

Antiviral and Preclinical Profiles of HCV NS5A Inhibitors IDX380 and IDX719

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BACKGROUND

- Inhibitors of NS5A have been identified through replicon screening and are in various stages of clinical development.
- Our HCV drug discovery program has identified a number of NS5A-targeted compounds from different structural series that inhibit the replication of multiple HCV genotypes with picomolar (pM) potencies.
- Two lead compounds, IDX380 and IDX719, were identified with favorable pharmacokinetic properties and low pM activity against HCV *in vitro*.
- Since this abstract was submitted, IDX719 was found to be slightly superior to IDX380 in preclinical assays.
- Therefore, IDX719 was selected as a clinical drug candidate while IDX380 remains a favorable backup compound.
- Here we describe the anti-HCV activity and early pharmacokinetic profile of IDX719.

METHODS

- Replicon assays:** The *in vitro* activities of IDX719 were evaluated against an infectious HCV virus and luciferase-reporter replicons bearing NS5A from multiple HCV genotypes following a 3-day incubation. The effects of human serum proteins \pm alpha-1 acid glycoprotein (AAG) were examined in the genotype 1b replicon model. The resistance profile was determined using site-directed mutant replicons and in replicon selection experiments. Sequences covering the first 100 amino acids of NS5A or the entire NS3-NS5B region were examined in drug-selected replicon cell lines.
- Stability and permeability assays:** Stability of IDX719 at 0.5 μ g/mL was assessed by incubation at 37°C in fresh whole blood from mouse, rat, dog, monkey or human for up to 4 h. Following protein removal by precipitation, samples were analyzed by reversed-phase LC-MS/MS. IDX719 was examined for cell permeability in Caco-2 cells and for intrinsic hepatic clearance in human cryopreserved hepatocytes using standard methods.
- CYP450 and UGT1A1 inhibition:** IDX719 was incubated with a panel of 7 human CYP450 cDNA-expressed isoenzymes according to the protocol (BD Bioscience). The potential inhibitory effect of IDX719 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.
- PK studies:** Overnight-fasted male cynomolgus monkeys (n=3) and male CD-1 mice (n=3 per time point) were given a single PO dose at 10 mg/kg of IDX719. Blood/plasma was collected at selected intervals up to 24 h after dose administration. Quantification in 50 μ L plasma (LLOQ=1.0 ng/mL) was made by LC-MS/MS after liquid-liquid extraction. PK parameters were derived using WinNonlin.

RESULTS

In Vitro Activity

- IDX719 EC₅₀ values ranged from 2-24 pM against an HCV infectious virus and replicons representing HCV genotypes 1-5 (genotype 6 has not been tested). This lead candidate compared favorably to BMS-790052 (Figure 1 and Table 1). The CC₅₀ value >100 μ M provides a selectivity index of >5x10⁷ for IDX719.
- Antiviral activity was reduced by <10-fold in the presence of 45% human serum. AAG had no significant effect (<3-fold) (data not shown).

