

## 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).<sup>1,2</sup>
- 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).<sup>3</sup> Densitometric quantitation of the bands on Northern blots was used to determine the IC<sub>50</sub> 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.<sup>4</sup>
- 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

We acknowledge Jacqueline Reeves and Andrew Galee at Monogram BioSci. for the selection of the panel of replicons encoding clinically-derived NS5B sequences and Anupriya Kulkarni for the mechanism of action studies.

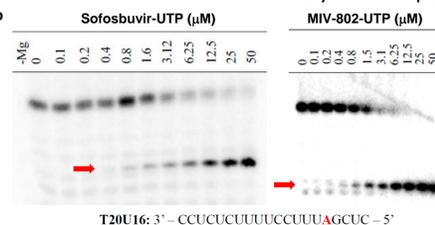
## In vitro Virology and Specificity

- 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<sub>50</sub>>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<sub>50</sub> 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<sub>50</sub> range of 17-58 nM (EC<sub>50</sub> 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<sub>50</sub> 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	hDNApol α	hDNApol β	hDNApol γ	hPOLMRT
IC <sub>50</sub> (μM)				
G1b	>200	>200	>200	>200
IC <sub>50</sub> (μM)				
0.71	>200	>200	>200	>200

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



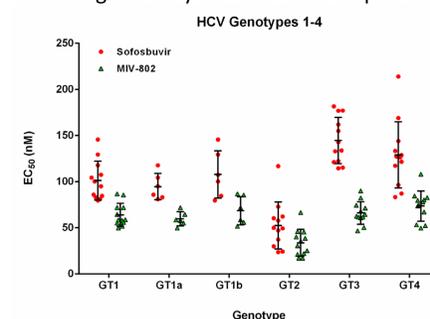
**Table 2.** *In vitro* activity of MIV-802 in HCV replicons encoding NS5B from GTs 1-6

HCV Assay: EC <sub>50</sub> (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 <sub>50</sub> (μM)	>100 (n=36)	>100 (n=37)

\*Chimeric replicons: HCV GT1b backbone with NS5B ORFs from specified GTs inserted. EC<sub>50</sub> values presented as geometric means.

## RESULTS

**Figure 2.** EC<sub>50</sub> 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.

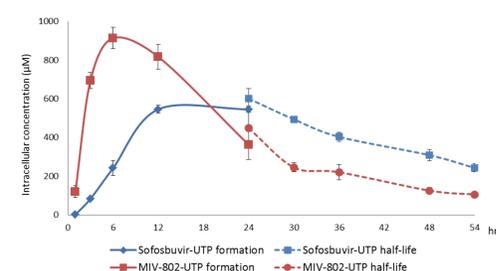
**Table 3.** Activities in HCV replicons harboring resistance mutations that confer loss of susceptibility to sofosbuvir

HCV Assay: EC <sub>50</sub> (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 T<sub>1/2</sub> 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 T<sub>1/2</sub> 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<sub>50</sub>: >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<sub>50</sub> >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<sub>2</sub> 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.

## REFERENCES

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