In vitro activity and pharmacological properties of IDX375, a novel HCV non-nucleoside inhibitor

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INTRODUCTION

- Hepatitis C virus (HCV) is a common blood-borne pathogen annually infecting three to four million people worldwide. Currently, an estimated 170 million people are infected globally, representing a nearly 5-fold greater prevalence than human immunodeficiency virus.[1]
- The current standard-of-care therapy, a combination of pegylated interferon and ribavirin, is effective in only 50% of patients infected with genotype 1 HCV and is associated with significant side effects. Thus, there remains a need for new, more effective and better tolerated HCV treatment options.
- The HCV polymerase has been an attractive antiviral target. Nucleoside analogs, or more recently pro-nucleotides such as IDX184, target the active site of the enzyme[2,3] while multiple classes of non-nucleoside polymerase inhibitors (NNIs) target different allosteric sites in the enzyme.
- IDX375 is a novel NNI developmental candidate that targets the palm pocket of the NS5B polymerase.
- This study evaluated the *in vitro* biochemical and cell-based activities of IDX375, and its pharmacokinetic profile in the rat and the monkey.

METHODS

Biochemical assays: IC_{50}, inhibitory constant (K_i) and Michaelis constants (K_M) were determined by standard methods. Human polymerase activity was determined by measuring the incorporation of α-[^{33}P] dNMP using activated calf thymus DNA as template.

HCV replicon assay: Huh-7 cells stably expressing a replicon containing the luciferase transgene were seeded onto 96-well plates, cultured for 3 days in the presence of drug and subjected to a luciferase assay. Cytotoxicity was measured by MTS in GS4.1, Huh-7 or HepG2 cells after 3 or 9 days of treatment.

HCV *in vitro* infection assays: HPC cells were infected with JFH-1 HCV (genotype 2a) and treated with serial drug dilutions. After 16 hours, virus inoculum was removed and cultures were incubated with drug for 3 days. Drug susceptibility was determined by ELISA using an anti-HCV core mAb.

Long-term treatment assay: GS4.1 cells stably expressing a bicistronic HCV replicon were cultured in the presence of drug, but without G418, for 14 days. Cells were split, RNA was collected every 3-4 days and replicon RNA levels were measured by RT-qPCR of the HCV 5'-UTR and normalized to GAPDH. Following 14-day treatment, cells were seeded onto 10 cm dishes in the absence of compound ± G418 for 21 days to determine the presence of the HCV replicon. The number of colonies in each plate was counted following staining with crystal violet in 50% ethanol.

Monkey and rat pharmacokinetics (PK): For the PK studies, serial plasma samples were obtained from male Sprague-Dawley rats and cynomolgus monkeys given single doses of IDX375 (2 mg/kg IV or 10 mg/kg PO; 2 animals per dose group). For tissue studies, samples of plasma and tissue were obtained from 2 cynomolgus monkeys. All samples were treated with acidified acetonitrile and concentrations of IDX375 were determined by reversed-phase LC-MS/MS.

RESULTS

Biochemical characterization of IDX375

Table 1: In vitro activity of IDX375 against HCV and human cellular polymerases

	HCV NS5B polymerases		Human cellular polymerases			
	HCV1a	HCV1b	DNA pol α	DNA pol β	DNA pol γ	RNA pol II
IC_{50} (µM)	0.030	0.018	> 100	> 100	> 100	> 100

Control inhibitors actinomycin D and ddGTP (DNA polymerase) and alpha amanitin (RNA polymerase II) gave expected results.

- As shown in **Table 1**, IDX375 inhibited HCV polymerases of genotypes 1a and 1b with submicromolar IC_{50} values, but did not inhibit human DNA polymerases α, β or γ or human RNA polymerase II.
- IDX375 did not inhibit HCV polymerases of genotypes 2a, 3a or 4a at concentrations up to 1 µM (data not shown).
- In biochemical experiments (data not shown), the choice of ^{33}P-NTP substrate did not affect the IC_{50} of IDX375; values ranged from 14 (^{33}P-ATP label) to 19 nM (^{33}P-UTP label).
- Kinetic analyses (data not shown) with the 1b HCV polymerase determined the K_i of IDX375 to be 40 nM, similar to the K_M values of the 4 nucleotides.
  - IDX375 was found to be a noncompetitive inhibitor with respect to the 4 nucleotide substrates.
  - Inhibition was mixed with respect to RNA template.

Activity against HCV in cell culture

Table 2: Activity of IDX375 in a standard HCV genotype 1b replicon assay

n	EC_{50} (nM)	n	CC_{50} (µM)	Selectivity Index
6	2.3	5	>100	>43,000

Luciferase reporter-based assay

- As seen in **Table 2**, IDX375 is a potent inhibitor of genotype 1b HCV replication with low cytotoxicity and excellent selectivity.
- In a variety of cell lines, IDX375 showed negligible cytotoxicity; in 9-day assays the CC_{50} of IDX375 was 88 µM in Huh-7 cells and >100 µM in HepG2 cells.
- The presence of 45% human serum increased the EC_{50} of IDX375 by 25-fold in the 1b luciferase replicon assay.
- The activity of IDX375 against the JFH-1 genotype 2a virus was lower, EC_{50} = 18.4 µM, using the core ELISA assay.

