426

A novel ERK1/2 inhibitor has potent activity in NRAS-mutant melanoma cancer models

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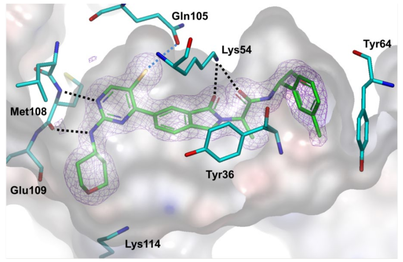
INTRODUCTION

- NRAS mutations occur in 15-20% of melanoma cancer patients. Currently there is no approved molecularly targeted therapy for NRAS-mutant melanoma, an indication which is associated with aggressive clinical outcome and a poor prognosis.
- The NRAS mutation leads to constitutive activation of the MAPK pathway. As ERK1/2 (ERK) is the primary downstream effector of the MAPK pathway, its direct inhibition may provide an attractive therapeutic approach for the treatment of NRAS-mutant melanoma.
- As previously described, using fragment-based drug discovery we have identified a novel and selective inhibitor of ERK which inhibits *in vitro* ERK catalytic activity as well as ERK phosphorylation[1].
- Here, we demonstrate the *in vitro* and *in vivo* activity of a novel, highly potent, selective ERK inhibitor in models of NRAS-mutant melanoma.

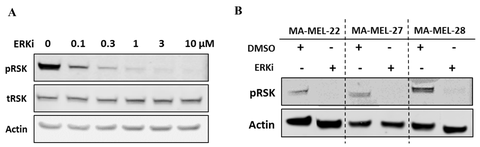
Fig 1. A novel potent and selective ERK inhibitor



Crystal structure of lead compound bound to human ERK2.

- The lead compound 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.
- It inhibits ERK catalytic activity with an IC_{50} of 3 nM (as determined in an ERK TRF Kinase assay).
- The lead compound was shown to be highly selective for ERK1/2 in a screen of 465 kinases.

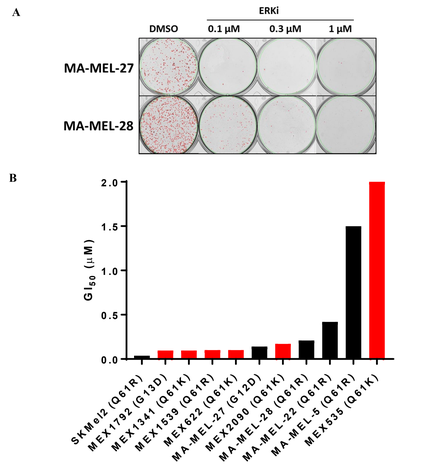
Fig 2. Inhibition of ERK catalytic activity of NRAS^{mut} melanoma cells



A) Dose-response inhibition of pRSK levels following 2 hour treatment with lead compound in MA-MEL-28 cell line.
B) Inhibition of pRSK levels following 4 hours treatment with 1 µM of lead compound in MA-MEL-22, MA-MEL-27 and MA-MEL-28 cell lines.

- The lead compound potently inhibited ERK catalytic activity in multiple NRAS-mutant melanoma cell lines.

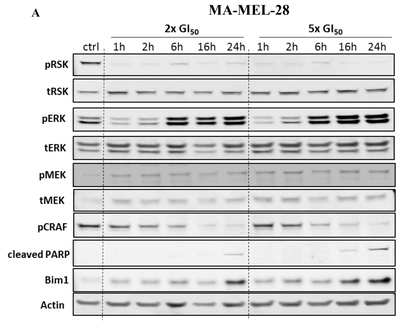
Fig 3. Inhibition of NRAS-mutant melanoma cell proliferation

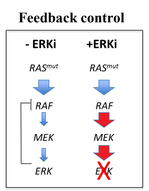


A) Colony formation assay (21 days).
B) 96-hour cell proliferation assay. Established cell lines (in black) and PDX-derived cell lines (in red) were analysed by alamarBlue® or CellTiter-Blue® Cell Viability Assay, respectively.

- Nine out of eleven established melanoma cell lines and patient-derived melanoma cells carrying an NRAS mutation showed sensitivity to the ERK inhibitor *in vitro*, with anti-proliferative GI_{50} values ranging from 37 to 420 nM.

