#1889

In Vitro and *In Vivo* Metabolism of INX-189, a Potent HCV Inhibitor, in Rat and Cynomolgus Monkey Hepatocytes

John Vernachio[1], Dawn Bryant[1], Stanley Chamberlain[1], Elena Gorovits[1], **Babita Ganguly[1,3]**, Andrea Hall[1], Geoffrey Henson[1], Damound Hunley[1], Jeff Hutchins[1], Jerry Muhammad[1], Joseph Patti[1], Robin Walters[1], Jin Wang[1], Karolina Madela[2], Mohamed Aljarah[2], Sarah Jones[2], Arnaud Gilles[2] and Christopher McGuigan[2]

[1]Inhibitex, Inc., Alpharetta, Georgia, USA; [2]Welsh School of Pharmacy, Cardiff University, Cardiff, UK; [3] Presenting Author

Introduction

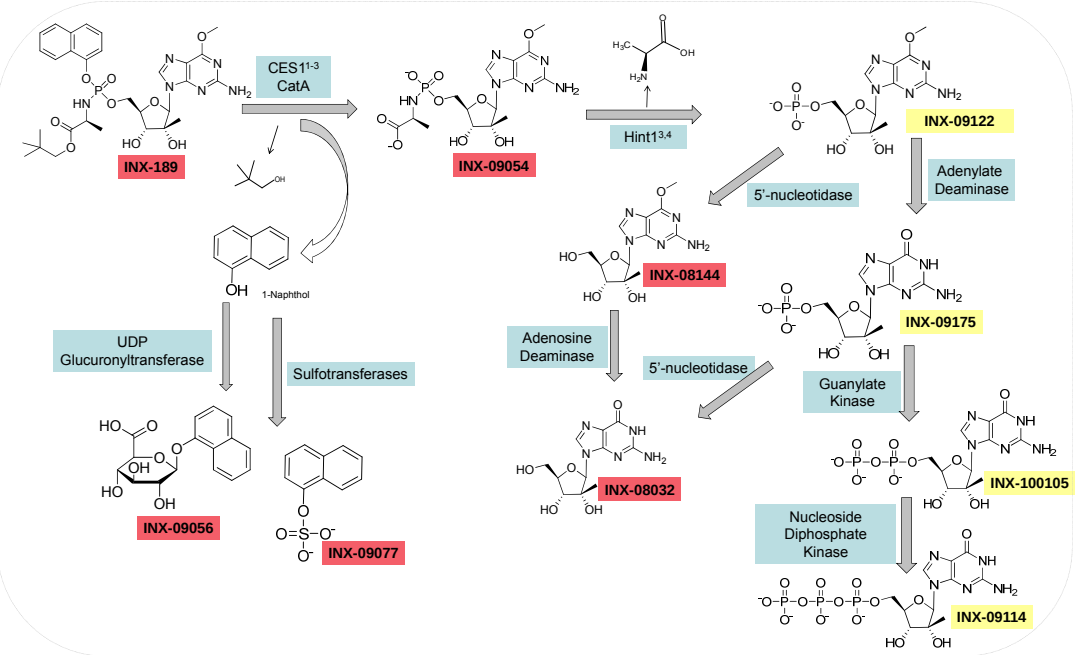
INX-189 is a novel potent phosphoramidate based pro-drug of an O6-methyl modified 2'-C-Methyl guanosine monophosphate currently in clinical development for the treatment of HCV. The compound is designed to deliver the monophosphate form of the nucleotide intracellularly, by-passing the first rate limiting phosphorylation step, facilitating rapid conversion to the active triphosphate. The aims of this study were to identify and quantitate the metabolites generated *in vitro* with primary rat and monkey hepatocytes and to compare the *in vivo* metabolism of INX-189 in the same species.

Methods

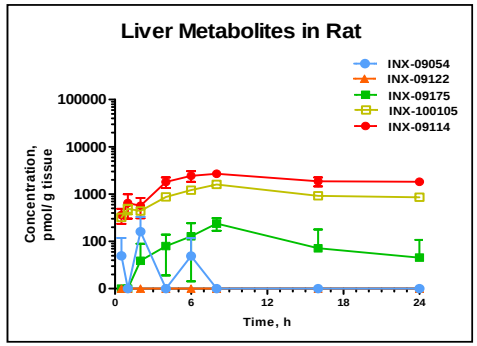
Fresh plated hepatocytes from Sprague-Dawley (SD) rat or Cynomolgus monkey were incubated at 37°C in the presence of 10 µM INX-189. The intracellular and extracellular metabolites were detected and quantified using LC-MS/MS methodology. *In vivo* samples (liver tissues and plasma) from SD rats and Cynomolgus monkeys dosed orally with INX-189 were analyzed for the presence of the same metabolites.

