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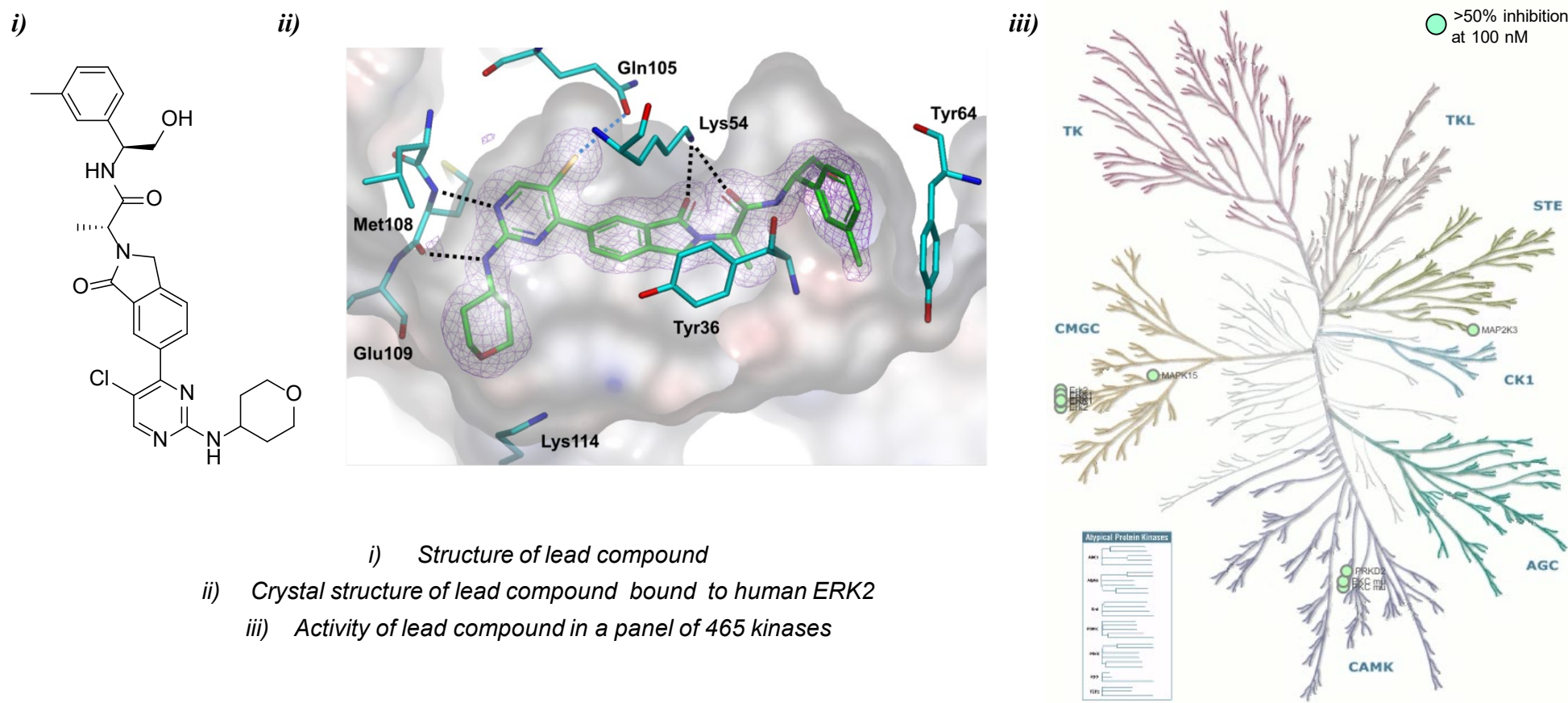
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INTRODUCTION

