PRECLINICAL CHARACTERISATION OF MIV-802, A NOVEL URIDINE NUCLEOTIDE HCV NS5B POLYMERASE INHIBITOR, FOR TREATMENT OF HEPATITIS C VIRUS INFECTION

Annelie Lindqvist [1] , Helen Kylefjord [1], Horst Wähling [1], Sanja Juric [1], Staffan Torssell [1], Gennadiy Kalayanov [1], Lotta Vrang [1], Emily Hoberg [2], Claes Gustafsson [2], Matthias Gotte[3], Anders Eneroth [1], Erik Lindström [1], Kristina Wikström [1], Paul Targett-Adams[1], Pedro Pinho [1], Richard Bethell [1], Åsa Rosenquist* [1]

[1]Medivir, Huddinge, Sweden. [2]Dept. of Biochem and Cell Biology, University of Gothenburg, Gothenburg, Sweden. [3]Department of Medical Microbiology & Immunology, University of Alberta, Edmonton (Alberta), Canada.

Contact: asa.rosenquist@medivir.se

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BACKGROUND

HCV NS5B polymerase nucleotide inhibitors are considered as a central part of current and future interferon-free combination therapies for treatment of hepatitis C virus infection.

The compounds have high pan-genotype activity and a high barrier to resistance making them highly attractive as part of shortened and simplified HCV treatment regimens.

OBJECTIVES

MIV-802 is a prodrug of a novel uridine analogue that is being developed for HCV therapy. The aim of this abstract is to summarize the *in vitro* anti-viral profile, early PK, safety and toxicology data of MIV-802 supporting the advancement of the compound into non-clinical development.

MATERIALS & METHODS

- Antiviral activity for MIV-802 was evaluated using HCV replicons expressing NS5B sequences from HCV genotypes 1-6, including variants conferring resistance to nucleotides, and clinical isolates (from genotypes 1-4; Monogram BioSciences).[1,2]
- The uridine nucleoside triphosphate (MIV-802-UTP) was tested for activity against purified HCV NS5B polymerase, and human RNA and DNA polymerases.
- The mechanism of action for MIV-802-UTP was elucidated through collaboration with Matthias Götte (University of Alberta).[3] Densitometric quantitation of the bands on Northern blots was used to determine the IC_{50} for chain termination (defined as the concentration of compound required to inhibit formation of full-length RNA product by 50%).
- In collaboration with Claes Gustafsson (University of Gothenburg), the potential for MIV-802-UTP to be incorporated into RNA by mitochondrial RNA polymerase (POLMRT), and the capacity of MIV-802-UTP to inhibit POLMRT-catalyzed transcription were evaluated. [4]
- The potential genotoxicity, together with the potential cellular/mitochondrial toxicities, of MIV-802 and its parent nucleoside (MIV-802-Nuc) were characterized using a panel of cell lines and human primary cells. Cardiovascular liabilities were evaluated *in vitro* using differentiated cardiomyocytes from human induced pluripotent stem cells (iPS). Compounds were evaluated on 4 electrophysiological outcomes after incubation up to 100 µM for 14 days .
- MIV-802-UTP levels were determined in fresh primary human hepatocytes *in vitro* and in dog liver after oral dosing. MIV-802 was evaluated in a 7 day toxicology study at oral doses of 500 mg/kg and 1000 mg/kg given once daily to CD-1 mice in order to assess the toxicity and toxicokinetics.