Results

Proposed Metabolic Pathway



Liver Metabolites in Rat



Pharmacokinetic Parameters for INX-08032

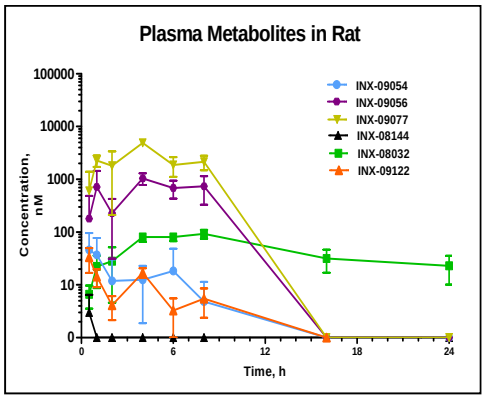
SPECIES (dose, mg/kg)	C_{max}	$t_{1/2}$	iv $AUC_{(0-24)}$	Po $AUC_{(0-24)}$	DNAUC	%F
	ng/mL	h	ng·h/mL	ng·h/mL	ng·h/mL	
RAT (10-po and iv)	27.5	7.9	705.2	354.6	35.5	50
MONKEY (25-po and 10-iv)	27.3	19.6	1805.5	349.2	14.0	8

Exposure of Analyzed Metabolites

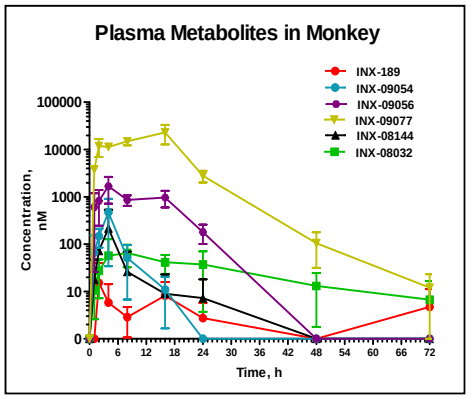
SPECIES (MATRIX)	Extracellular (nm·h) or Plasma (ng·h/mL) Exposure[a]						Intracellular (pmol·h/1.0E+6 cells) or Liver (ng·h/g) Exposure[a]			
	INX-0189	INX-09054	INX-09056	INX-09077	INX-08144	INX-08032	INX-09122	INX-09175	INX-100105	INX-09114
RAT HEPATOCYTE	29,533	299,263[b]	99,838	262,935	187,844	157,871	1,504	1,104	NE	11,011
RAT	NE	61[c]	6,536	1,156	<LOQ[d]	355	<LOQ[e]	700	9,243	24,558
MONKEY HEPATOCYTE	368,934	68,413	66,279	46,464	47,722	18,345	482	<LOQ[f]	NE	<LOQ[g]
MONKEY	110	927	6,552	84,166	191	615	NE	NE	NE	NE

[a]$AUC_{(0-t)}$, area under the curve from time 0 to the last time if observed concentration
[b]Intracellular Levels seen (exposure-18159 pmol·h/1.0E+6 cells)
[c]liver levels observed as well (exposure-139 ng·h/g)
[d]<LOQ-below limit of quantitation, 1.22 ng/g
[e]<LOQ-below limit of quantitation, 39.2 ng/g
[f]<LOQ-below limit of quantitation, 7.95 pmol/1.0E+6 cells
[g]<LOQ-below limit of quantitation, 1.88 pmol/1.0E+6 cells
NE-not estimated

Plasma Metabolites in Rat



Plasma Metabolites in Monkey



Conclusions

1. Analytical methods have been established to identify and quantitate the key metabolites of the proposed metabolic pathway of INX-189.
2. These methods have been applied to the analyses of samples from *in vivo* and *in vitro* studies to confirm this pathway.
3. These results support the use of rats and monkeys in the non-clinical development of INX-189.

References

1. Saboulard, D., et al. *Mol. Pharm.* 56, 693-704 (1999).
2. McGuigan, C., et al. *Bioorg. Med. Chem. Lett.* 19 (15), 4316-4320 (2009).
3. Murakami, E., et al. J. Biol. Chem. M110.161802 (2010).
4. Congiatu, C., et al. *Nucleosides Nucleotides Nucleic Acids* 26, 1121-1124 (2007).

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