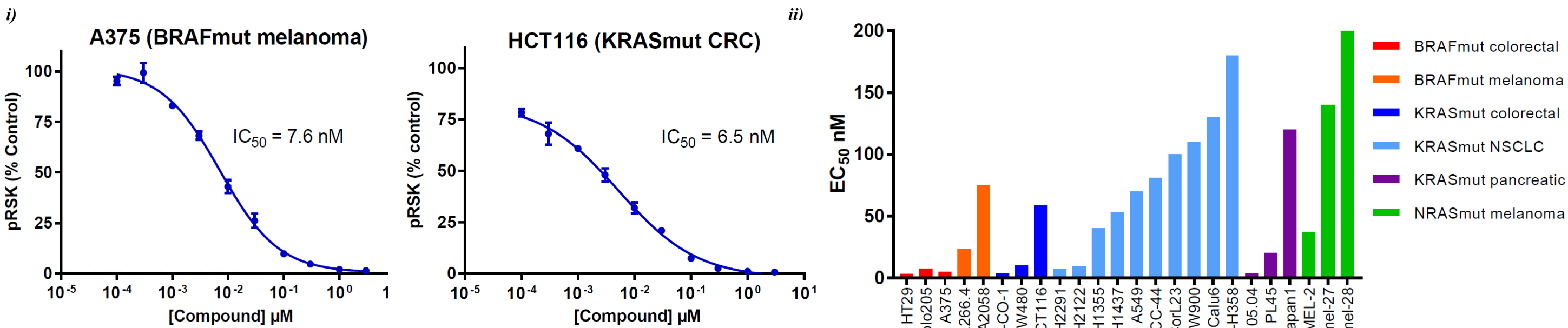
- The MAPK pathway is commonly hyper-activated in human cancers due to the occurrence of oncogenic mutations in RAF and RAS, and multiple studies have demonstrated that MAPK pathway inhibition suppresses the growth of such cancer cells.
- MAPK inhibition has been clinically validated by BRAF and MEK inhibitors, which are approved for the treatment of BRAF^{V600E}-mutant melanoma and NSCLC. However, response to such agents is often short-lived due to the onset of resistance mechanisms that result in re-activation of ERK1/2 (ERK) signalling^{1,2}.
- RAF and MEK inhibitors have also been clinically tested in other cancers, including BRAF-mutant colorectal (CRC) and KRAS-mutant Non-small cell lung cancer (NSCLC), where they had limited clinical activity^{3,4}.
- As 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 RAF and MEK inhibitors and therefore have the potential to overcome the limitations of RAF and MEK inhibitors.
- 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 IC₅₀ value and has strong anti-proliferative effects in a wide range of MAPK-activated 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 both BRAF-mutant and KRAS-mutant 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 *in vivo* models.
- These data support the further optimisation of this series of compounds for clinical development.

1. A novel, potent and selective ERK inhibitor

- The lead compound inhibits ERK1/2 catalytic activity with an IC₅₀ 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.
- The lead compound was highly selective for ERK1/2 in a screen of 465 kinases.