Figure 1: Activity of IDX719 Compared to BMS-790052

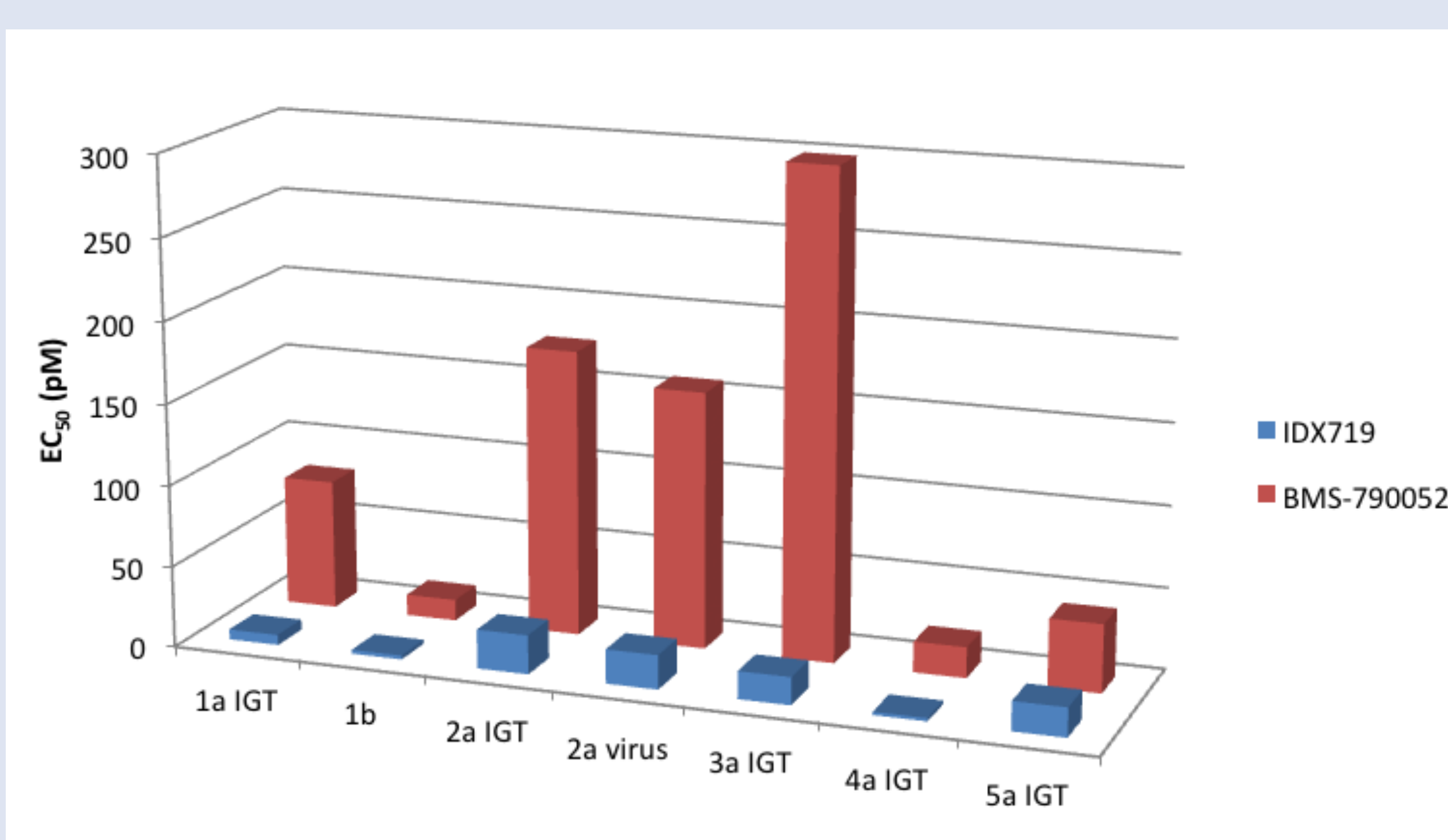


Table 1: Antiviral Potency and Cytotoxicity of IDX719 and BMS-790052

	EC ₅₀ (pM)						CC ₅₀ (μ M)
	1a IGT	1b	2a IGT	2a virus	3a IGT	4a IGT	
IDX719	6.2	2.4	24	21	17	2.0	18
BMS-790052	80	13	176	158	299	19	42

n = 3-5
IGT = NS5A intergenotypic replicon

- IDX719 was examined for activity against genotype 1a and 1b replicons bearing NS5A mutations that have been reported by others to confer resistance to the NS5A inhibitor class of compounds.¹
 - IDX719 was generally more active against mutations in genotype 1b replicons than against mutant genotype 1a replicons. Fold-change and absolute EC₅₀ values compared favorably to those of BMS-790052 for all mutants (Tables 2 & 3).
 - The nucleotide prodrug IDX184 retained full activity against all NS5A mutant replicons (Tables 2 & 3). Similarly, genotype 1b replicons bearing representative resistance mutations to nucleoside, protease and non-nucleoside inhibitors were fully-susceptible to IDX719 (data not shown).

Table 2: Activity of IDX719 Against Genotype 1a Replicons Bearing Mutations in NS5A

1a Replicon Mutant	Fold-change			Replicative Capacity (% of WT)
	IDX719	BMS-790052	IDX184	
M28T	155	165	1.1	66
Q30E	422	2935	1.1	119
Q30H	24	155	1.1	100
Q30K	310	752	0.9	100
Q30R	10	95	1.0	119
L31F	68	43	1.1	85
L31M	311	115	1.0	143
L31V	425	899	1.3	210
P32L	173	142	0.9	16
Y93C	40	287	1.1	92
Y93H	4427	1392	1.1	43
Y93N	14,363	7842	1.0	60

n = 3
Fold-change values are relative to wild-type (WT) replicon EC₅₀ determined in parallel with mutant EC₅₀ values.

