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A triple combination of fostrox (MIV-818) with immune checkpoint and kinase inhibition shows increased anti-tumor efficacy in vivo

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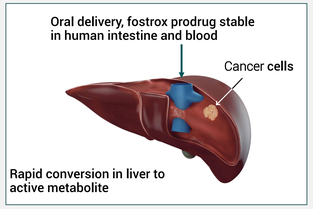
Background

Fostrox (fostroxacitabine bralpamide) is an orally administered, liver-directed, nucleotide prodrug that has completed an open-label, multi-centre phase 1 monotherapy clinical trial and is currently progressing in a phase 1/2a trial in hepatocellular carcinoma (HCC), in combinations 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

Materials & Methods

CT26.WT syngeneic mouse model

Each mouse was inoculated subcutaneously with 5 x 10^5 CT26.WT cells in 100µl of PBS into the right rear flank. Mice were randomly allocated to study groups (n=10).

Treatment was started on Day 1. Vehicle (20% HPBCD, p.o.) BID, fostroxabine bralpamide (30mg/kg, p.o.) BID, aPD1 (Biocell CD279 3mg/kg, i.p.) QD, lenvatinib (5mg/kg, p.o.), or combinations thereof. Tumours were measured three times weekly during the dosing phase. Tumour volumes were estimated using the formula 0.5 (LxW2) by measuring the tumour in two dimensions using electronic callipers.

Immuno-histochemistry (IHC) analysis of tumour marker expression

For IHC tumours were excised on Day 5 and prepared for FFPE blocking and IHC staining to evaluate the level of expression of CD4, CD8, CD11b, CD20, FOXP3, PD-L1, LAG-3, and pH2AX.

Tissue slides were stained with the following antibodies: Phospho-Histone H2A.X (Ser139) (20E3, 1:2000 dilution), CD4 (CellSignalling 64988, 1:100 dilution), CD8a (D4W2Z, 1:200 dilution), CD11b (Abcam EPR1344, 1:50000), CD20 (Abcam 64088, 1:200 dilution), FOXP3 (CellSignalling D608R, 1:400 dilution), PD-L1 (D5V3B, 1:50 dilution) and Cell Signaling (64988) Rabbit IgG mAb.

For CD4, CD8, CD211b, CD20 and FOXP3 the positive staining cell number were counted, and viable tissue surface area were measured. IHC scores were presented as the ratio of positive cell counts against the viable tissue surface area. For pH2AX and PD-L1 the intensity of IHC staining were scored at four levels, 0(negative), 1(weak staining), 2(medium staining), 3(strong staining). The percentages of tumor cells at different intensity levels were evaluated. The H-Score were calculated as the IHC score for each sample. H-Score = (% at 0) ×0 + (% at 1) ×1 + (% at 2) ×2 + (% at 3) ×3.

[1]The study was 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)

High base-line DNA-damage (pH2AX) in syngeneic models

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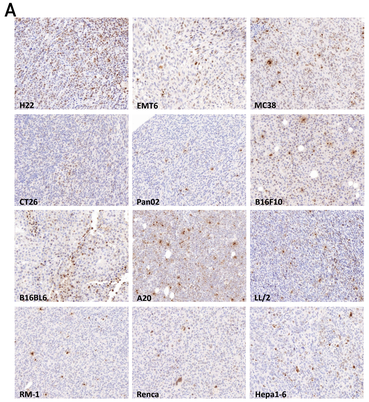

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Syngeneic Model	Cancer Type
H22	Liver cancer
EMT6	Breast cancer
MC38	Colon cancer
CT26	Colon cancer
Pan02	Pancreatic cancer
B16F10	Melanoma
B16BL6	Melanoma
A20	Lymphoma
LL/2	Lung cancer
RM-1	Prostate cancer
Renca	Kidney cancer
Hepa1-6	Liver cancer

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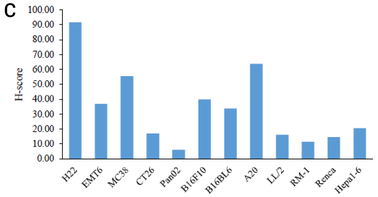

A significant level of DNA-damage measured as pH2AX was observed across 12 different syngeneic tumor models in untreated mice. Notably the HCC models, H22 and Hepa1-6, showed a high baseline pH2AX. This is in stark contrast to patients with HCC (see below).

