

Pharmacokinetic and Safety Profile of IDX320, a Novel and Potent HCV Protease Inhibitor

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INTRODUCTION

- Hepatitis C virus (HCV) infection remains a significant health problem, annually infecting three to four million people worldwide. Currently, an estimated 170 million people worldwide are infected.¹
- The current standard-of-care therapy, a combination of pegylated interferon and ribavirin, is curative in approximately 40-50% of patients infected with HCV genotype 1 and is frequently associated with significant side effects.² Thus, there is a need to develop additional therapies, including potent and safe direct-acting antiviral agents (DAAs), to provide more effective and tolerable HCV treatment options.
- We have identified a novel macrocyclic compound, IDX320, which is a specific, multi-genotypic inhibitor of the HCV NS3/4A protease with subnanomolar to nanomolar potency.
- The studies reported here evaluated the preclinical pharmacokinetic and ADME properties and the *in vitro* and *in vivo* safety profiles of IDX320.
- A phase I clinical trial evaluating single and multiple ascending doses of IDX320 in healthy volunteers is ongoing. Pharmacokinetic data from the single 200 mg oral dose cohort are presented.

METHODS

PK studies: Male mice (3/time point) and monkeys (3/dose group) were given a single IV or PO dose of IDX320 in a PEG-based vehicle. IDX320 was quantified in liver (LLOQ=25 ng/g) and heart (LLOQ=50 ng/g) tissue (mice only) and in plasma samples (LLOQ=5 ng/mL) by HPLC-MS/MS after liquid-liquid extraction. Six healthy volunteers were given a single 200 mg dose of IDX320 using the selected tablet formulation and plasma concentrations were similarly determined (LLOQ=2 ng/mL). PK parameters were calculated using WinNonlin.

Formulation development studies: Various prototype formulations were prepared as capsules, tablets or elixirs containing 25 mg IDX320. Male monkeys (n=3/formulation) were orally administered a single 25 mg dose (4.7-10 mg/kg) and IDX320 was quantified in plasma as described above.

***In vitro* cytotoxicity assays:** Freshly isolated hepatocytes were incubated with various concentrations of IDX320 for 48 h. Intracellular ATP content was measured (CellTiter-Glo luminescent cell viability assay) to determine cell cytotoxicity (CC₅₀).

CYP450 and UGT1A1 inhibition assays: IDX320 was incubated with human CYP450 cDNA-expressed isoenzymes according to the protocol (BD Bioscience). For CYP2C9, a luminogenic substrate was used with the P450-Glo™ kit. In addition, the effect of IDX320 on CYP2C9-mediated diclofenac metabolism was measured by LC-UV. The potential inhibitory effect of IDX320 on human UGT1A1 was examined using human liver microsomes and bilirubin as substrate. The metabolites (mono- and di-glucuronidated bilirubin) were measured by LC-UV.

Permeability in Caco-2 cell monolayers: Caco-2 cells were grown to confluence on collagen-coated, microporous, polycarbonate membranes in 12-well Costar Transwell plates. 5 μM IDX320 was prepared in Hanks balanced salt solution containing 10 mM HEPES and 15 mM glucose at a pH of 7.4. Cell monolayers were dosed on the apical side (A-to-B) or basolateral side (B-to-A). Samples were taken from the receiver chambers at 120 minutes and quantified by LC-MS/MS.

Safety pharmacology studies: One *in vitro* GLP safety pharmacology study evaluated the effects of IDX320 on hERG channel currents in voltage-clamped human embryonic kidney cells (HEK293) stably expressing the human *ether-à-go-go*-related gene (hERG) channel. Four *in vivo* GLP safety pharmacology studies evaluated the potential effects of IDX320 on the cardiovascular and respiratory systems in the cynomolgus monkey and on the central nervous, renal and gastrointestinal systems in the CD-1 mouse at single oral doses up to 250 mg/kg IDX320.

Genotoxicity studies: The potential for IDX320 to induce mutations in a bacterial system (*S. typhimurium* and *E. coli* – at concentrations up to 5000 μg/plate), to induce chromosomal aberrations in cultures of human peripheral blood lymphocytes (at concentrations up to 84 μg/mL), and to induce clastogenic and/or aneugenic activity in CD-1 mice (at oral doses up to 2000 mg/kg) was evaluated. The two *in vitro* studies were performed with and without the addition of a mammalian metabolic activation system (rat liver S9 subcellular fraction).

Repeat-dose toxicology and toxicokinetic studies: Daily oral doses of IDX320 (up to 250 mg/kg/day) in PEG 400 were administered to both mice and monkeys for 28 consecutive days. Toxicity was evaluated based on mortality, clinical observations, body weights, food consumption, ophthalmological examination, hematology and serum chemistry, organ weights, gross and microscopic examinations. ECG, coagulation and urinalysis were also performed in the monkey study.