Table 3: Activity of IDX719 Against Genotype 1b Replicons Bearing Mutations in NS5A

1b Replicon Mutant	Fold-change			Replicative Capacity (% WT)
	IDX719	BMS-790052	IDX184	
L28T	74	25	0.5	2.7
R30E	1.1	7.3	0.5	17
L31F	4.0	6.9	0.9	90
L31M	3.6	3.2	0.9	86
L31V	15	23	0.9	61
P32L	6.7	9.6	0.7	12
Y93C	2.7	2.1	0.7	33
Y93H	93	21	0.8	25
Y93N	160	35	0.7	23

n = 3
Fold-change values are relative to wild-type (WT) replicon EC₅₀ determined in parallel with mutant EC₅₀ values.

- Three genotype 1a HCV replicon cell lines resistant to IDX719 (719R) were generated by selection with compound for >90 days.
 - By population sequencing, only mutations at Y93 in NS5A became dominant in all three cell lines, with the primary mutation being Y93H.
 - Clonal sequencing (100 clones) of one IDX719-resistant cell line revealed that the Y93H mutation never achieved complete penetrance; 58 of 100 clones contained mutations at Y93, with 51 of the 58 mutations being Y93H. Every one of the 100 clones examined had at least one mutation in the first 100 amino acids of NS5A, the region reported to confer resistance to known NS5A inhibitors.¹
 - The IDX719-selected cell lines showed high resistance to IDX719 and BMS-790052, but remained fully susceptible to the nucleotide prodrug IDX184 (Table 4).

Table 4: Extent of Resistance After Selection of Genotype 1a Replicons with IDX719

Cell Line	Fold-change		
	IDX719	BMS-790052	IDX184
719R-A	3086	1703	0.8
719R-B	1132	631	0.9
719R-C	1291	2181	1.6

n = 3
Fold-change values are relative to EC₅₀ values of untreated cells determined in parallel with EC₅₀ values of selected cells.

Metabolism and Pharmacokinetic Characteristics

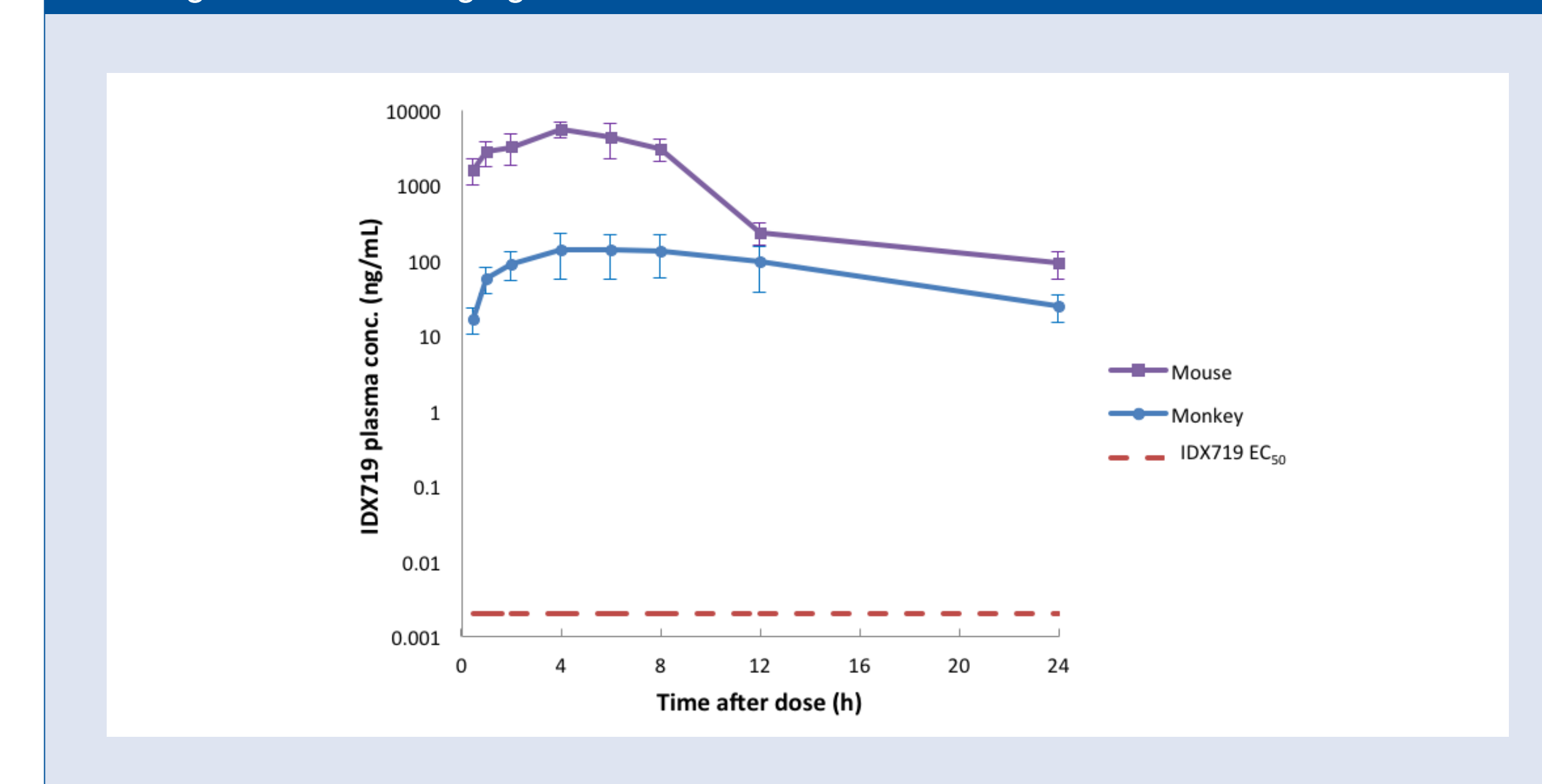
- Testing in human hepatocytes showed moderate clearance, similar to BMS-790052; the Caco-2 assay showed a lower permeability of IDX719 relative to BMS-790052, but no P-glycoprotein efflux (Table 5).

