P01-05: The biomarker potential of Ki67 and pH2AX immunohistochemistry in guiding use of the liver-targeting nucleotide MIV-818 in patients with hepatocellular carcinoma

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INTRODUCTION

MIV-818 has been developed as an orally administered troxacitabine-based prodrug for the treatment of HCC and other liver cancers.

MIV-818 is a nucleotide prodrug of troxacitabine monophosphate (MP), and was designed to target the active metabolite to the liver after oral dosing through first-pass uptake, while minimizing systemic exposure.

MIV-818 is rapidly metabolised in human hepatocytes, generating high levels of the chain-terminating nucleotide troxacitabine triphosphate (TRX-TP) metabolite. TRX-TP is expected to be generated in normal and tumour liver tissue, but only to be toxic to replicating (tumour) cells.

We have demonstrated marked anti-proliferative effects of MIV-818 in cancer cells *in vitro*, and high selectivity for HCC cell lines relative to primary human hepatocytes

Compound	CC_{50} (µM)			
	Primary Hepatocyte (5d)	Hep 3B (5d)	HepG2 (5d)	Window
MIV-818	>100	0.12	0.013	>10,000
Sorafenib	40	2.6	0.99	<20

AIM

To evaluate effects of MIV-818 *in vivo* in an HCC mouse xenograft model

To assess the baseline levels of proliferation and DNA damage in non-tumour vs tumour tissues in HCC patients with chronic liver disease

METHODS

In vivo xenograft study

HepG2 ($1x10^7$) cells were implanted subcutaneously into the left flank of Balb/C nude female mice. Treatment was initiated when a tumour volume of ~200 mm^3 was reached. MIV-818 was dosed via oral gavage BID for 5 days at 160 µmol/kg. Tumours were measured using electronic callipers and volumes were estimated using the formula 0.5 (LxW2). The mice were injected IP with a BrdU/pimonidazole (600 mg/kg; 60 mg/kg) mixture 2 hrs prior to being terminated and tumours were collected for histology.

Quantitative immuno-fluorescence histology on mouse xenograft tumours

Tumour cryosections (10 µm) were immunostained for vasculature using a hamster-anti-mouse-PECAM/CD31, hypoxia using mouse-anti-pimonidazole-FITC (1:500), anti-phospho-Histone H2A.X (Ser139) using mouse-anti-human-pH2AX, BrdU using a monoclonal rat-anti-BrdU. Cellular DNA was counter-stained with Hoechst 33342.

HCC liver tissue study

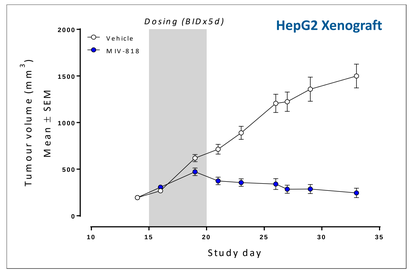
Patients with paired formalin fixed paraffin embedded tumour and non-tumour tissues were selected from those stored in the ethically approved Newcastle Academic Health Partners Bioresource [NAHPB Project 48; REC 12/NE/0395; R&D 6579; HTA license 12534].

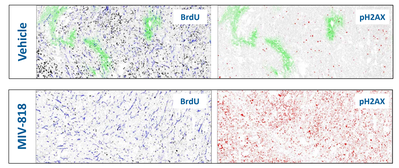
Quantitative immuno-fluorescence histology on HCC liver tissue

Matched tumor and NT biopsy (n=30) or resection tissues (n=10) from 40 patients with HCC were assessed with Ki67 (proliferation) and pH2AX IHC, quantified using Aperio digital analysis.

MIV-818 anti-tumour effects in HCC mouse xenograft model

- Tumour growth inhibition (96%) was observed in the HepG2 xenograft model after treatment with MIV-818 at 160 µmol/kg twice daily for 5 days.
- MIV-818 treatment was well tolerated in mice with no adverse effects (body weight loss or clinical signs).
- The anti-tumour effects were associated with significant inhibition of proliferation (by 94% vs. vehicle) and induction of DNA damage (>20-fold vs. vehicle) in the tumours after treatment with MIV-818.

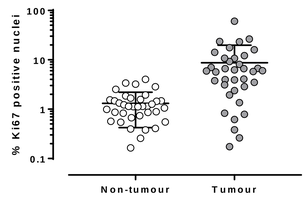


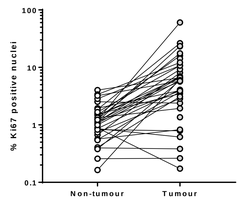


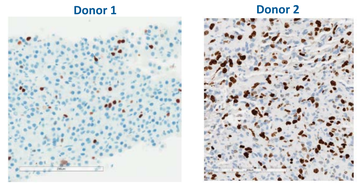
Photoimages of sections stained for BrdU (black), pH2AX (red), mouse vasculature (blue) and hypoxia (green) from control (vehicle) and MIV-818 treated mice

Baseline Ki67 expression in HCC liver tissue

- In patient tissues, the % of Ki67 positive hepatocytes (%Ki67) was significantly higher in tumour compared to non-tumour tissues (Table 1).
- The range of %Ki67 was 0.16-4.02% in non-tumour tissues and 0.17-60.56% in tumour tissue.
- There were no significant differences in %Ki67 in tumour or non-tumour tissues in the presence of cirrhosis (Table 2).







Images at 20x magnification from sections stained for Ki67. Donor 1 with a Ki67 tumour score of 5.6%. Donor 2 with a Ki67 tumour score of 26%.