Fig 4. Modulation of MAPK signalling and induction of apoptosis in NRAS^{Q61R} melanoma cells

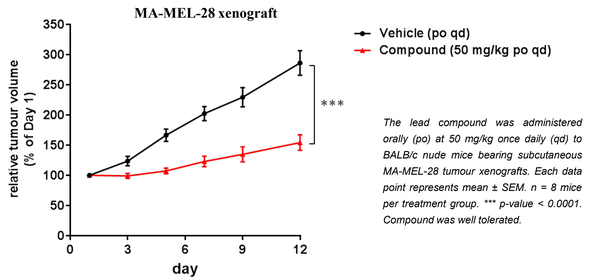




A) MA-MEL-28 cells were treated for up to 24 hours with 2x or 5x GI_{50} (0.2 or 1 µM) of lead compound.
B) ERK directly phosphorylates and inhibits wild-type RAF. In RAS-mutant cell lines, ERK inhibitors therefore confer an increase in RAF activity and increase in phosphorylation of MEK and ERK.

- The lead compound inhibited ERK catalytic activity in MA-MEL-28 cells. This was indicated by a decrease in phosphorylation of the ERK substrates RSK and CRAF.
- An increase in pMEK is consistent with previous observations that the inhibition of ERK-dependent negative feedback confers an increase in MAPK signalling in RAS-mutant cells[2].
- The lead compound conferred a decrease in the phosphorylation of ERK. pERK levels were restored by 6 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.
- An increase in apoptosis was observed in response to the lead compound, as indicated by an increase in cleaved PARP and BIM1 (a pro-apoptotic protein, which is negatively regulated by ERK).

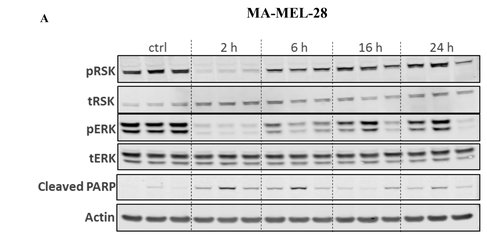
Fig 5. Anti-tumour activity in NRAS^{Q61R} melanoma xenograft

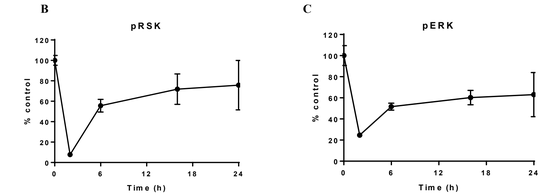


*The lead compound was administered orally (po) at 50 mg/kg once daily (qd) to BALB/c nude mice bearing subcutaneous MA-MEL-28 tumour xenografts. Each data point represents mean ± SEM. n = 8 mice per treatment group. *** p-value < 0.0001. Compound was well tolerated.*

- Once daily oral administration of 50 mg/kg of lead compound conferred significant anti-tumour activity in the MA-MEL-28 NRAS-mutant melanoma xenograft model.

Fig 6. Inhibition of ERK catalytic activity and ERK phosphorylation in NRAS^{Q61R} melanoma xenograft





A-B) A single dose of 50 mg/kg lead compound was administered orally to BALB/c mice bearing subcutaneous MA-MEL-28 tumor xenografts. Animals were sacrificed and tumors removed at indicated time-points. Tumor lysates were analysed by western blotting (A), by MSD (B) or ELISA (C) assays. Each data point represents mean ± SEM. n = 3 mice per treatment group.

- A single dose of 50 mg/kg inhibited ERK catalytic activity in MA-MEL-28 tumour xenografts (indicated by the decrease in phosphorylation of the ERK substrate RSK). Furthermore, the compound conferred a decrease in the phosphorylation of ERK itself and increase in apoptosis as indicated by increase in cleaved PARP.

DISCUSSION

- The direct targeting of ERK is an attractive therapeutic approach for the treatment of NRAS-mutant melanoma. 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 NRAS-mutant melanoma cell lines. *In vivo*, the compound confers significant anti-tumour activity in the MA-MEL-28, NRAS-mutant melanoma, xenograft model.
- These data support future clinical development of ERK inhibitors in NRAS-mutant melanoma.
- Candidate molecule ASTX029 is now in Ph1/2 clinical trial for patients with advanced solid tumours.

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References: 1) Heightman *et al.*, (2018) J. Med. Chem. 61 (11), 4978-4992. 2) Hatzivassiliou *et al.*, (2013) Nature 501 (7466): 232-236