# P02-04: Liver targeting and anti-tumour efficacy of the nucleotide prodrug MIV-818 in nonclinical models of hepatocellular carcinoma

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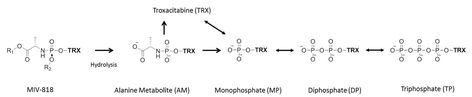
## INTRODUCTION

- Troxacitabine (TRX) is a chain terminating nucleoside analogue with preclinical anticancer activity against hepatocellular carcinoma (HCC). Clinical development of TRX (given IV) was halted due to systemic toxicity.
- MIV-818, a nucleotide prodrug of troxacitabine monophosphate (TRX-MP), has been designed to direct high levels of the chain-terminating nucleotide troxacitabine triphosphate (TRX-TP) to the liver after oral dosing through first-pass uptake, while minimizing systemic exposure
- In the liver, the membrane permeable prodrug undergoes fast conversion into the poorly permeable alanine metabolite (AM), which is then converted to the active TRX-TP metabolite via a series of charged and poorly permeable metabolites:




- MIV-818 is rapidly hydrolysed to the AM in rodent blood ($CL_{int}$ >150µL/min/mg) due to its high levels of esterase activity. This limits the utility of mouse and rat models. MIV-818 is stable in human and non-rodent blood ($CL_{int}$ <2µL/min/mg)
- Liver targeting of MIV-818 was investigated in rats after oral dosing and anti-tumour efficacy was evaluated *in vivo* in HCC mouse xenograft models

## METHODS

***In vivo* rat PK**
MIV-818 ( 80 µmol/kg, PO) and TRX (80 µmol/kg, PO and IV) were administered to male Wistar rats and plasma and liver were collected at different time points after dosing (up to 24 h) for bioanalysis.

***In vivo* mouse xenograft models**
HCC subcutaneous xenograft models were established by inoculation of Hep3B ($2x10^6$) or HepG2 ($1x10^7$) cells (0.1 mL in 1:1 PBS:Matrigel) subcutaneously into the left or right flank of Balb/C nude female mice. Treatment was initiated when a tumour volume (TV) of ~200 $mm^3$ was reached. MIV-818 was dosed via oral gavage (PO) twice daily (BID) for 5 days at doses 48, 80 and 160 µmol/kg. Tumours were measured using electronic callipers and volumes were estimated using the formula 0.5 ($LxW^2$). For histological analyses, the mice were injected intraperitoneally with a BrdU/pimonidazole (600mg/kg / 60mg/kg) mixture 2 hrs prior to being terminated and the tumour was 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-gH2AX, BrdU using a monoclonal rat-anti-BrdU. Cellular DNA was counter-stained with Hoechst 33342.

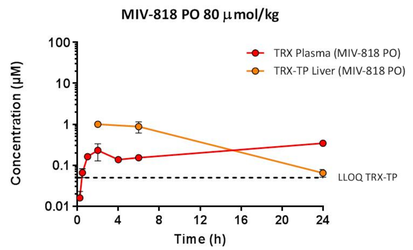
**AFP ELISA**
AFP levels were measured in plasma using the Quantikine ELISA human α-fetoprotein kit from R&D systems (catalogue number DAFP00, Lot 343004), according to the manufacturer's instructions.

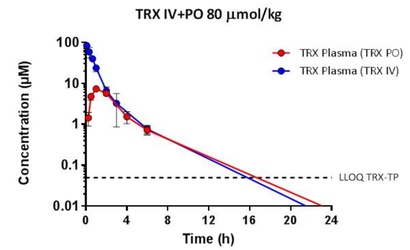
**Bioanalysis**
Determination of TRX in plasma and TRX-TP concentrations in tumour and liver homogenates was performed using LC-MS/MS.

## RESULTS

### Liver targeting in rat

- Troxacitabine (TRX) at 80 µmol/kg was dosed IV or PO and MIV-818 at 80 µmol/kg was dosed PO to rats and the exposures to TRX in plasma and TRX-TP in liver were assessed



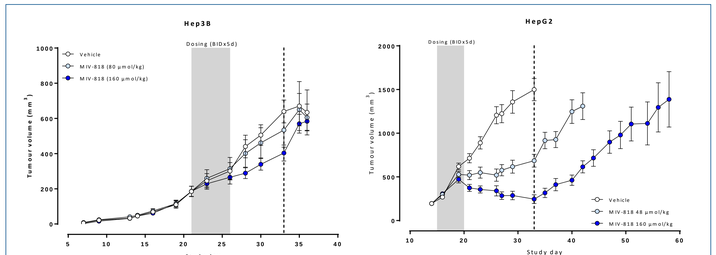




| Drug | Dose (µmol/kg) | Adm Route | Analyte | Tissue | $C_{max}$ (µM) | $AUC_{0-24h}$ (µM.h) | $TP_{liver}$ / $TRX_{plasma}$ ratio |
|---|---|---|---|---|---|---|---|
| MIV-818 | 80 | PO | TRX<br>TRX-TP | Plasma<br>Liver | 0.35<br>1.0 | 5.4<br>10 | **1.9** |
| TRX | 80 | IV | TRX<br>TRX-TP | Plasma<br>Liver | 102<br><0.05 | 76<br><1.2 | **<0.016** |
| TRX | 80 | PO | TRX<br>TRX-TP | Plasma<br>Liver | 7.4<br><0.05 | 22<br><1.2 | **<0.055** |