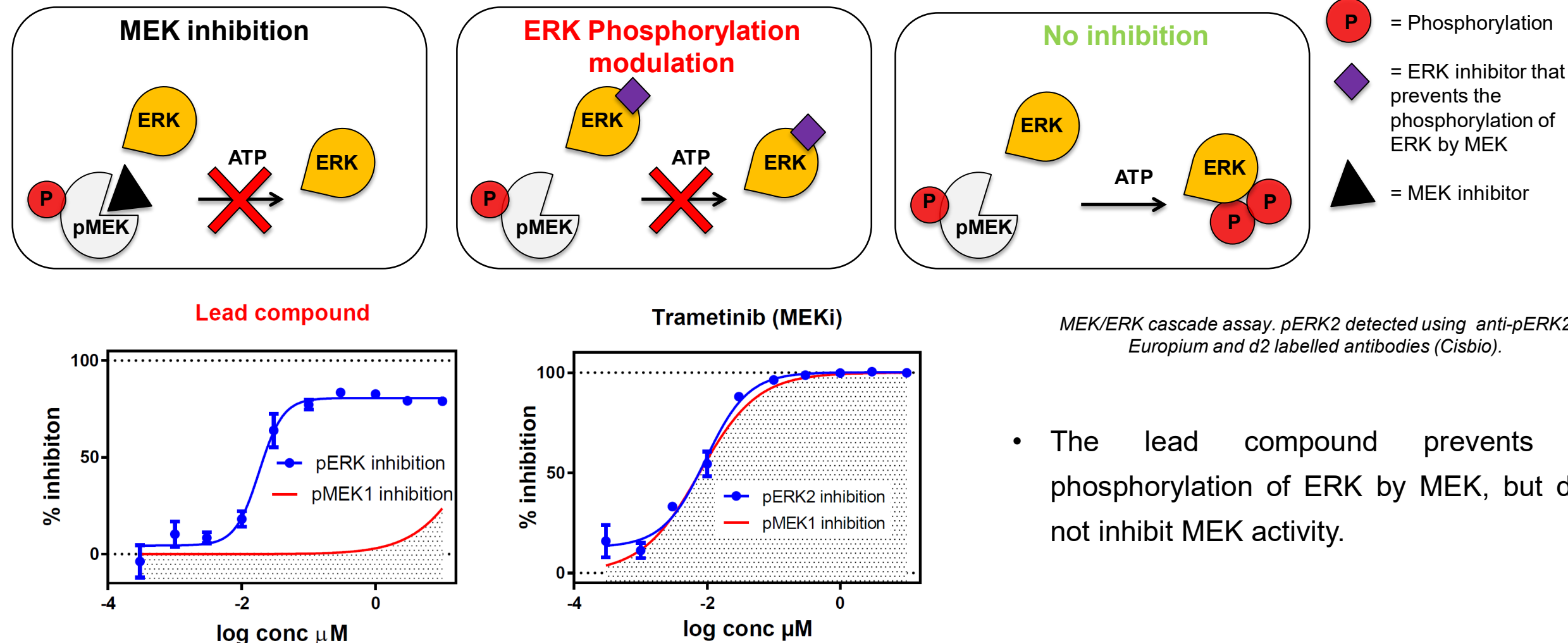
2. Inhibition of ERK catalytic activity and the proliferation of MAPK-activated cells



- The lead compound potentially inhibited ERK catalytic activity in A375 (BRAF-mutant melanoma) and HCT116 (KRAS-mutant CRC) cells, with an IC₅₀ value of 7.2 nM and 5.2 nM, respectively.
- The lead compound potentially inhibited the proliferation of cell lines harbouring a range of MAPK aberrations. EC₅₀ values ranged from 3.4 nM in HT29 cells (BRAF-mutant melanoma) to 210 nM in Ma-mel-28 cells (NRAS-mutant melanoma).