A majority of HCC patients have DNA-damage (pH2AX) <1% in liver biopsies

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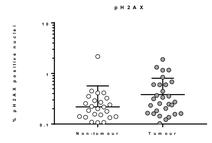

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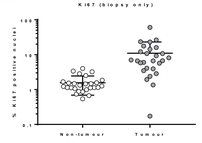
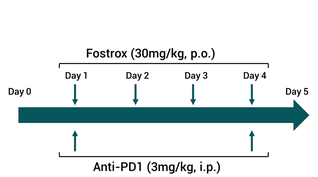
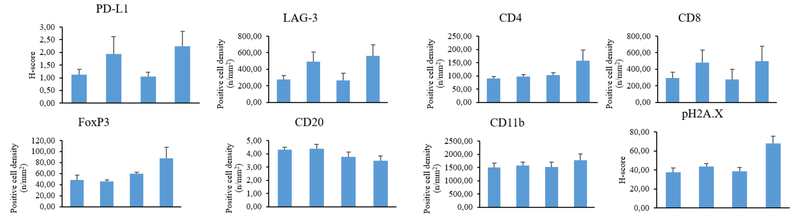


(A) Low baseline DNA damage (%pH2AX) was observed in both normal (mean 0.22%) & tumour cells (mean 0.38%) in the liver. (B) Significantly higher proliferation rate (%Ki67) observed in tumor tissue vs normal liver. Range 0.16-4.02% in non-tumour tissues and 0.17-60.56% in tumour tissue.

Data from Albertella, M. et al EASL Summit P01-05, 2017

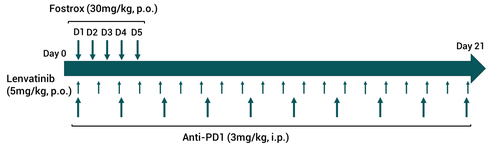
fostrox + anti-PD1 induces changes in the CT26 TME

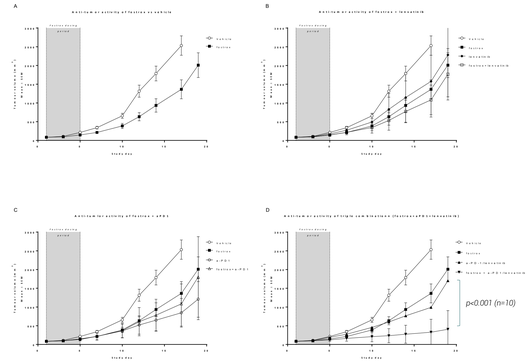




An increase in CD8 positive T-cells in the tumour is shown for mice treated with fostroxacitabine bralpamide as a single agent, also increased expression of PD-L1 and LAG-3. These data indicate that fostroxacitabine bralpamide treatment of tumour bearing mice induces changes in the tumour microenvironment (TME) conducive of an enhanced anti-tumour response with checkpoint inhibitor therapy. An increase in CD4 and FOXP3 (Treg cells) was noted for the combination of fostrox and anti-PD1. No changes in CD20 (B-cells) or CD11b (monocytic cells) was observed. DNA-damage (pHAX) was enhanced with the combination of fostrox + anti-PD1

A triple combination of fostrox + lenvatinib + anti-PD1 shows synergistic anti-tumour efficacy





Each mouse was inoculated subcutaneously in the right lower flank region with CT26 tumor cells. Mice were randomly allocated to 8 study groups with a mean tumour volume of ~84mm^3 at start of the indicated treatments. Notably, although a moderate anti-tumour response is shown for each agent alone or with dual combinations in the CT26 mouse model (Figure A, B, and C), a clearly superior anti-tumour activity is observed for the triple combination of fostroxacitabine bralpamide plus lenvatinib plus anti-PD1 (Figure D).

Conclusions

- **Fostrox induced an increased intra-tumoral PD-L1, LAG-3, and CD8 expression, changes that are consistent with increased immune-mediated anti-tumor activity.**
- **The triple combination of fostrox with anti-PD1 and lenvatinib showed enhanced efficacy in the CT26 syngeneic tumor model**
- **The results indicate a potential for increased anti-tumor efficacy using a triple combination of fostrox plus checkpoint inhibition and anti-angiogenic therapy**

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