- MIV-818 administered PO resulted in a liver TRX-TP $C_{max}$ of 1.0 µM and a liver TRX-TP $AUC_{(0-24)}$ of 10 µM.h
- The same dose of TRX dosed IV and PO resulted in liver TRX-TP concentrations below the limit of quantification (0.05 µM) at all time points
- The $C_{max}$ liver TRX-TP was >20 times higher for MIV-818 after PO dosing than for TRX after IV and PO dosing
- The $AUC_{(0-24)}$ liver TRX-TP vs. $AUC_{(0-24)}$ plasma TRX ratio for MIV-818 after PO dosing was >100 times higher than for TRX after IV dosing, demonstrating the substantially improved liver targeting by MIV-818 in rat, despite low stability in rat blood

### Tumour growth inhibition

- MIV-818 was given at 48, 80 or 160 µmol/kg (PO) twice daily for 5 days to mice bearing Hep3B or HepG2 tumours
- Dose-dependent tumour growth inhibition (TGI) was demonstrated in both HCC xenograft models despite the expected poor delivery to the tumour due to rapid metabolism in mouse blood
- The largest effects were observed in the HepG2 model
- In these models, higher tumour TP levels are associated with greater anti-tumour effects (Albertella et al, 2017)



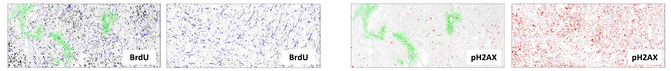

*Data expressed as mean±SEM, n=10/group. Vertical dashed line indicates from which study day TV data were used for the TGI calculations.*

| | Hep3B | | | HepG2 | | |
|---|---|---|---|---|---|---|
| **Group** | **TGI** | **TGD** | **TP tumour Cmax (µM)** | **TGI** | **TGD** | **TP tumour Cmax (µM)** |
| MIV-818 (48 µmol/kg) | - | - | - | 63% (P<0.0001) | ~12d | 0.06 |
| MIV-818 (80 µmol/kg) | 25% (ns) | ND | 0.25 | - | - | - |
| MIV-818 (160 µmol/kg) | 40% (P<0.001) | ND | 0.41 | 96% (P<0.0001) | ~23d | 0.11 |
| Troxacitabine* (117 µmol/kg) | 101% (P<0.0001) | ~26d | 1.6 | 111% (P<0.0001) | >48d | 0.34 |

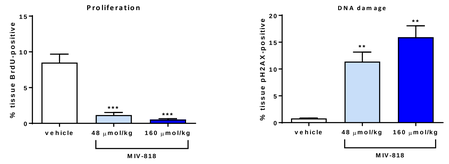
*TGI = [1-[Tt/T0] / [Ct/C0] / 1-[C0/Ct]] x 100; where Tt and T0 are TV of treated mouse X at day t or 0. Ct and C0 are the mean TV of the control group at day t and 0*
*Tumour growth delay (TGD) is calculated as T-C where T and C are times in days for mean relative TV in the treated and control groups to reach 4x the initial relative TV*
P values indicate significant difference compared to control (vehicle) group; ND=Not determined
*Albertella et al, 2017

### Inhibition of proliferation and induction of DNA damage

- Dose-response effects of MIV-818 on proliferation (BrdU) and DNA damage (pH2AX) were evaluated in the HepG2 tumours after dosing at 48 and 160 µmol/kg (PO) twice daily for 5 days



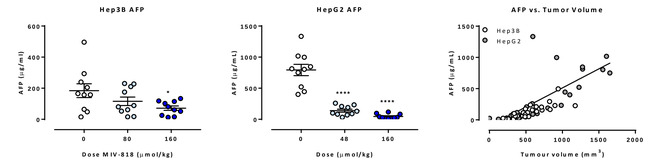

*Photoimages of sections stained for BrdU (black), pH2AX (red), mouse vasculature (blue) and hypoxia (green) from control (vehicle) and MIV-818 (160 µmol/kg) treated mice*




*Data are presented as mean±SEM (n=3). **P<0.01, ***P<0.001, compared to vehicle.*

### Reductions in plasma AFP

- Dose-dependent reductions in plasma AFP levels were observed in the Hep3B and HepG2 models after treatment with MIV-818




*Data are presented as individual plots and mean±SEM (n=10/group). *P<0.05, ****P<0.0001, compared to vehicle.*

- The largest reductions were observed in the HepG2 model (by up to 94%) at the highest dose (160 µmol/kg), in line with the largest anti-tumour effects seen in this model
- The plasma AFP levels correlated strongly with the corresponding tumour volume ($r^2$=0.56, P<0.0001)

## CONCLUSIONS

- MIV-818 is a nucleotide prodrug of troxacitabine with improved liver targeting in rat and anti-tumour effects in mouse xenograft models of HCC, even despite the low blood stability in these species
- MIV-818 has completed the nonclinical toxicology package and is currently in preparation for the first clinical trials in patients with advanced HCC and other liver cancers

## REFERENCES

Albertella et al, AACR Annual meeting 2017, Abstract 5101
http://www.medivir.se/v5/images/pdf/2017/AACR-2017-Poster-MIV-818-Albertella-final.pdf