Table 5: Human Hepatocyte Clearance and Caco-2 Studies

	IDX719	BMS-790052
Human hepatocytes CL _i (μ L/min/10 ⁶ cells)	2.6	2.6
Caco-2 P _{app} (10 ⁻⁶ cm/sec) (B→A/A→B ratio)	0.98 (0.4)	7.2 (5.8)

- IDX719 was found to be stable (\geq 95% remaining) in fresh whole blood from mouse, rat, dog, monkey and human at 37°C for at least 4 hours (data not shown).
- With IC₅₀ values > 20 μ M, there is a low potential for IDX719 to inhibit a panel of 7 CYP450 isozymes (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A4). The IC₅₀ value for inhibition of UGT1A1, an enzyme involved in bilirubin glucuronidation, was in the low micromolar range (1.8 μ M).
- The pharmacokinetic profile of IDX719 was favorable in mice and monkeys after an oral dose of 10 mg/kg (Figure 2).
 - The terminal half-life was estimated at 5.4 h in mice and 6.8 h in monkeys, modestly longer than that of IDX380 (data not shown).
 - Exposures in mice exceeded those in monkeys
 - IDX719 levels in mice and monkeys remained far above *in vitro* genotype 1b EC₅₀ values 24 h after dose (42,600- and 11,000-fold, respectively).

Figure 2: IDX719 Pharmacokinetics in Mouse and Monkey Following Oral Administration of a Single Dose at 10 mg/kg



EC₅₀ value for genotype 1b replicons (Table 1).
Mouse (n=3/time point): Vehicle PEG200
Monkey (n=3): Vehicle VitE TPGS/PEG200 (1:9)

CONCLUSIONS

- IDX719 showed pan-genotypic activity against HCV *in vitro* as evidenced by EC₅₀ values ranging from 2-24 pM against HCV genotypes 1-5.
- The *in vitro* selectivity index for IDX719 was >5x10⁷.
- Our results suggest similar overall resistance profiles for IDX719 and BMS-790052. There was no cross-resistance to other classes of DAAs.
- In >90-day selection studies, only the Y93H mutant emerged as a dominant mutation in NS5A in all three genotype 1a replicon-bearing cell lines.
- Oral dosing with IDX719 in mice and monkeys resulted in favorable exposures and plasma concentrations far above the *in vitro* EC₅₀ values at 24 h post dose.
- Based on its favorable preclinical activity and pharmacokinetic characteristics, IDX719 has entered full IND-enabling studies, including one month toxicology analyses in the mouse and monkey. Completion of these studies is expected before the end of 2011.

References

- Fridell RA, Qiu D, Wang C, *et al.* (2010). Antimicrob. Agents Chemother. 54:3641-3650.

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Disclosures

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