Selective targeting of the liver with nucleotide prodrugs for the treatment of liver cancers

P-035

Richard Bethell, Björn Classon, Anders Eneroth, Fredrik Öberg, Pedro Pinho, Sanja Juric, Annelie Lindqvist, Susanne Sedig, Karin Tunblad, Biljana Rizoska, and Mark Albertella
Medivir AB, Huddinge, Sweden

Background

Current standards of care in hepatocellular carcinoma all involve liver targeting…

Systemically administered chemotherapeutic agents typically have low liver access

- Systemic toxicity typically precedes efficacy on HCC – many trial failures
- Not a good alternative as neoadjuvant to curative treatment

Topographic invasive methods only proven way to hit therapeutic window (TACE)

- Transarterial administration of doxorubicin, oxaliplatin or radiation scaffolds in presence or absence of concomitant embolization
- Costly and risky as well as tech demanding
- Limited by AV-shunt, portal vein tumor thrombosis, arteritis reaction, tumor burden etc.
- Not suitable as neoadjuvant to curative treatment

Sorafenib, an oral tyrosine kinase inhibitor with proven efficacy in HCC

- Primarily hepatic pharmacokinetics, with oral BD dosing - 51% parent eliminated in bile
- This "passive" liver targeting potentially contributes to the efficacy of sorafenib in this indication

Strategy for the development of an orally administered liver-targeted prodrug to treat HCC

Nucleotide prodrugs have been shown to selectively deliver high levels of active metabolites to the liver after oral delivery, e.g. sofosbuvir and MIV-802

Troxacitabine selected as the nucleoside to prodrug

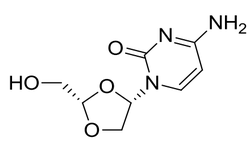
- Troxacitabine is active in preclinical cancer models, including HCC, with evidence of clinical activity
- Troxacitabine has low permeability, limiting its oral bioavailability and intracellular penetration
- Unique mechanism of conversion of diphosphate to triphosphate expected to lead to enhanced triphosphate formation in hypoxic cells
- Clinical development halted due to systemic toxicity

Optimization Objectives

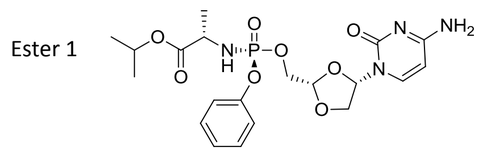
- Improve delivery of troxacitabine triphosphate to the liver through first-pass uptake and rapid intracellular conversion to non-permeable charged metabolites
- Optimize intestinal stability to minimize GI exposure to active metabolites
- Improve permeability to enable oral administration
- Minimize systemic exposure to troxacitabine

