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Fostrox (MIV-818) in combination with anti-PD-1 shows increased efficacy in nonclinical tumor models in vivo

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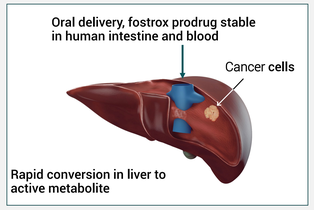
Background

- 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

H22 syngeneic mouse model

Mice, female BALB/c, were inoculated subcutaneously with mouse hepatocellular carcinoma H22 (10^6 cells). At a mean tumor size of 100mm^3 mice were randomized into groups (n=10), and treated with vehicle, anti-PD-1 Ab Biocell (CD279), 3 mg/kg ip BIW for 3 weeks, and fostrox, 30 mg/kg po BID for 5 days, or the combination. Tumor volumes were measured three times per week after randomization

H460 xenograft CAM model

Human H460 lung cancer cells (10^6 cells) were transplanted to the chorioallantoic membrane (CAM) of each egg on day E9. Eggs were then randomized into 4 groups (n=16-22). Treatment with pembrolizumab 2 mg/kg (Carbosynth) was on days E10, E12, E14, E15, E17, and fostrox 0.005 mg/kg on days E10 - E14. Tumors were cut out and weighed on day E18

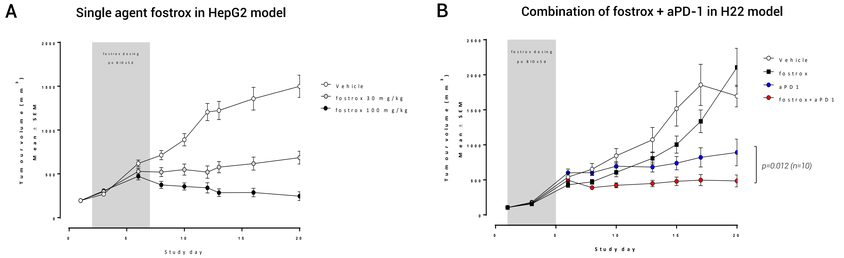
Gene expression analysis

For gene expression analysis a satellite group of tumor bearing mice (n=5) were treated with fostrox (30 mg/kg, po BID x3days) and aPD-1 (10 mg/kg Biocell (CD279), ip on days 1 and 4) or the combination. On day 6 tumors were snap-frozen and targeted RNA-sequencing of a panel of 1080 genes representing different immune cell types was performed (CrownBio mouse I/O RNA Seq Panel)

Histology and IHC

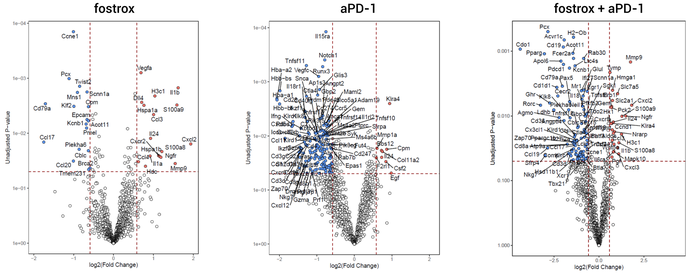
Hematoxylin and eosin (H&E) stained sections were examined by a pathologist. Microscopically 10 non-overlapping highpower fields (HPF) from each section of the tumor were evaluated and graded by a semi-quantitative scoring system for tumor infiltrating lymphocytes (TILs), (0) absence of lymphocytes, (1) < 5, (2) >5 to < 20, and (3) > 20 lymphocytes per HPF. Immunohistochemistry (IHC) scoring for CD3+ and CD8+ cells was 0= No positive reaction, 1=(<5 positive cells), 2=(5-15 positive cells), 3=(15-25 positive cells), 4=(25-50 positive cells), 5=(>50 positive cells)

fostrox + aPD-1 anti-tumor efficacy in the mouse H22 tumor model in vivo



Significant anti-tumor growth activity was observed in the HCC xenograft model HepG2 when treated with fostrox at 30 mg/kg and 100 mg/kg (BID for 5 days, n=10) (**A**). To test the impact of fostrox in combination with a checkpoint inhibitor the syngeneic HCC mouse model H22 was treated with aPD-1 (Biocell CD279, 3mg/kg ip BIW for 3 weeks), fostrox (30 mg/kg po BID for 5 days) or the combination (**B**). A significant enhancement of tumor growth inhibition was observed for the combination (p=0.012, n=10)

Gene expression changes in the mouse H22 tumor model in vivo

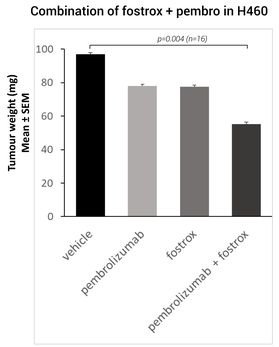


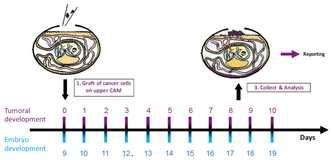
Differentially regulated Gene Ontology (GO) terms by treatments