RESULTS

Favorable Pharmacokinetics in Mice and Monkeys

Figure 1: Mean (SD) Plasma Concentration-Time Profiles for IDX320 in CD-1 Mice

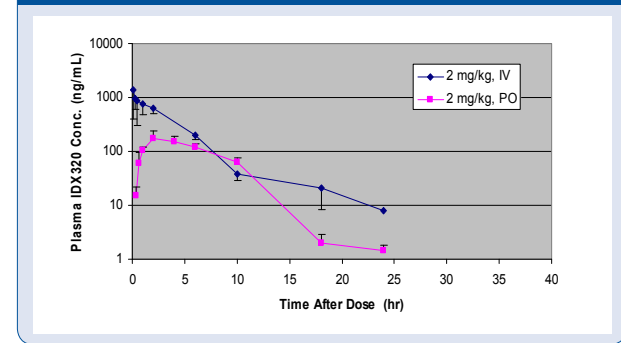


Figure 2: Mean (SD) Plasma Concentration-Time Profiles for IDX320 in Cynomolgus Monkeys

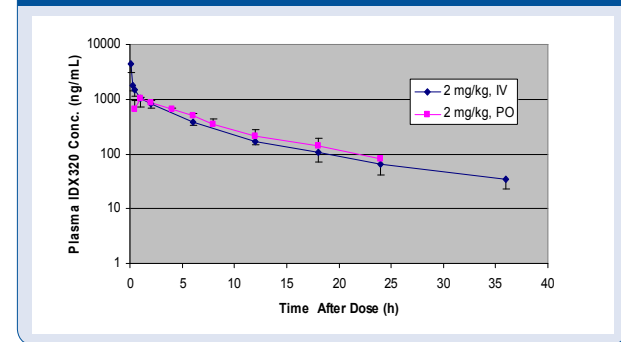


Table 1. IDX320 Pharmacokinetic Parameters in Mice and Monkeys Administered a Single 2 mg/kg Dose*

Species	Dose Route	Cl (L/h/kg)	V _d (L/kg)	t _{1/2} (h)	C _{max} (ng/mL)	t _{max} (h)	C _{24h} (ng/mL)	AUC _{0-24h} (ng·h/mL)	F (%)
CD-1 mouse	IV	0.48	4.4	6.3	n/a	n/a	7.9 ± 11	4060	n/a
	PO	n/a	n/a	n/c	174	2	1.5 ± 0.3	1410	34.7
Cynomolgus Monkey	IV	0.23 ± 0.03	3.5 ± 0.5	10.3 ± 1.0	n/a	n/a	66 ± 26	9580 ± 1420	n/a
	PO	n/a	n/a	7.6 ± 2.0	1030 ± 270	1.0	81 ± 47	8920 ± 2190	107 ± 40

*vehicle: 70% PEG300/30% D5W for IV dose and PEG400 for PO dose
 *AUC_{0-24h} for mouse and AUC_{0-24h} for monkey
 n/a: not applicable
 n/c: not calculable

- Favorable oral bioavailability of IDX320 was observed in the mouse and monkey, with substantial plasma concentrations observed 24 h after a single 2 mg/kg oral dose.
- Low clearance (~9% of hepatic blood flow) and relatively long plasma half-lives in both the mouse and monkey support the potential for once-daily dosing in patients.

Table 2. IDX320 Mean Concentrations in Plasma, Liver and Heart of CD-1 Mice Given a Single 2 mg/kg Oral Dose

Time (h)	Mean IDX320 concentration (ng/mL or ng/g)			Mean concentration ratio*	
	Plasma	Liver	Heart	Liver/Plasma	Heart/Plasma
2	174	4410	144	27	0.79
6	121	3540	93.0	30	0.78
24	1.45	57.6	BLQ ^b	39	n/c

*Mean values calculated from individual tissue/plasma ratios (n=3/time point)
^bBLQ: all 3 samples were below the lower limit of quantification (50 ng/g in heart tissue)
 n/c: not calculable

- IDX320 is selectively concentrated in the liver of orally dosed mice and is cleared at approximately the same rate from liver and plasma.

Favorable Preclinical Safety Profile

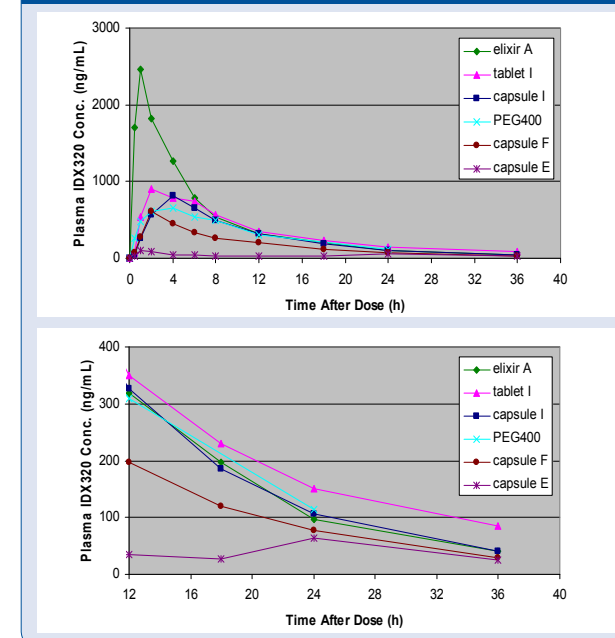
- IDX320 was not cytotoxic to fresh mouse, rat, monkey and human hepatocytes, with CC₅₀ values > 10 μM.
- IDX320 showed no significant inhibition of human CYP450 1A2, 2B6, 2C9, 2D6, 3A4 or human UGT1A1 (IC₅₀ ≥ 10 μM).
- IDX320 did not affect the cardiac hERG potassium channel current in HEK293 cells.
- At single oral doses up to 250 mg/kg, IDX320 had no effects on the cardiovascular and respiratory systems of monkeys or on the central nervous system, renal function or gastrointestinal motility of mice.
- IDX320 demonstrated no genotoxicity in the bacterial mutation, human lymphocyte chromosomal aberration and mouse micronucleus tests.
- In 4-week GLP toxicology studies in mice and monkeys, IDX320 was well tolerated and all in-life and post-mortem parameters were generally unremarkable at oral doses up to 250 mg/kg/day.

