Liver-targeting with the novel nucleotide prodrug MIV-818 designed for the treatment of liver cancers

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R BETHELL, K TUNBLAD, B RIZOSKA, A LINDQVIST, S JURIC, S SEDIG, F ÖBERG, B CLASSON, A ENEROTH, J ÖHD AND M ALBERTELLA
Medivir AB, Huddinge, SWEDEN

SAT-123

INTRODUCTION

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 was developed as a dioxalane nucleoside which was not subject for enzymes conferring resistance to other nucleoside analogues such as cytidine deaminase.

Troxacitabine was active in preclinical cancer models and in clinical studies, but ultimately failed in the clinic due to systemic dose limiting toxicities.

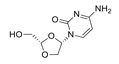
MIV-818 is a novel nucleotide prodrug of troxacitabine-monophosphate (TRX-MP), that has been designed to deliver high levels of the chain-terminating nucleotide troxacitabine-triphosphate (TRX-TP) to the liver after oral dosing while minimizing systemic exposure – offering multiple advantages over troxacitabine itself:

- Oral bioavailability and increased permeability
- Directed delivery to the liver and reduced systemic toxicity
- Increased cancer cell killing

We compare MIV-818 and troxacitabine using *in vitro* and *in vivo* models in order to demonstrate liver targeting and a superior anti-cancer profile.

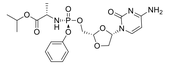
Metabolic activation of troxacitabine prodrugs

Troxacitabine



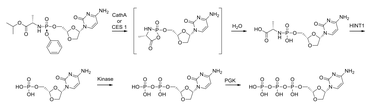
Example prodrug

Ester 1



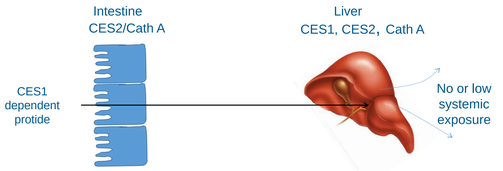
Ester 2

Variations in prodrug esters modulate multiple parameters, e.g. 1st step rate, potency, phys chem properties, liver/intestinal metabolism



Monophosphate (MP) | Diphosphate (DP) | Triphosphate (TP)

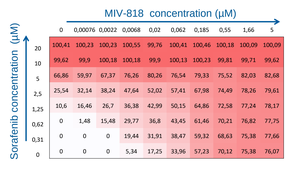
LIVER-TARGETING CONCEPT



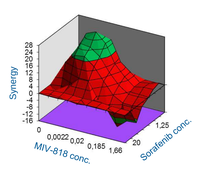
RESULTS

Assay	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
Human hepatocytes, *mean EC_{50}*	>100 µM	>100 µM
Human intestinal S9 fraction *(µL/min/mg)*	7	Stable
Human liver S9 fraction *(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
Mouse, rat whole blood CL_{int} *(µL/min/mg)*	Very unstable (>150)	Stable

The combination of MIV-818 and sorafenib is synergistic



Hep3B Percent growth inhibition for each concentration combination (WST-8 assay, Kit8)



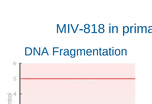
Synergy plot (MacSynergy II software)
Hills indicate synergistic interactions

- MIV-818 shows synergy *in vitro* with sorafenib and regorafenib (not shown) in Hep3B cells
- Consistent effect – seen in other cell lines

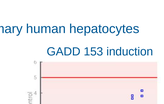
Low toxicity in human primary hepatocytes suggests potential for tumour selectivity

MIV-818 in primary human hepatocytes

DNA Fragmentation



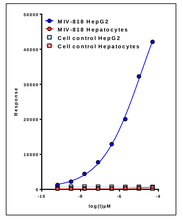
GADD 153 induction



Compound	CC_{50} (µM)		
	1° Hepatocyte (2d)	Hep 3B (5d)	Window
MIV-818	>100	0.024	>4,000
Sorafenib	40	2.6	<20

- Prodrugs show high selectivity for HCC cell lines relative to primary human hepatocytes compared to sorafenib in viability assays

HepG2 vs. primary human hepatocytes



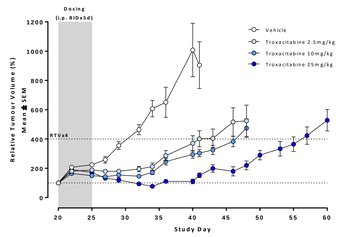
- Large selective index in terms of DNA-damage response observed between HepG2 and fresh human hepatocytes (24h)
- Dramatic induction of DNA damage a potential surrogate biomarker of clinical efficacy

Anti-tumour activity and exposure to troxacitabine triphosphate

Due to the instability of MIV-818 in mouse blood (see Table) the efficacy studies were performed with troxacitabine.

The aim was to establish exposures to TRX-TP required for effective tumor growth suppression in different HCC *in vivo* models in order to define target therapeutic concentrations in the clinic.

Dose-response effects of troxacitabine on tumour growth inhibition (TGI) were demonstrated in the Hep3B (below), Huh-7 and HepG2 (not shown) xenograft models.