Table 1 Cohort Summary	
Age (Median)	71
Sex M/F	32/8
Surgical/Biopsy	10/30
Cirrhosis N/Y	20/20
Etiology	
none	4
ALD	7
NAFLD	20
HCV	3
Other	6
Histological Grade (1/2/3)	15/20/4
Tumour size (largest cm) (median)	3.4
Tumour number (meadian)	1.5
Portal Vein invasion N/Y	36/4
Extrahepatic disease N/Y	39/1
TNM Stage (1/2/3+4)	18/4/8
Ascites No/Yes	32/8
encephaloptahy No/Yes	40/0
Childs Pugh Stage A/B/C	34/3/3
BCLC stage 1/2/3/4	17/4/17/2

Table 1. Ki67 staining in tumour vs. non-tumour tissue

Ki67 % +ve nuclei	Non-tumour	Tumour
Mean±SD (n)	1.31 ± 0.89 (37)	8.72 ± 11.04 (37)
Significance	P<0.001	

Wilcoxon Rank test on paired data

Table 2. Ki67 staining in cirrhotic vs non-cirrhotic tissue

Ki67 % +ve nuclei		Non-tumour	Tumour	Non-tumour vs. Tumour
No cirrhosis	Mean±SD (n)	1.47 ± 0.88 (19)	9.21 ± 6.97 (17)	P<0.001
Cirrhosis	Mean±SD (n)	1.15 ± 0.90 (18)	8.31 ± 13.77 (20)	P=0.005
No cirrhosis vs. cirrhosis		P=0.075	P=0.069	

Non-tumour vs Tumour: Wilcoxon Rank test on paired data; No cirrhosis vs cirrhosis: Mann Whitney test

Baseline pH2AX expression in HCC liver tissue

- Baseline % pH2AX positive hepatocytes were low in both tumour (0.24-1.89%) and non-tumour (0.14-2.17%) tissues (Table 3), although the % pH2AX was significantly higher in tumour tissue.
- There were no significant differences in % pH2AX in tumour or non-tumour tissues in the presence of cirrhosis (data not shown).

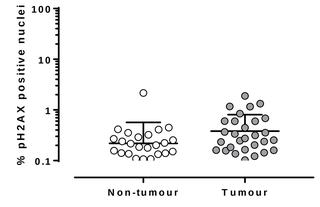


Table 3. pH2AX staining in tumour vs. non-tumour tissue

pH2AX % +ve nuclei	Non-tumour	Tumour
Mean±SD (n)	0.22 ± 0.35 (37)	0.38 ± 0.42 (36)
Significance	P<0.001	

Wilcoxon Rank test on paired data

Ki67 expression and correlation to demographic and HCC stage or grade

- In tumour tissues, %Ki67 correlated with tumour size, portal vein thrombosis (PVT) & TNM staging.
- %Ki67 above 6% (median) was associated with poorer survival (17.6 vs. 80.8 months, p=0.001).
- %Ki67 was independently associated with survival in multivariate cox regression including tissue type (biopsy/resection), tumour number, size, age and PVT.

Spearman's Rho Correlations (Biopsy Tissues only)

		Age	Sex	Etiology	Cirrhosis	Number	Size	EHD	PVT	TNM	CPS	BCLC	GRADE
Ki67_NT	Correlation Coefficient	0.188	0.054	-0.331	-0.135	-0.249	0.184	-0.126	0.287	-0.032	0.043	0.082	0.218
	Sig. (2-tailed)	0.349	0.791	0.091	0.500	0.211	0.359	0.532	0.146	0.873	0.831	0.683	0.285
	N	27	27	27	27	27	27	27	27	27	27	27	26
Ki67_Tumour	Correlation Coefficient	0.222	0.171	-0.051	-0.143	0.288	.438*	0.076	.509**	.566**	-0.153	0.205	0.261
	Sig. (2-tailed)	0.265	0.393	0.799	0.478	0.145	0.022	0.708	0.007	0.002	0.447	0.304	0.197
	N	27	27	27	27	27	27	27	27	27	27	27	26
H2AX_NT	Correlation Coefficient	-0.081	0.214	0.170	-0.271	-0.188	-0.184	-0.101	0.151	-0.065	-0.122	-.412*	-.516**
	Sig. (2-tailed)	0.687	0.283	0.398	0.172	0.348	0.359	0.617	0.451	0.748	0.544	0.033	0.007
	N	27	27	27	27	27	27	27	27	27	27	27	26
H2AX_Tumour	Correlation Coefficient	0.387	-0.298	-0.222	-.446*	-0.126	0.192	-0.120	0.201	-0.046	-0.197	-0.087	-0.052
	Sig. (2-tailed)	0.051	0.139	0.275	0.022	0.539	0.348	0.559	0.326	0.822	0.336	0.672	0.805
	N	26	26	26	26	26	26	26	26	26	26	26	25

Bivariate correlation using a Spearman Rho test

CONCLUSIONS

MIV-818 is highly toxic to proliferating cells *in vivo* without adverse effects in mice.

Using the surrogate marker Ki67, HCC tumour tissue showed marked elevations in proliferation relative to normal liver tissues.

MIV-818 may show a therapeutic window by preferentially targeting proliferating tumour cells in HCC with limited impact on normal hepatocytes

%Ki67 may have value to identify patients with a poorer prognosis who also may be most likely to respond to MIV-818

Low baseline pH2AX levels in both normal and tumour tissues supports the further investigation of pH2AX as a potential clinical biomarker of MIV-818 pharmacodynamics