Acknowledgements

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RESULTS

In vitro Virology and Specificity

- MIV-802-UTP was a competitive inhibitor of the NS5B polymerase competing with natural UTP with a Ki of 0.71 µM and displayed excellent selectivity against the human DNA polymerases α, β and γ as well as the mitochondrial RNA polymerase with IC_{50}>200 µM (Table 1). Also, MIV-802-UTP was not a substrate for POLMRT-catalyzed incorporation into RNA at concentrations up to 200 µM.
- The mechanism of action for MIV-802-UTP was revealed to be inhibition of NS5B-catalyzed RNA polymerization through chain termination. The IC_{50} was 2.63 µM for MIV-802-UTP and 2.10 µM for sofosbuvir-UTP (Figure 1).
- MIV-802 displayed pan-genotypic potency in HCV replicons GTs 1-6 with an EC_{50} range of 17-58 nM (EC_{50} range for sofosbuvir: 48-210 nM) (Table 2).
- The antiviral profile of MIV-802 on a series of clinical isolates was also studied. For each GT, EC_{50} values obtained using MIV-802 were lower than those obtained using sofosbuvir, e.g. MIV-802 was 2.2-fold more potent than sofosbuvir against the GT3 panel (Figure 2).
- MIV-802 was evaluated for inhibition of HCV replicons encoding sofosbuvir-associated resistance substitutions in NS5B. The data revealed that, like sofosbuvir, S282T confers low-level resistance to MIV-802, while L159F/L320F confers a small change in susceptibility (Table 3).

Table 1. *In vitro* inhibition of HCV polymerase and cellular human polymerases by the triphosphate derived from MIV-802

HCV NS5B Pol G1b IC50 (µM)	hDNApol α IC_{50} (µM)	hDNApol β IC_{50} (µM)	hDNApol γ IC_{50}0 (µM)	hPOLMRT IC_{50} (µM)
0.71	>200	>200	>200	>200

Figure 1. Chain termination of HCV NS5B-catalyzed RNA polymerization by MIV-802-UTP

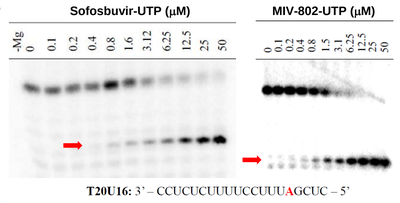
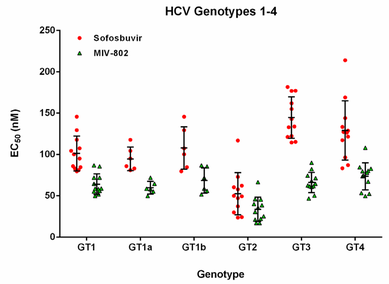


Table 2. *In vitro* activity of MIV-802 in HCV replicons encoding NS5B from GTs 1-6

HCV Assay: EC_{50} (µM)	Sofosbuvir	MIV-802
HCV GT1b (stable)	0.098 (n=128)	0.045 (n=65)
HCV GT1b (transient)	0.081 (n=31)	0.044 (n=22)
HCV GT1a*	0.13 (n=18)	0.050 (n=18)
HCV GT2a replicon	0.048 (n=2)	0.023 (n=2)
HCV GT2a virus (JFH1)	0.054 (n=4)	0.017 (n=3)
HCV GT3a*	0.13 (n=8)	0.046 (n=8)
HCV GT4a*	0.21 (n=9)	0.058 (n=9)
HCV GT5a*	0.12 (n=6)	0.042 (n=9)
HCV GT6a*	0.17 (n=5)	0.055 (n=7)
Cellular toxicity Huh-7: CC_{50} (µM)	>100 (n=36)	>100 (n=37)

*Chimeric replicons: HCV GT1b backbone with NS5B ORFs from specified GTs inserted. EC_{50} values presented as geometric means.

Figure 2. EC_{50} values MIV-802 versus sofosbuvir against a panel of replicons encoding clinically-derived NS5B sequences.



The panel of replicons encompassing GTs 1 to 4 were selected for sequence diversity and decreased susceptibility to sofosbuvir but without known sofosbuvir associated mutations. In total, 12 isolates were selected for each genotype.

