P-37

Combinations of fostrox (MIV-818), a novel nucleotide prodrug, with lenvatinib or sorafenib shows increased efficacy in nonclinical hepatocellular carcinoma (HCC) models in vivo

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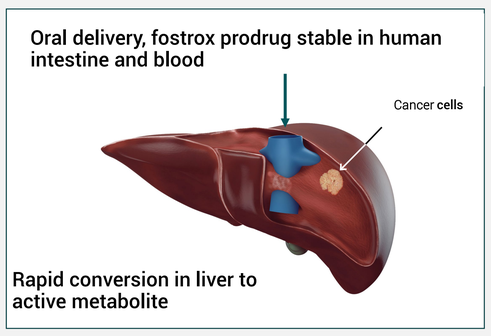
Background

Fostrox (fostroxacitabine bralpamide) is an orally administered, liver-directed, nucleotide prodrug that is currently in a phase 1b/2a trial in 2nd and 3rd line hepatocellular carcinoma (HCC), in combination with Keytruda® or Lenvima® (NCT03781934).



Fostrox is designed to deliver high levels of the chain-terminating nucleotide to the liver after oral dosing while minimizing systemic exposure.

Hypoxic conditions have been shown to increase cytotoxicity via increased formation of the active metabolite of fostrox. Tyrosine kinase inhibitors (TKIs) such as lenvatinib or sorafenib have, by induced hypoxia resulting from their anti-angiogenic activity, the potential to synergize with fostrox.

Materials & Methods

In vitro HCC models

- Human patient-derived HCC cell line (PDCs) representing a range of proliferation rates were incubated with a 10-point, 3-fold serial dilution of fostrox. Assay time ranged from 4 to 10 days, and IC_{50} was determined by CellTiter-Glo Reagent

In vivo tumour models

HCC xenografts were established by inoculation HepG2 ($1x10^7$) cells (0.1mL in1:1PBS:Matrigel) subcutaneously into the left flank of Balb/C nude female mice.

For the syngeneic model BALB/c were inoculated subcutaneously with mouse hepatocellular carcinoma H22 ($1x10^6$ cells). Treatment was initiated at a tumour volume of 100-200mm3 and randomized to study groups (n=10). Fostrox was dosed via oral gavage BID for 5 days at 48 and 160 µmol/kg. Sorafenib was dosed at 10 and 30 mg/kg, lenvatinib at 5 mg/kg, both PO QD for 21days. Tumours were measured 3 times weekly using electronic callipers and volumes were estimated using the formula 0.5(LxW2).

For PD studies mice were injected ip with a BrdU/pimonidazole (600mg/kg/60mg/kg) mixture 2h prior to being terminated at 2h after the last dose. Tumour was collected for histology. Tumour growth inhibition (TGI) = ((1-(Tt/T0) / (Ct/C0)) / (1-C0/Ct)) x 100; where Tt and T0 are tumour volume (TV) of compound treated mouse X at day t and 0 (prior to start of treatment). Ct and C0 are the mean TV of the control group at day t and 0.

Pharmacodynamics and histology

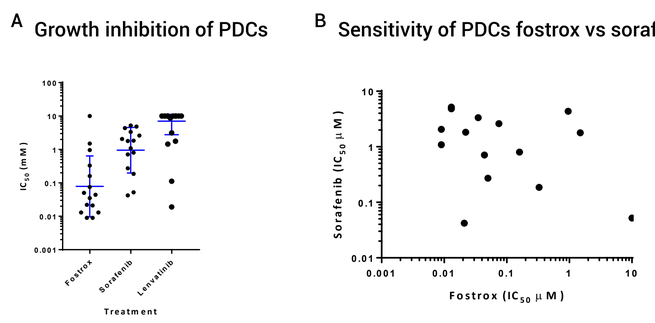
Tumour cryosections (10µm) were immunostained for hypoxia using mouse-anti-pimonidazole-FITC (1:500), anti-phospho-HistoneH2A.X (Ser139) mouse-anti-human-gH2AX (CloneJBW301) tagged with Alexa647, BrdU using a monoclonal rat-anti-BrdU (cloneBU/175;1:500) and anti-mouse Alexa750 secondary (1:500). Cellular DNA was counter-stained with Hoechst33342.