Additional Findings

- IDX320 showed high permeability (P_{app} = 1.8x10⁻⁶ cm/s) and a low efflux ratio (ER = 2.7) in Caco-2 cell monolayers.
- IDX320 is highly protein bound in plasma of mouse (99.2%), monkey (99.9%) and human (99.6%).

Development of a Clinical Formulation

Figure 3: Mean Dose-normalized* Monkey Plasma Profiles for IDX320 in Selected Formulations



*normalized to a dose of 10 mg/kg (n=3/formulation; actual doses ranged from 4.7 to 10 mg/kg)

- In monkeys, the selected formulation (tablet I) provided better oral bioavailability of IDX320 than a solution of IDX320 in PEG400.
- The selected tablet formulation produced a lower C_{max} compared to an elixir formulation and favorable 24-h plasma concentrations compared to those obtained with all other formulations tested.

Favorable Pharmacokinetics in Healthy Volunteers

Figure 4: Mean (SD) Plasma Concentration-Time Profiles for IDX320 in Monkeys and Healthy Volunteers

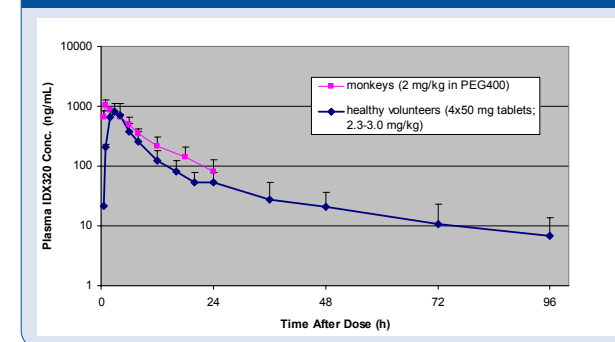


Table 3. IDX320 Pharmacokinetic Parameters in Healthy Volunteers Given a Single 200 mg Oral Dose

Dose (mg)	C _{max} (ng/mL)	T _{max} (h)	C _{24h} (ng/mL)	AUC _{0-24h} (ng·h/mL)	t _{1/2} (h)	Cl/F (L/h/kg)	V _d /F (L/kg)
200	982 ± 463	2.0-3.0	53 ± 26	7240 ± 2930	26.0 ± 7.1	0.40 ± 0.17	14.1 ± 4.8

Results represent mean ± SD or observed range (n=6)
 Single doses were administered as 50 mg tablets (formulation I)
 Full pharmacokinetic results for all dose groups will be presented at a future scientific meeting

- IDX320 was generally safe and well tolerated in healthy volunteers.
- In healthy volunteers given a single 200 mg oral dose of IDX320, the tablet formulation produced a pharmacokinetic profile similar to that observed after a comparable dose in monkeys.
- The plasma elimination half-life of IDX320 in healthy volunteers confirms its potential for once-daily treatment of HCV-infected patients.
- The mean 24-h plasma concentration after administration of a single 200 mg dose to healthy volunteers was >100-fold above the EC₅₀ value for IDX320 (1b replicon).

CONCLUSIONS

- Favorable pharmacokinetics of IDX320 were observed in preclinical species and in humans, suggesting the potential for once-daily oral administration of IDX320 in HCV-infected patients.
- No significant inhibition of human CYP450s and UGT1A1 by IDX320 *in vitro* suggests low potential for drug-drug interactions in patients.
- IDX320 showed no effects in several *in vitro* and *in vivo* GLP safety pharmacology and genotoxicity studies.
- No adverse effects were observed in 4-week GLP toxicology studies in mice and monkeys administered daily oral doses of IDX320 up to 250 mg/kg/day.
- Results from a pharmacokinetic study of IDX320 in healthy volunteers given a single 200 mg oral dose in a tablet formulation indicated a plasma half-life of 26 h and a 24-h plasma concentration that was orders of magnitude above the *in vitro* EC₅₀ values.
- These data, together with those presented by Lalloo et al. (Poster #768) and La Colla et al. (Poster #769), support the ongoing clinical evaluation of IDX320 in HCV-infected patients.

REFERENCES

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DISCLOSURES

All authors except J.v.d. Wetering de Rooij are current employees of Idenix Pharmaceuticals, Inc.