Metabolic activation of troxacitabine prodrugs

Troxacitabine



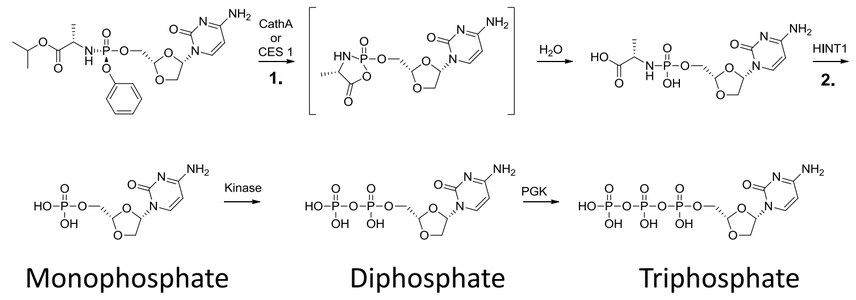
Example prodrug



Ester 1

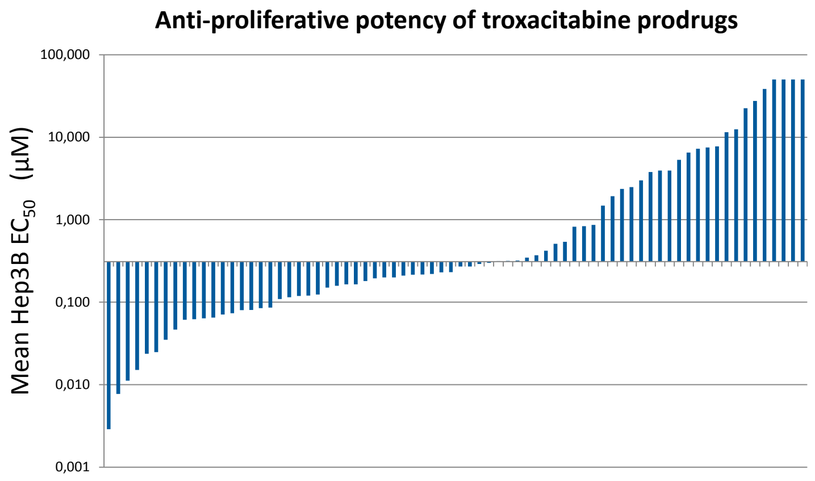
Ester 2

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



Monophosphate Diphosphate Triphosphate

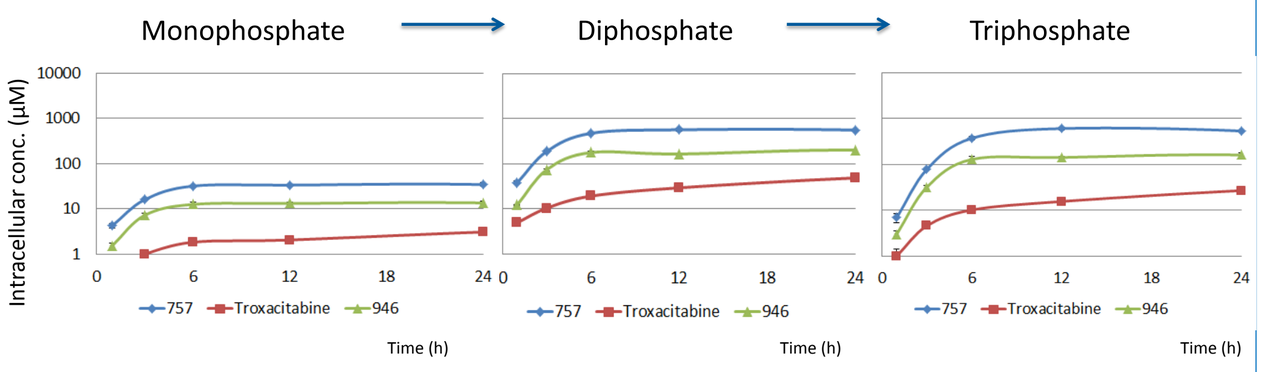
Prodrugs can show increased potency compared to troxacitabine



Many prodrugs have significantly increased potency compared to troxacitabine. Similar data obtained for Huh7 and HepG2 (not shown)

Waterfall plot of prodrugs ranked by potency vs .Hep3B cells. X-axis centered on troxacitabine potency of ~0.3 µM

Formation and elimination time curves of metabolites in human hepatocytes



Compounds incubated at 10 µM concentration for times indicated

- Prodrugs show increased rate and extent of conversion to mono-, di- and triphosphate compared to troxacitabine
- Similar data obtained in multiple HCC cell lines (not shown)
- 10-fold increase in CaCo-2 cell permeability relative to troxacitabine

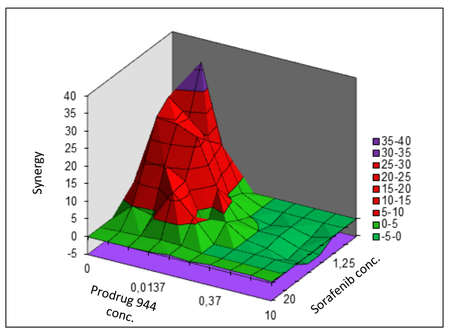
Prodrug combinations with sorafenib are synergistic

Prodrug 944 concentration (µM)

Sorafenib concentration (µM)

	0	0,0015	0,0045	0,0137	0,041	0,12	0,37	1,11	3,33	10
20	100,4	100,8	100,8	100,9	101,0	100,9	101,0	100,9	101,0	100,9
10	99,0	99,9	99,8	100,1	100,3	100,2	100,3	99,7	100,2	99,5
5	52,7	59,0	63,6	71,5	77,3	80,7	84,1	89,3	90,2	90,4
2,5	24,8	36,7	43,4	51,7	63,3	68,2	76,1	85,9	88,6	88,8
1,25	4,9	23,0	30,0	41,6	52,4	61,7	71,3	84,3	88,5	88,6
0,62	-10,9	10,8	20,8	29,1	43,5	53,2	66,2	80,7	87,7	88,4
0,31	-19,9	4,9	16,9	24,4	39,7	49,7	64,7	80,9	87,5	88,8
0	0,0	-10,1	-1,2	13,4	36,3	52,8	72,0	84,7	90,5	91,6