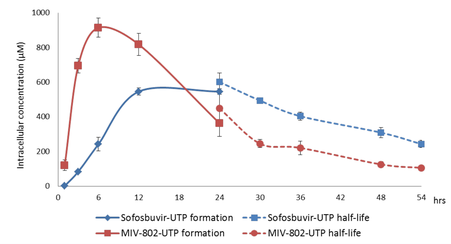
Table 3. Activities in HCV replicons harboring resistance mutations that confer loss of susceptibility to sofosbuvir

HCV Assay: EC_{50} (µM)	Sofosbuvir	MIV-802
HCV GT1b S282T	0.74 (n=18)	0.30 (n=9)
FC vs WT	*9.1*	*6.8*
HCV GT1b L159F/L320F	0.20 (n=5)	0.069 (n=5)
FC vs WT	*2.5*	*1.6*
HCV GT1a* S282T	1.05 (n=6)	0.30 (n=6)
FC vs WT	*8.1*	*6.4*
HCV GT3a* S282T	0.52 (n=6)	0.122 (n=6)
FC vs WT	*2.5*	*2.7*
HCV GT3a* L159F/L320F	0.19 (n=1)	0.062 (n=1)
FC vs WT	*1.5*	*1.3*

Formation of MIV-802-UTP *in vitro* and *in vivo*

- High levels of MIV-802-UTP (100- fold above its Ki against HCV NS5B polymerase) were rapidly formed in primary human hepatocytes during incubation with 10 µM MIV-802. After 24h incubation with MIV-802, following removal of extracellular MIV-802, the MIV-802-UTP decayed with a T½ of 14 hours, supporting once daily dosing in human (Figure 3).
- Hepatic MIV-802-UTP levels in dog, 4 hours post-dose (oral dosing 50 mg/kg, once daily for 4 days), were 40-fold above the HCV NS5B polymerase Ki. The mean UTP T½ was estimated to 12 hours.

Figure 3. Triphosphate formation in fresh human hepatocytes after incubation with 10 µM MIV-802.



Safety and toxicology

- MIV-802 and MIV-802-Nuc were negative in Ames, Green Screen™ and micronucleus assays.
- MIV-802 and MIV-802-Nuc did not interact with a panel of 30 molecular targets at 10 µM nor with hERG function at >30 µM
- MIV-802 did not affect erythroid proliferation but inhibited myeloid proliferation at 100 µM (47% inh, 14 days) which was similar to sofosbuvir (45% inh, 14 days). MIV-802-Nuc did not affect bone marrow progenitors at any concentration tested (IC_{50}: >100 µM).
- MIV-802 had mild effects (40% inh) on cardiomyocyte function when incubated up to 100 µM for 14 days but had no effect at 50 µM. The effects were similar to sofosbuvir (22% inh). MIV-802-Nuc did not affect cardiomyocyte function. For comparison, clear inhibitory effects could be seen for INX-189 at 80 nM.
- MIV-802 and MIV-802-Nuc did not affect the viability of human primary cells such as dermal fibroblasts, renal proximal tubuli, HUVEC and HUMSC (IC_{50} >100 µM).
- No specific effects on mtDNA were detected when incubating HepG2 or Huh7 cells with MIV-802 and MIV-802-Nuc at up to 100 µM for up to 14 days.
- Unlike many cancer cell lines, differentiated hepatocyte-like HepaRG® cells are highly dependent on mitochondria for survival and were chosen to investigate potential long-term mitochondrial toxicity (12 days). MIV-802 and sofosbuvir reduced ATP production and O_2 consumption in a similar fashion.
- MIV-802 was evaluated in a 7 day toxicology study in mice. There were no treatment-related findings i.e. no adverse clinical signs nor any organ weight changes, macroscopic or microscopic pathology findings. The NOAEL was 1000 mg/kg/day in this study. MIV-802-UTP was present in mouse liver. There were high levels of MIV-802-Nuc in mouse plasma.

CONCLUSIONS

- MIV-802 is a potent, pan-genotypic and selective nucleotide analogue with favorable resistance profile.
- MIV-802 displays high potency against replicons encoding NS5B sequences derived from HCV-infected patients with improved antiviral activity relative to sofosbuvir.
- MIV-802 shows good safety margins *in vitro and in vivo* and delivers pharmacologically relevant amounts of UTP to human hepatocytes, and to dog liver after oral administration.
- Given its favorable preclinical profile, MIV-802 is currently being advanced towards clinical development.

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