5101: Defining exposure-PD and efficacy relationships with the novel liver-targeting nucleotide prodrug MIV-818 for the treatment of liver cancers

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Background

- Many systemic chemotherapeutics have failed to show efficacy in hepatocellular carcinoma (HCC), often because systemic toxicity prevents efficacious liver levels of the drug from being reached.
- Troxacitabine is a dioxalane nucleoside that is not metabolized by enzymes such as cytidine deaminase that confer resistance to other nucleoside analogues. It was active in preclinical cancer models and in clinical studies, but ultimately failed in the clinic due to systemic dose limiting toxicities.
- MIV-818 is a novel nucleotide prodrug of troxacitabine-monophosphate (TRX-MP), that was designed as a novel approach to deliver high levels of the chain-terminating nucleotide troxacitabine-triphosphate (TRX-TP) to the liver after oral dosing while minimizing systemic exposure.
- We investigated MIV-818 and troxacitabine using several *in vivo* models in order to identify therapeutic levels of TRX-TP required in the tumour for efficacy.

Methods

***In vivo* xenograft models**

HCC subcutaneous xenograft models were established by inoculation of Hep3B ($2x10^6$), Huh-7 ($1x10^7$) or HepG2 ($1x10^7$) cells (0.1 mL in 1:1 PBS:Matrigel) subcutaneously into the left flank of Balb/C nude female mice. Treatment was initiated when a tumour volume of ~200 mm3 was reached. Troxacitabine was dosed intraperitoneally (i.p.) BID for 5 days at doses, 2.5, 10 and 25 mg/kg. Tumour were measured using electronic callipers and volumes were estimated using the formula 0.5 (LxW^2). For efficacy studies the animals were monitored until a terminal size tumour was reached or until a relative tumour volume (RTV) times four the initial TV at start of treatment was reached. For PK/PD studies the mice were injected i.p. with a BrdU/pimonidazole (600mg/kg/60mg/kg) mixture 2 hrs prior to being terminated at pre-defined time-points after the last dose. Tumour was collected for bioanalysis and histology.

Histology

Tumour cryosections (10 µm) were immunostained for vasculature using a hamster-anti-mouse-PECAM/CD31 (1:500) and fluorescent Alexa 546 secondary (1:500), hypoxia using mouse-anti-pimonidazole-FITC (1:500), anti-phospho-Histone H2A.X (Ser139) using mouse-anti-human-gH2AX (Clone JBW301) tagged with Alexa 647, BrdU using a monoclonal rat-anti-BrdU (clone BU/175; 1:500) and anti-mouse Alexa 750 secondary (1:500). Cellular DNA was counter-stained with Hoechst 33342. The imaging system consisted of a robotic fluorescence microscope with a PCO Edge 4.2 camera and customized ImageJ software. Images of CD31, BrdU, pH2AX, pimonidazole & Hoechst 33342 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 area excluding necrosis and empty regions.

Bioanalysis

Determination of TRX-TP concentrations in tumour homogenates was performed using LC-MS/MS.

In vitro properties

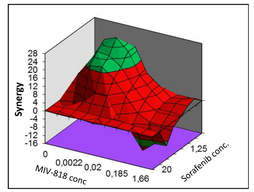
MIV-818 has a superior *in vitro* profile to troxacitabine:

- 10x increased potency of inhibition of HCC cell line growth
- 9x increased conversion to its active metabolite TRX-TP
- Optimized for oral bioavailability and liver targeting, including permeability and intestinal stability
- Stable in human, dog and cynomologus whole blood although being unstable in rodent blood