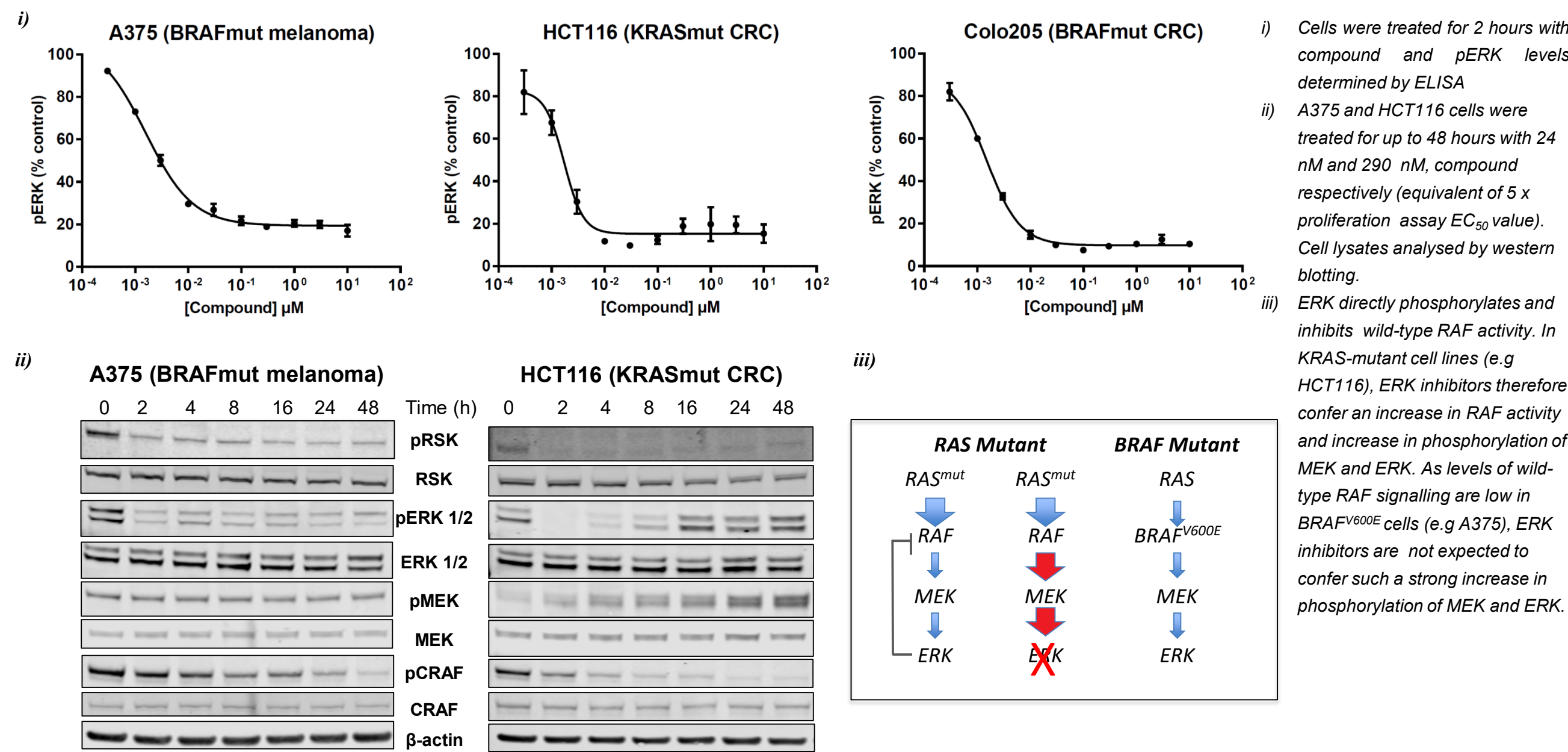
3. Modulation of the *in vitro* phosphorylation of ERK by MEK

- A MEK/ERK cascade assay was used to evaluate the effects of the lead compound on the phosphorylation of ERK by MEK.
- Assay: Active, phosphorylated MEK incubated with non-phosphorylated ERK in the presence of ATP and compound, and the degree of phosphorylation of ERK determined. Compounds were also tested in a MEK binding assay in order to distinguish between a) ERK inhibitors that prevent the phosphorylation of ERK by MEK and b) MEK inhibitors.



