MIV-802, a uridine nucleotide prodrug, is a more potent inhibitor of HCV genotype 3 replication than sofosbuvir.

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INTRODUCTION

Nucleotide inhibitors of the hepatitis C virus (HCV) NS5B polymerase are a central component of direct-acting antiviral (DAA) combination therapies for the treatment of HCV infection. Their potent and pan-genotypic activities, and their high barrier to resistance make them highly attractive components of shortened and simplified HCV treatment regimens. Sofosbuvir is the only current nucleotide inhibitor approved for the treatment of HCV-infected patients and DAA regimens that include sofosbuvir have demonstrated high sustained virological response rate (SVR) in many populations. However, when combined with suboptimal background therapies, e.g. ribavirin, SVR rates of sofosbuvir-based regimens are lower against HCV genotype (GT) 3 relative to other HCV GTs.

MIV-802 is a liver-targeted uridine nucleotide prodrug. Its active metabolite, the uridine triphosphate (802-UTP), is a selective and potent chain-terminating inhibitor of the HCV NS5B polymerase. MIV-802 is in preclinical development for treatment of HCV infection in combination with other DAAs.

AIMS

Early preclinical data with MIV-802 indicated superior antiviral activity against HCV GT3 compared to sofosbuvir. Our aims were to compare the *in vitro* antiviral activities of MIV-802 with sofosbuvir in HCV GT3 replicon and polymerase assays.

METHODS

- Pan-genotype antiviral activities were evaluated in Huh7 cells transiently transfected with chimeric subgenomic HCV replicons encoding NS5B sequences from HCV GTs 1a-6 (in a GT1b con1 background). The S282T resistance substitution was created in the GT3a NS5B sequences using standard molecular biology techniques and its presence confirmed by nucleotide sequencing.
- Assessments of antiviral activities against replicons encoding clinically-derived HCV GT3 NS5B sequences were performed following transient transifection of Huh7 cells by Monogram Biosciences.
- UTP levels were determined in Huh7 cells following incubation of compounds for 24 hours at 0.1-100 μM.
- An *in vitro* biochemical assay for HCV GT3a NS5B (consensus sequence) polymerase activity was established using purified GT3a protein and a RNA template corresponding to the HCV GT1b 5' IRES.

RESULTS

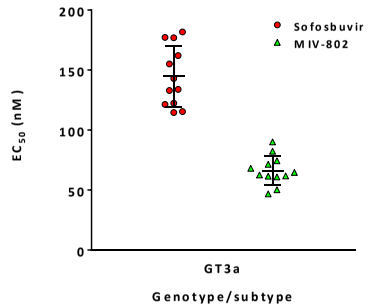
Table 1. *In vitro* activities in HCV replicons encoding NS5B from GTs 1-6.

HCV genotype/subtype	EC_{50} (μM)	
	Sofosbuvir	MIV-802
HCV GT1b	0.081 (n=31)	0.044 (n=22)
HCV GT1a	0.131 (n=16)	0.050 (n=16)
HCV GT2a replicon	0.048 (n=2)	0.023 (n=2)
HCV GT2a virus	0.054 (n=4)	0.017 (n=3)
HCV GT3a	0.129 (n=8)	0.046 (n=8)
HCV GT4a	0.218 (n=8)	0.059 (n=8)
HCV GT5a	0.114 (n=5)	0.044 (n=8)
HCV GT6a	0.179 (n=4)	0.058 (n=6)
Cellular toxicity Huh-7 (6d): CC_{50} (μM)	>100 (n=36)	>100 (n=47)

EC_{50} data presented as geometric means

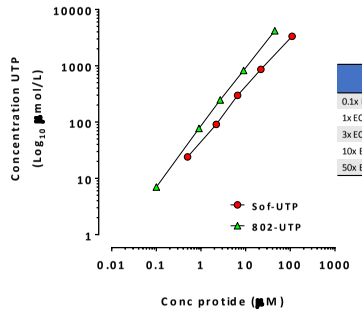
The antiviral activities of MIV-802 were consistently greater than sofosbuvir against subgenomic HCV replicons (and GT2a virus) encoding the NS5B gene from GTs 1-6. Of interest, MIV-802 was 2.8-fold more active than sofosbuvir against a replicon encoding NS5B from GT3a; EC_{50} values were 0.046 μM and 0.129 μM respectively.