Table 1	MIV-818	Troxacitabine
Hep3B cell line, mean EC_{50}	0.029 µM	0.24 µM
HepG2 cell line, mean EC_{50}	0.017 µM	0.17 µM
HUH-7 cell line, mean EC_{50}	0.043 µM	0.46 µM
TRX-TP conversion human hepatocytes (AUC_{0-t})	13,579 µM*h	1576 µM*h
Human intestinal S9 (µL/min/mg)	7	Stable
Human liver S9 (µL/min/mg)	42	Stable
Human hepatocytes CL_{int} (µL/min/10^6 cells)	58	Stable
Human, dog, cyno whole blood CL_{int} (µL/min/mg)	Stable (<2)	Stable (<2)
Mouse, rat whole blood CL_{int} (µL/min/mg)	Very unstable (>150)	Stable (<2)

Synergy with sorafenib

- MIV-818 shows synergy *in vitro* with sorafenib in Hep3B cells (Fig. 1)



Hep3B proliferation WST-8 assay

Fig. 1. Synergy plot generated using MacSynergy II software. Hills indicate synergistic interactions.

Toxicity in HCC cells vs. hepatocytes

- MIV-818 shows high selectivity for HCC cell lines relative to primary human hepatocytes in proliferation assays (>2000x)
- MIV-818 demonstrates a large selectivity index in terms of DNA-damage response (phospho-p53, Fig.2; pH2AX, not shown) in HepG2 compared to human hepatocytes
- Low toxicity in human hepatocytes suggests potential for tumour selectivity

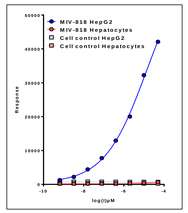


Fig. 2. p-p53 response

Tumour growth inhibition

- Efficacy studies to determine therapeutic TRX-TP levels were performed using troxacitabine due to the instability of MIV-818 in mouse blood (see Table 1)
- Dose-and TRX-TP exposure-dependent tumour growth inhibition (TGI) was demonstrated in the Hep3B, Huh-7 and HepG2 xenograft models (Fig. 6)
- The largest effects were observed in the HepG2 model (Fig. 6C) with a complete tumour regression for a prolonged period of time

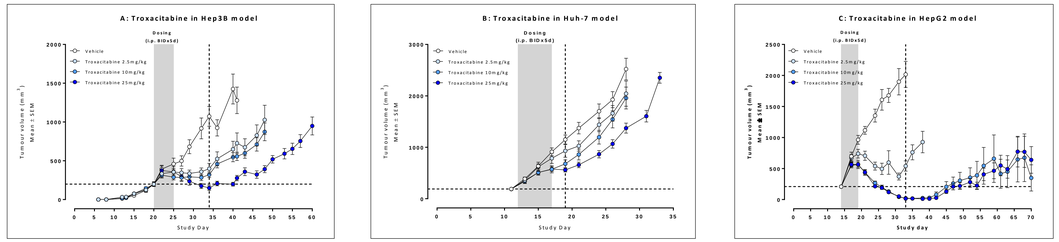


Fig. 6: Effects of troxacitabine on tumour growth inhibition in the Hep3B (A), Huh-7 (B) and HepG2 (C) models. Data expressed as mean±SEM, n=10/group.
Horizontal dashed line indicates tumour volume at start of treatment; Vertical dashed line indicates from which study day TV data were used for the TGI calculations below.

Group / dose	Hep3B TGI	Hep3B TGD
Troxacitabine 2.5mg/kg	70%	~11d
Troxacitabine 10mg/kg	81%	~16d
Troxacitabine 25mg/kg	101%	~26d

Huh-7 TGI	Huh-7 TGD
20%	~0.5d
50%	~3d
60%	~6d

HepG2 TGI	HepG2 TGD
81%	~18d
110%	>48d
111%	>48d

%TGI = (1-[Tt/T0] / [Ct/C0] / 1-[C0/Ct]) x 100; where Tt and T0 are TV of treated mouse X at day t or 0. Ct and C0 are the mean TV of the control group at day t and 0.
Tumour growth delay (TGD) is calculated as T-C where T and C are times in days for mean TV in the treated and control groups to reach 4x the initial TV.