The imaging system consisted of a robotic fluorescence microscope with a PCOEdge4.2 camera. Images of BrdU, pH2AX, pimonidazole&Hoechst33342 staining from each tumour section were overlaid and areas of necrosis, acellular cavities and staining artifacts manually removed. Positive regions for each marker were identified by selecting all pixels above tissue background levels. Analysis of whole tissue averages for each marker were determined by dividing the total number of positive pixels by the total tissue are a excluding necrosis and empty regions

[1]The in vivo studies were performed at CrownBiosciences UK, approved by the Institutional Animal Care and Use Committee (IACUC), and conducted in accordance with the regulations of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)

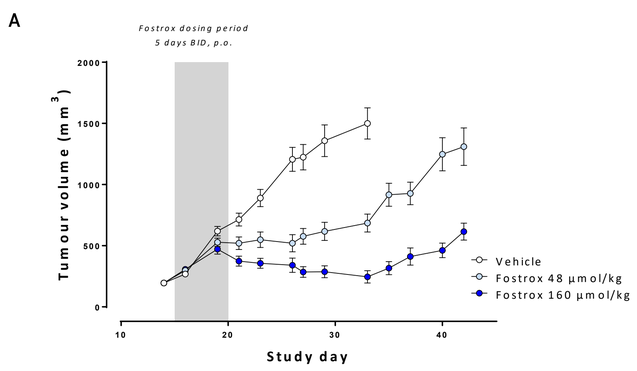
[2] Rizoska B et al. et al AACR #2930, 2018

Fostrox potently inhibits primary patient-derived cell lines (PDCs)



- Fostrox potently inhibited growth of a panel of 15 primary patient derived HCC cell lines (PDCs), with an IC_{50} below 0.1 uM for 10 out of the 15 PDCs. The anti-proliferative potency in vitro of fostrox compared favourably with sorafenib and lenvatinib (A).
- No correlation of sensitivity to fostrox and sorafenib was observed among the 15 PDCs, consistent with distinct mechanisms of action of fostrox and TKIs (B)

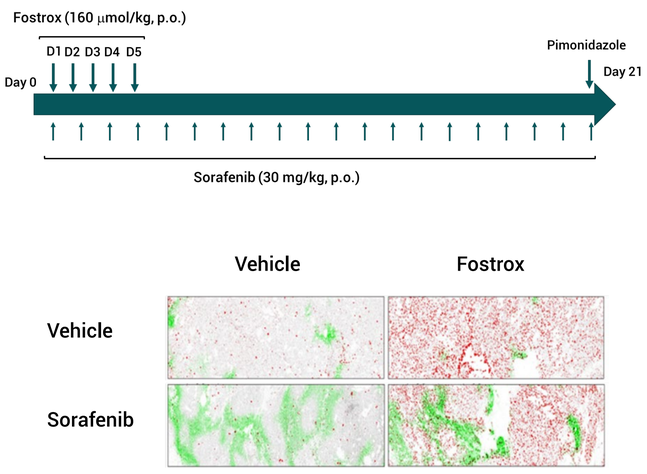
Fostrox inhibits HCC tumour (HepG2) growth in vivo



- In the HepG2 tumours in vivo, dose-dependent tumour growth inhibition (TGI) by fostrox at day 33 was 63% (48 µmol/kg) and 96% (160 µmol/kg) (A).
- Proliferation (BrdU incorporation) was reduced by 86% (48 µmol/kg) and 94% (160 µmol/kg), compared to vehicle (B). DNA damage (pH2AX) was induced 14-fold (48 µmol/kg) and 20-fold (160 µmol/kg), compared to vehicle (C). The effects were statistically significant for both doses

The combination of fostrox and sorafenib induces DNA-damage and hypoxia[2]

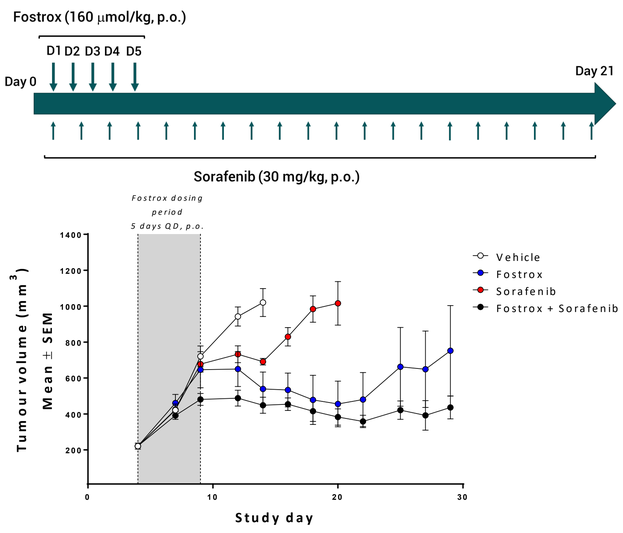
PD effects in foxtrox + sorafenib in the HepG2 xenograft model