Hep3B xenograft model
Dosing initiated at mean tumour volumes of 200 mm^3

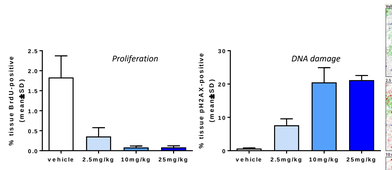
%TGI = (1-[Tt/T0] / [Ct/C0] / 1-[C0/Ct]) x 100; where Tt and T0 are TV of treated mouse X at day t or 0. Ct and C0 are the mean TV of the control group at day t and 0.
Tumour growth delay (TGD) is calculated as T-C where T and C are times in days for mean TV in the treated and control groups to reach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

Group	Maximal TGI (Day 34)	TGD (at RTVx4)	TP $_{tumour}$ (AUC_{0-t})
Troxacitabine (25 mg/kg)	101%	~26d	62 µMxh
Troxacitabine (10 mg/kg)	81%	~16d	51 µMxh
Troxacitabine (2.5 mg/kg)	70%	~11d	8 µMxh

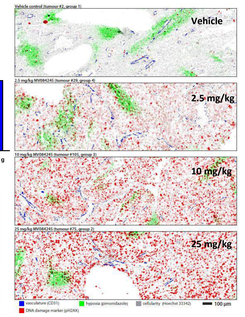
- Dose-dependent effects on tumour growth inhibition (TGI) and tumour growth delay (TGD)
- TRX-TP exposures in tumour leading to significant tumour growth delay were defined

Dramatic induction of DNA damage in treated tumours

The effect of the different exposures to TRX-TP on DNA damage (pH2AX) and proliferation (BrdU) was examined in Hep3B tumours, excised after the treatment period (bidx5d ip).



DNA damage (pH2AX)



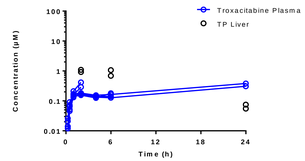
Dose	BrdU (% of vehicle)	pH2AX (fold change vs. vehicle)
2.5 mg/kg	19%	12-fold
10 mg/kg	4%	31-fold
25 mg/kg	5%	32-fold

- Dose-dependent inhibition of proliferation (BrdU) and induction of DNA damage (pH2AX) following troxacitabine treatment (2h after last dose)
- Note induction of DNA damage and inhibition of proliferation within hypoxic areas (green) indicating good penetration and conversion to TP in these hard to treat regions

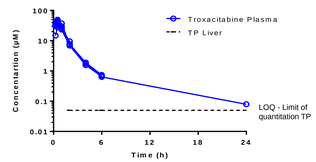
MIV-818 delivers high concentrations of active triphosphate to liver

Despite the instability of MIV-818 in rat blood (see Table) the liver delivery of MIV-818 was examined *in vivo* and compared to ip administration of troxacitabine.

MIV-818 po (80 µmol/kg)



Troxacitabine ip (80 µmol/kg)



MIV-818 po (80 µmol/kg)	Tissue	C_{max} µM	AUC µM*hr
Troxacitabine	Plasma	0.35	5.4
Troxacitabine triphosphate	Liver	1.0	10
$TP_{liver}/trox_{plasma}$ ratio			1.9

Troxacitabine ip (80 µmol/kg)	Tissue	C_{max} µM	AUC µM*hr
Troxacitabine	Plasma	42	67
Troxacitabine triphosphate	Liver	<0.05	<1.2
$TP_{liver}/trox_{plasma}$ ratio			<0.018

- Single dose oral administration of MIV-818 to rat gave significant liver exposure to the TRX-TP
- Low systemic exposure to troxacitabine was seen, consistent with aim of reduced systemic toxicity
- In contrast, troxacitabine administration did not yield quantifiable TRX-TP in liver, and gave high systemic exposure to troxacitabine, which is known to cause significant toxicity
- >100-fold increased delivery of TRX-TP to liver from MIV-818 compared to troxacitabine
- Efficacious liver exposure to troxacitabine triphosphate from MIV-818 but not troxacitabine

CONCLUSIONS

MIV-818 is a novel phosphoramidate prodrug of troxacitabine that shows greatly improved *in vitro* properties compared to the parent nucleoside, including

- Potent inhibition of HCC cell line growth and selective induction of DNA damage relative to primary human hepatocytes
- Increased conversion to the active metabolite, troxacitabine triphosphate, TRX-TP
- Orally bioavailable and targeted for metabolism and activation in the liver

MIV-818 is synergistic with sorafenib *in vitro*, suggesting that it might prove particularly efficacious in combination treatment

Efficacious exposures to TRX-TP were defined *in vivo*, that demonstrate dramatic induction of DNA damage defining target concentrations for future clinical studies

Oral dosing of MIV-818 results in a >100-fold increased delivery of the active metabolite, TRX-TP, to the liver compared to troxacitabine

MIV-818 is in preclinical development for the treatment of HCC and other liver cancers

CONTACT INFORMATION

Richard.Bethell@medivir.com
Mark.Albertella@medivir.com

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