Figure 1. Inhibition of HCV replicons encoding clinically-derived GT3a NS5B sequences (scatter plot; mean ± SD).



The antiviral activities of MIV-802 and sofosbuvir were evaluated against a panel of 12 chimeric HCV replicons encoding NS5B sequences from HCV GT3-infected patients. Again, MIV-802 was consistently more potent than sofosbuvir; it had a mean EC_{50} value of 0.066 ± 0.012 μM compared to 0.145 ± 0.025 μM for sofosbuvir (p = $2x10^{-9}$; two-tailed T test).

Figure 2. UTP generation in Huh7 cells.



	MIV-802 (μM)	Sofosbuvir (μM)
0.1x EC_{90}	0.1	0.5
1x EC_{90}	0.9	2.2
3x EC_{90}	2.7	6.6
10x EC_{90}	9.0	22
50x EC_{90}	45	110

At given protide concentrations, MIV-802 generated 2-3 fold more intracellular UTP than sofosbuvir in Huh7 cells.

Table 2. Inhibition of HCV GT3a NS5B polymerase activity in an *in vitro* biochemical assay.

Experiment	Sof-UTP		802-UTP	
	IC_{50} (μM)	Ki (μM)	IC_{50} (μM)	Ki (μM)
n=1	4.14	1.96	2.04	0.97
n=2	3.09	1.46	1.46	0.69
n=3	3.41	1.62	1.63	0.77
Average	**3.55**	**1.68**	**1.71**	**0.81**
Std Dev	0.54	0.26	0.30	0.14

On average, 802-UTP was 2.1-fold more active than Sof-UTP against HCV GT3a polymerase as judged by both IC_{50} and Ki value determinations (p = 0.007 for IC_{50} and Ki; two-tailed T test).

Table 3. *In vitro* activities in an HCV replicon encoding a S282T resistance substitution in HCV GT3a NS5B.

HCV Assay: EC_{50} (μM)	Sofosbuvir	MIV-802
HCV GT3a	0.129 (n=8)	0.046 (n=8)
HCV GT3a S282T	0.521 (n=6)	0.122 (n=6)

EC_{50} data presented as geometric means

The greater activity of MIV-802 compared to sofosbuvir against wild-type (WT) GT3a HCV was maintained against a replicon encoding GT3 NS5B engineered to contain the nucleotide-resistance associated substitution S282T. Further, the fold-change in activity from WT to mutant replicon was lower for MIV-802 (2.7) than for sofosbuvir (4.0).

SUMMARY AND CONCLUSIONS

- MIV-802 demonstrated superior potency compared with sofosbuvir aginst a panel of subgenomic HCV replicons encoding NS5B from GTs 1-6.
- MIV-802 showed superior potency compared with sofosbuvir against a panel of 12 subgenomic HCV replicons encoding NS5B isolated from GT3-infected patients.
- MIV-802 generated more UTP in Huh7 cells compared to sofosbuvir, which was consistent with its superior replicon efficacies.
- 802-UTP was also a more potent inhibitor of HCV GT3 NS5B than Sof-UTP in an *in vitro* biochemical assay for polymerase activity, which likely also contributed to its enhanced efficacy against replicons encoding NS5B sequences from GT3.
- Finally, the superior activity of MIV-802 against WT GT3 translated to enhanced activity against a replicon engineered to express a S282T resistance-associated mutation in GT3a-derived NS5B.

MIV-802 has greater antiviral activity than sofosbuvir against HCV GT3 in replicon cells consistent with its higher conversion to the active metabolite, UTP, and greater inhibition of the GT3 NS5B polymerase by the UTP. These data support its further development as a component of pan-genotypic DAA regimens.

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REFERENCES

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