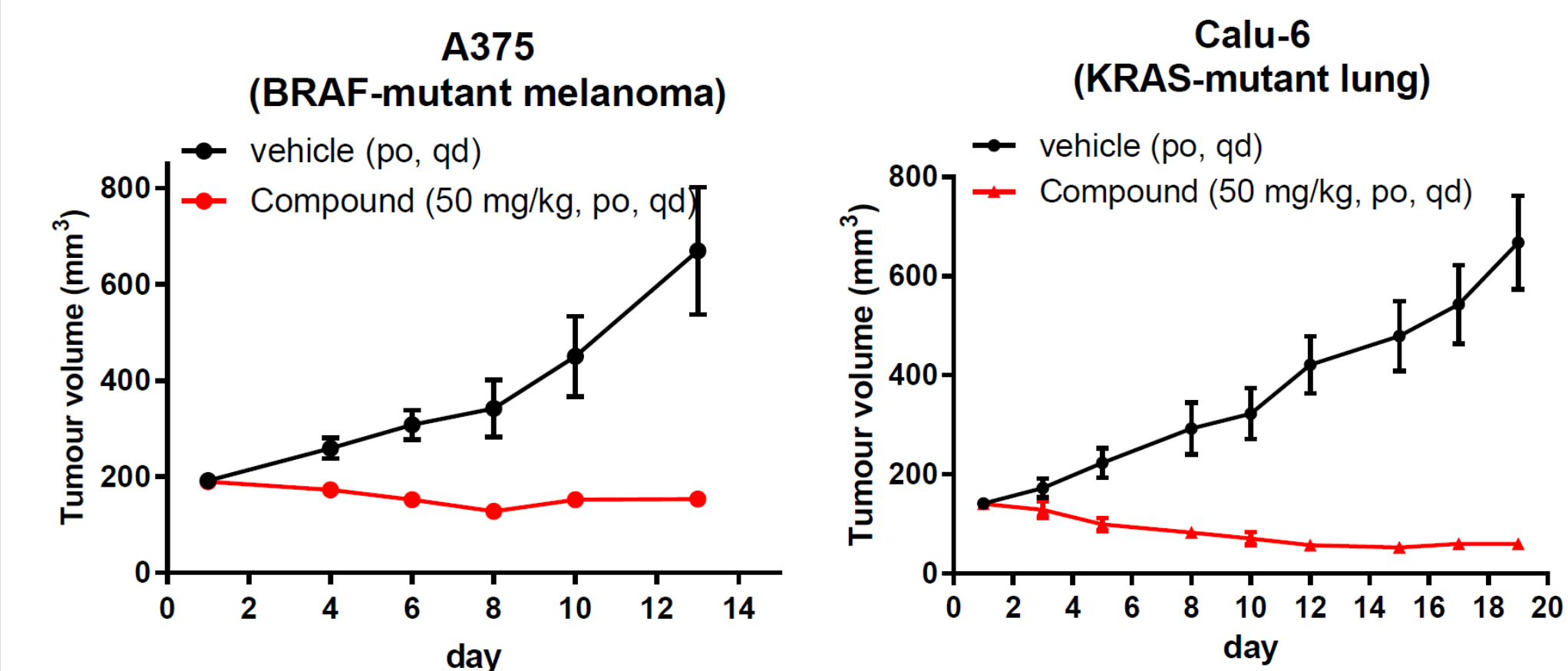
- The lead compound prevents the phosphorylation of ERK by MEK, but does not inhibit MEK activity.

4. Modulation of pERK levels in BRAF- and KRAS-mutant cell lines



- The lead compound inhibited ERK catalytic activity in both A375 and HCT116 cell lines (indicated by the decrease in phosphorylation of the ERK substrates RSK and CRAF).
- Furthermore, it conferred a decrease in the phosphorylation of ERK itself in both cell lines (confirmed by ELISA and western blotting).
- An increase in pMEK was observed in HCT116 cells only. This is consistent with previous observations that the inhibition of ERK-dependent negative feedback has a greater impact in KRAS-mutant than BRAF-mutant cell lines⁵.