Long-term treatment of replicon cells with IDX375

- As shown in **Table 3** and **Figure 1**, longer term treatment with IDX375 achieved a 1.0 log_{10} reduction in replicon RNA at a 1x EC_{50} of IDX375 and a 3 log_{10} reduction at 20x EC_{50} of IDX375.

Figure 1: Long-term inhibition of replicon replication by IDX375

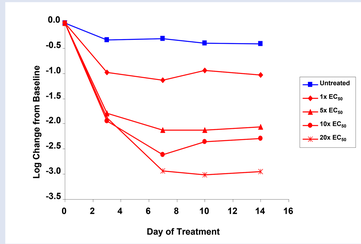


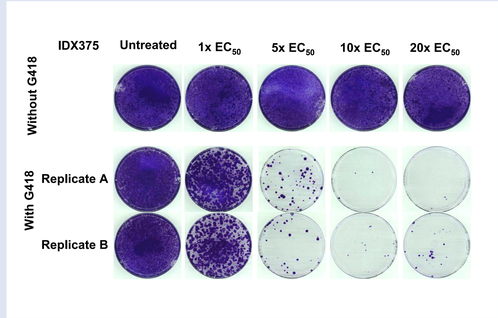
Table 3: Effect of 14-day treatment with IDX375 on replicon RNA

Concentration (x EC_{50})	Replicon RNA (log_{10} reduction)
-	0.42
1	1.03
5	2.05
10	2.29
20	2.95

Values represent log_{10} reduction derived from averages from four independent experiments. Log_{10} reduction was calculated by subtracting the average log_{10} copies HCV/GAPDH RNA of the sample at Day 14 from the average log_{10} copies HCV/GAPDH RNA of the untreated control at Day 0.

- This decline in HCV replicon was confirmed by further culture of the 14-day treated cells with G418 in the absence of compound (**Figure 2**).
- Without G418 selection pressure, replicons were eliminated and cells remained viable (top row). In the presence of G418, only HCV replicon-expressing colonies survived (bottom rows). The number of colonies reflects the efficacy of each treatment.

Figure 2: Confirmation of replicon reduction after long-term treatment with IDX375



Quantitation of colonies shown above

G418	IDX375 concentration (x EC_{50})				
	Untreated	1	5	10	20
-	>600	>600	>600	>600	>600
+	>600	>600	63	3	3
+	>600	>600	32	8	18

- As seen in **Figure 2**, the number of replicon colonies was reduced in a dose-dependent manner after 14-day treatment with IDX375.
- At 1x EC_{50}, the reduction in colonies was already visible and became marked at ≥ 5x EC_{50}.

Pharmacokinetic profile of IDX375

- Since the submission of the abstract, the PK profile of IDX375 has been refined in more detailed studies. The recent data are presented below.

Table 4: Plasma PK profile of IDX375 in the rat and monkey

Dose and Route	Parameter	Sprague-Dawley Rat	Cynomolgus Monkey
2 mg/kg IV	Cl (L/h/kg)	2.5	0.24
	Vd (L/kg)	17	0.77
	$T_{1/2}$ (h)	4.6	2.3
10 mg/kg PO	C_{max} (nM)	860	4160
	T_{max} (h)	0.5 - 4.0	4.0
	Bioavailability (%)	101	28

- The oral bioavailability of IDX375 was good to excellent in rats and monkeys.
- After a 10 mg/kg oral dose, 8 h plasma levels in rats were 200-fold above the EC_{50} value obtained in the HCV genotype 1b replicon. At 24 h, drug levels remained 10- and 30-fold above the EC_{50} in rats and monkeys, respectively.
- IDX375 was selectively concentrated in liver; in monkeys, liver concentrations of IDX375 were approximately 50-fold greater than the corresponding plasma concentrations 24 h post-dose.
  - This accumulation was not observed in other tissues.

CONCLUSIONS

- IDX375 is a potent and selective noncompetitive inhibitor that targets the palm domain of the HCV NS5B enzyme.
- IDX375 inhibited HCV replication in an *in vitro* replicon assay with an EC_{50} value of 2.3 nM and a selectivity index of >43,000.
- IDX375 was not cytotoxic in test cell lines.
- Treatment of replicon cells with 20x EC_{50} of IDX375 for 14 days resulted in a 3 log_{10} reduction in HCV replicon RNA and reduced the number of replicon-containing foci in cell culture.
- The PK profile of IDX375 in the rat and the cynomolgus monkey shows adequate drug exposure in the systemic circulation. Moreover, the drug selectively concentrated in the liver.
- The preclinical PK profile of IDX375 suggests the potential for once-a-day dosing. After 24 h, plasma levels remained 10- to 30-fold above the EC_{50} in both rats and monkeys given single 10 mg/kg oral doses.
- Based on the *in vitro* antiviral potency and the exposure seen in animal PK studies, IDX375 is a promising candidate for clinical evaluation.

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Disclosures: All authors are current employees of Idenix Pharmaceuticals, Inc.

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