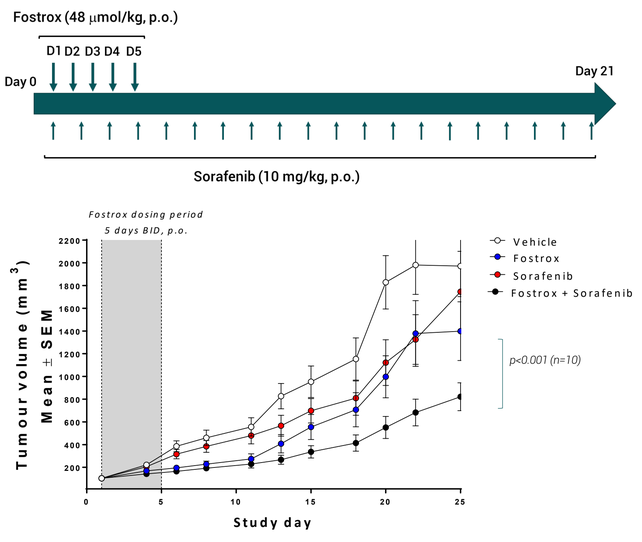
- A clear increase in hypoxia (pimonidazole-green) was seen in the sorafenib-treated HepG2 tumour bearing mice only, consistent with the anti-angiogenic mode of action
- Substantial induction of DNA damage (pH2AX-red) in animals receiving fostrox alone or in combination with sorafenib, also in hypoxic regions, while no DNA-damage induction was observed with sorafenib as single agent

The combination of fostrox and sorafenib show enhanced efficacy in HepG2 xenograft and H22 syngeneic tumour models in vivo

Foxtrox + sorafenib in HepG2 xenograft model

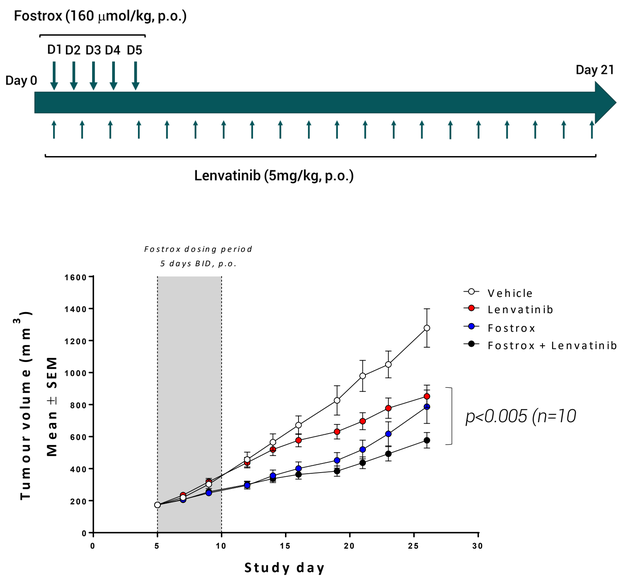


Foxtrox + sorafenib in H22 syngeneic model



- A substantial increase in growth inhibition was observed when sorafenib was combined with a 5 day dosing of fostrox
- The enhanced efficacy was observed in both HepG2 xenograft and H22 syngeneic mouse tumour models

The combination of fostrox and lenvatinib show enhanced efficacy in HepG2 xenograft model in vivo



- A significant increase in growth inhibition was observed when lenvatinib was combined with a 5 day dosing of fostrox

Conclusions

- The combination of fostrox with tyrosine kinase inhibitors (TKIs), lenvatinib or sorafenib, enhances anti-tumour efficacy in both human HCC xenografts and syngeneic mouse HCC models
- The different sensitivity profiles for fostrox vs sorafenib, in a panel of primary patient-derived HCC cell lines, suggest distinct and complementary mechanisms of anti-tumour activity
- The results indicate that the combination of TKIs with fostrox could potentially improve tumour response in HCC

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