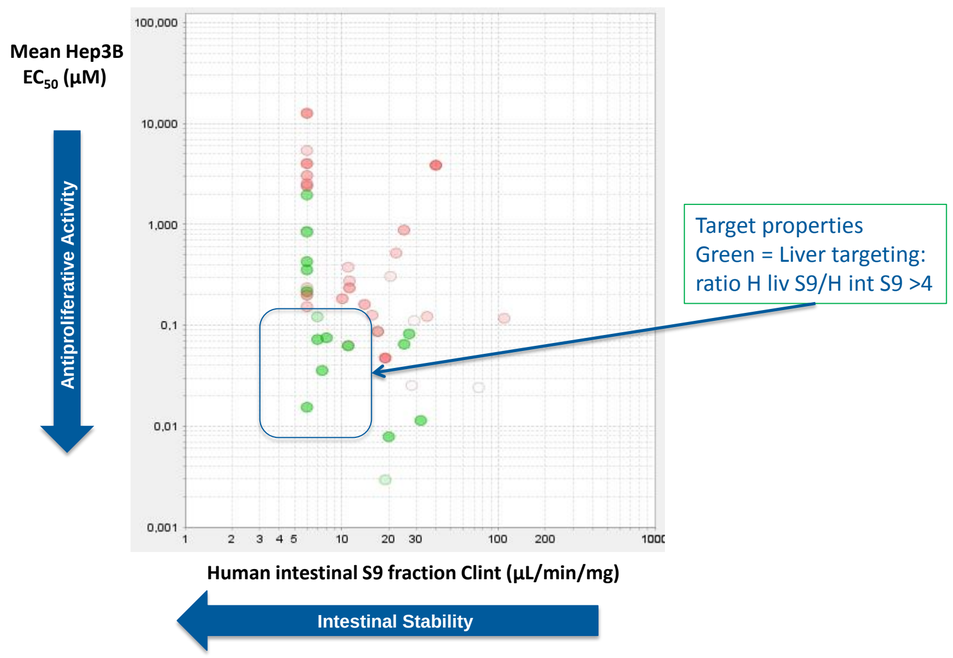
Percent growth inhibition for each concentration combination (WST-8 assay, Kit8)



Synergy plot (MacSynergy II software)
Red hills indicate synergistic interactions

- New therapy will need to be considered in a combination with sorafenib
- Prodrugs of troxacitabine including 944 show synergy with sorafenib in Hep3B cells

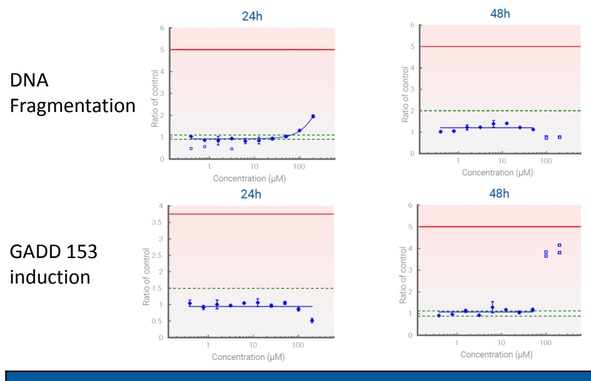
Prodrugs are optimised for potential for liver targeting



- The optimal drugs should be stable in the intestine and yet rapidly converted in the liver to the active triphosphate metabolite
- Prodrugs for progression are selected based on potency, stability in human intestinal S9 and having a good ratio vs. human liver S9

Low toxicity in human primary hepatocytes suggests potential tumour selectivity

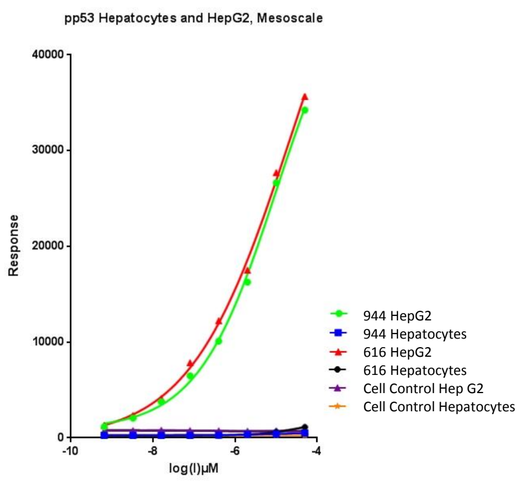
Prodrug 757 in primary human hepatocytes



Compound	CC_{50} (µM)	
	1° Hepatocyte (2d)	Hep 3B (5d)
Troxacitabine	>100	0.31
Prodrug 757	>100	0.014
Sorafenib	40	2.6

- 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

Conclusions

- **Phosphoramidate prodrugs of troxacitabine have been identified that show 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 formation of the active metabolite, troxacitabine triphosphate**
- **A number of these compounds have properties that enable them to be orally bioavailable and targeted for metabolism and activation in the liver**
- **These compounds are synergistic with sorafenib, suggesting that they might prove efficacious in combination treatment**
- **Further preclinical profiling of the best compounds is ongoing. GLP safety studies are expected to start later this year with the intention to develop this class of compounds for the treatment of HCC and other liver cancers**