Effects on pharmacodynamic (PD) end-points

- Dose-response effects of troxacitabine on PD end-points were evaluated in the Hep3B, Huh-7 and HepG2 models
- Troxacitabine was administered at doses 2.5, 10 and 25 mg/kg (i.p.) BID for 5 days. Tumours were dissected after the last dose and processed for histological analyses of markers of proliferation (BrdU; Fig. 3A-B) and DNA damage (pH2AX; Fig. 3C-D)
- Dose-dependent inhibition of proliferation (Fig. 3E) and dramatic induction of DNA damage (Fig. 3F) was observed in all three models

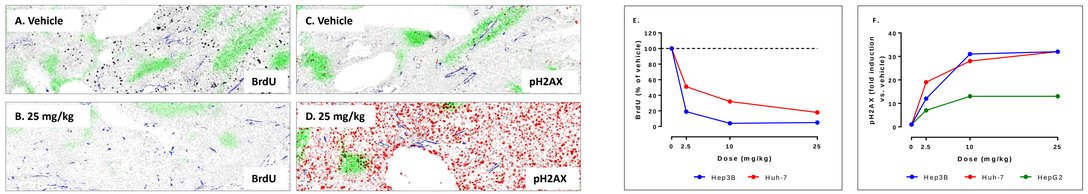


Fig. 3: Representative photomicrographs stained for BrdU (A-B) and pH2AX (C-D) from Hep3B tumours treated with vehicle or troxacitabine (25 mg/kg). Black: BrdU, Red: pH2AX, Blue: Vasculature (CD31), Green: Hypoxia (pimonidazole). Dose-dependent effects of troxacitabine on proliferation (E) and DNA damage (F) markers.

- Time-response effects were evaluated in the Hep3B model after single and repeated dosing with troxacitabine
- Reduced proliferation (Fig. 4A) and induction of DNA damage (Fig. 4B) was observed for up to 168 hrs after last dose of troxacitabine at 25 mg/kg (BIDx5d)
- Following a single dose of troxacitabine, reduced proliferation was observed up to 24 hrs for both doses (Fig. 5A). A time-dependent induction of DNA damage was seen after a single dose of 25 mg/kg troxacitabine (Fig. 5B)

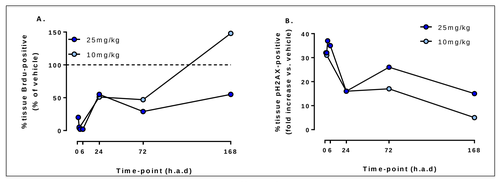


Fig. 4: Time-response effects after repeated dosing

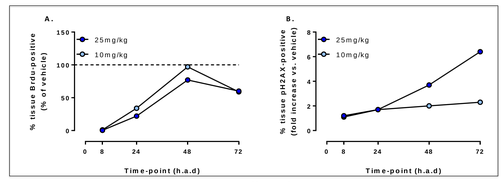
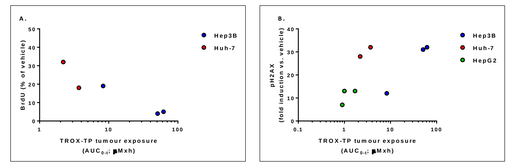


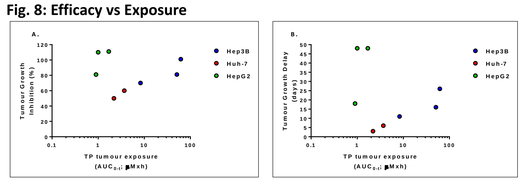
Fig. 5: Time-response effects after a single dose

Exposure-PD-Efficacy Relationships

- Fig. 7: PD vs Exposure



- Fig. 8: Efficacy vs Exposure



Conclusions

- TRX-TP exposures required for pronounced anti-tumor effects are informing a comprehensive understanding of PK-PD-efficacy relationships for the active metabolite of MIV-818 and are expected to guide dosing and dose selection in clinical studies.
- MIV-818 is currently in nonclinical development in preparation for the initiation of clinical trials in patients with advanced HCC and other liver cancers.