Treatment (n=5)	Upregulated genes[1]	GO id	Description	p-value (adj)[2]	Genes (upregulated)
fostrox	18	GO:0048247 GO:0006261 GO:1903671	lymphocyte chemotaxis DNA-dependent DNA replication negative regulation of sprouting angiogenesis	0.0007 0.0054 0.0092	Ccl17/Ccl20 Ccne1/Brca2 Klf2
aPD1	156	GO:0050870	positive regulation of T cell activation	3.34E-30	Tnfsf11/Runx3/Cd40lg/Cd274/Cd28/Ifng/Prdm1/ Tnfrsf14/Ctla4/Il1rl2/Sirpa/Syk/Ptprc/Ikzf1/Myb/Icos/ Cd4/Zap70/Vcam1/Lck/Fgl2/Il21/Itgal/Lag3/Tgfbr2/ Foxp3/Cd3e/Cd5/Cd27/Il2ra/Tbx21/Stat5a/Xcl1
fostrox + aPD1	98	GO:0030098	lymphocyte differentiation	2.73E-23	Cd19/Cd79a/Cd1d1/Egr1/Mr1/Rorc/Il18/Tnfsf8/Tnfsf18/ Ctla4/Zap70/Cd8a/Tnfsf4/Ccl19/Lck/Klrc1/Myb/Irf4/ Cd3d/Il18r1/Stat5a/Cd28/Cd27/Mertk/Ikzf3/Tbx21
Treatment (n=5)	**Down-regulated genes[1]**	**GO id**	**Description**	**p-value (adj)[2]**	**Genes (downregulated)**
fostrox	17	GO:0097529 GO:0045766	myeloid leukocyte migration positive regulation of angiogenesis	1.17E-14 1.28E-07	Vegfa/Il1b/S100a9/Ccl3/Cxcl2/S100a8/Cxcr2/Il1a/Ccl4 Vegfa/Il1b/Cxcr2/Il1a/Mmp9
aPD1	9	GO:0007259 GO:0014066	receptor signaling pathway via JAK-STAT regulation of PI3-kinase signaling	4.36E-5 0.0007	Il24/Csf2/Egf F2rl1/Egf
fostrox + aPD1	25	GO:0030595 GO:0050729	leukocyte chemotaxis positive regulation of inflammatory response	3.52E-11 2.33E-07	Cxcl2/S100a9/Ccl3/S100a8/Wnt5a/Il1b/Cxcl3/Pgf S100a9/Ccl3/S100a8/Wnt5a/Il1b

[1] Fold change >1.5, p<0.05
[2] Benjamini-Hochberg false discovery rate adjusted p-value

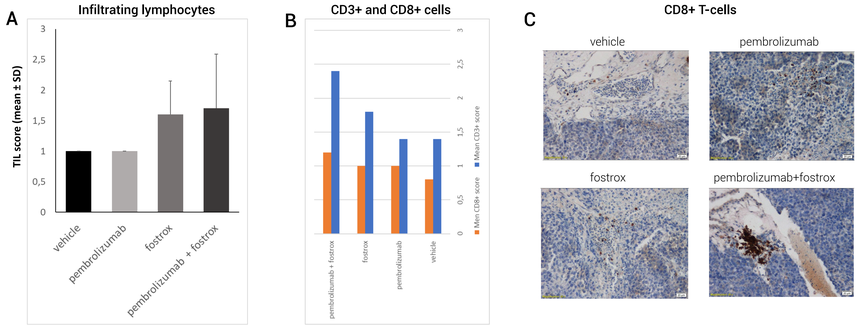
fostrox + pembrolizumab anti-tumour efficacy in the H460 CAM model in vivo





Anti-tumor efficacy of fostrox in combination with pembrolizumab was investigated in the chicken chorioallantoic membrane (CAM) model (Inovotion SAS) using H460 human lung carcinoma cells. Treatment with pembrolizumab (2 mg/kg) or fostrox (0.005 mg/kg) lead to a reduction of tumor weight of 20% and 20%, respectively. The combined treatment resulted in an additive tumor reduction of 43%

fostrox + pembrolizumab induced changes in the H460 TME



Changes in lymphocyte infiltration was scored on H&E stained tumor sections (n=5) from the H460 efficacy study (**A**). Infiltration of CD3 and CD8 positive cells were assessed by IHC (**B**). Representative images of CD8 IHC (brown stain) for the different treatments are shown in (**C**)

Conclusions

- The combination of a checkpoint inhibitor (anti-PD-1) with fostrox showed enhanced tumor growth inhibition
- Fostrox-induced changes in the tumor microenvironment (TME), gene expression and lymphocyte infiltration, are consistent with increased immune-mediated anti-tumor activity
- The results indicate a potential for enhanced efficacy when combining anti-PD-1 with fostrox in the treatment of HCC

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