/kg qd	21	40	2.7
20 mg/kg tid	15	37	5.3

*i) The lead compound was administered orally either at 50 mg/kg once daily (qd) or at 20 mg/kg three times daily (tid) to BALB/c Nude mice bearing subcutaneous Colo205 tumor xenografts. Each data point represents mean ± SEM of the tumor volume relative to volume on day 1. ****, $p < 0.0001$; one-way ANOVA. Dotted lines indicate 100% RTV (···) and dashed lines indicate 50% RTV (---). ii) Table summarising tumor growth inhibition and body weight loss for both dosing schedules.*

- Three times daily dosing at lower doses of the lead compound results in similar, significant anti-tumor activity to once daily dosing at a higher dose.
- The total dose administered daily is similar in both schedules, suggesting that the total dose administered may drive anti-tumor activity.
- Both dosing schedules are well tolerated with minimal body weight loss and no clinical signs observed.

SUMMARY AND CONCLUSIONS

- The direct targeting of ERK is an attractive therapeutic approach being actively pursued in the clinic.
- Using fragment-based drug discovery we have developed a novel, potent and selective ERK inhibitor which displays target engagement following dosing in vivo.
- ERK activity remains inhibited following a single dose of the lead compound for up to 6 h.
- More frequent dosing of the lead compound results in lower maximal inhibition at 2 h but prolonged suppression of ERK activity beyond 12 h.
- Similar anti-tumor activity is observed in vivo with a similar total daily dose administered either as a single, high daily dose of the lead compound or three times daily dosing with a lower dose.
- Prolonged pathway suppression is not required for maximum anti-tumor activity, which can be achieved with a single dose.

These data support investigation of a once daily dosing schedule in the clinic for this series.

References: 1. Heightman et al., 2018. J Med Chem 61(11): 4978-4992. 2. Kidger et al., 2020. Mol Cancer Ther 19(2): 525-539.

Poster presented at AACR Annual Meeting 2020, Virtual Meeting II. Jun 22 – 24 2020