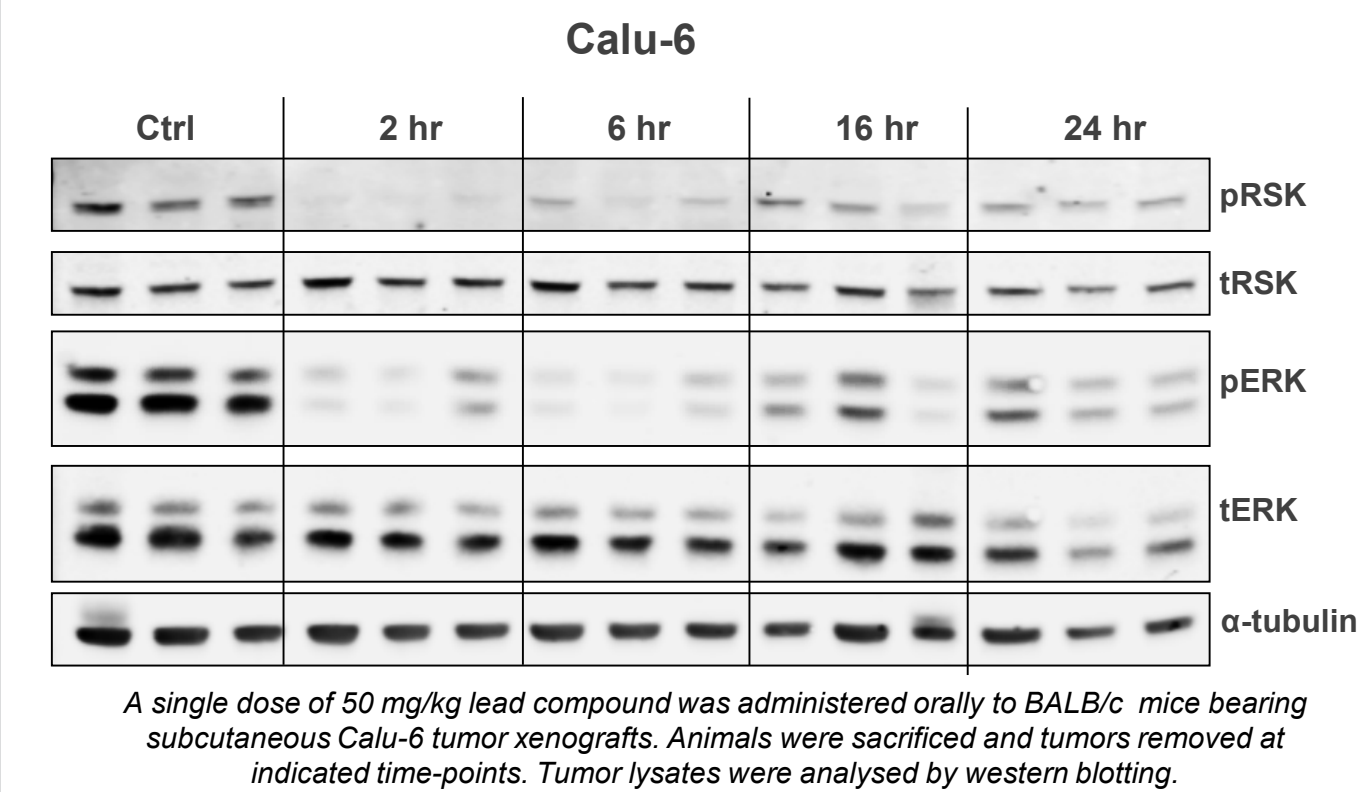
5. Anti-tumor activity in BRAF- and KRAS-mutant tumor xenografts



The lead compound was administered orally at 50 mg/kg once daily (qd) to BALB/c nude mice bearing either sub-cutaneous A375 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 in A375 and Calu-6 xenograft models (p < 0.0001).

6. Inhibition of ERK catalytic activity and the phosphorylation of ERK in xenograft tumor tissue



- A single dose of 50 mg/kg inhibited ERK catalytic activity in 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 24 hours.

SUMMARY AND CONCLUSIONS

- The direct targeting of ERK is an attractive therapeutic approach to overcoming the limitations of RAF or MEK inhibitors.
- 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 has *in vivo* activity, conferring significant anti-tumor activity in A375 (BRAF-mut melanoma) and Calu-6 (KRAS-mut lung) xenograft models.
- These data support the further optimisation of this compound series for future clinical development.
- For more information on the discovery of this compound series, please visit **Poster Board Number 161** Heightman et al., *Fragment-based discovery of a highly potent orally bioavailable ERK1/2 inhibitor which modulates the phosphorylation and catalytic activity of ERK1/2* (Abstract B161).

