

Characterization of *in vitro* selected Hepatitis C Virus Replicon Mutants Resistant to the Phosphoramidate Analog of 2'-C-Methylguanosine, INX-189

#1888

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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. In order to determine potential resistant genotypes that may be selected in the clinical setting, INX-189 resistant HCV replicons were selected and characterized *in vitro*. HCV genotype 1b (HCV 1b) and genotype 1a (HCV 1a) replicon cells were cultured in the presence of INX-189 and G418 an antibiotic selection agent which is used to maintain expression of the recombinant replicon construct. The establishment of stable surviving colonies was significantly delayed, requiring selection over 4 to 7 weeks in culture. Two NS5B mutations, S282T and I585T, were identified in HCV 1b surviving clones and one mutation, A540T, was identified in one HCV 1a clone. These mutations were engineered into the HCV 1b replicon and their effects on INX-189 potency were characterized.

Methods

A luciferase-reporter genotype 1b subgenomic replicon cell line, was obtained from Apath, LLC, Brooklyn, NY. HCV replication was measured using the Renilla luciferase reporter assay (Promega, Madison, WI).

All replicon constructs carrying NS5B mutant sequences were transfected into cured Huh-7 cells by electroporation and tested for sensitivity to INX-189 in a transient replication assay. A stable replicon expressing the S282T NS5B mutant was also established and used for the 14 day clearance studies.

The effects of combining INX-189 and Ribavirin (Rbv) on inhibition of the HCV 1b S282T replicon was tested in a transient replication assay. Analysis of drug combination studies was carried out with MacSynergyII software (Pritchard 1990 Antiviral. Res. 14:181-206).

Figure 1: INX-189 Structure

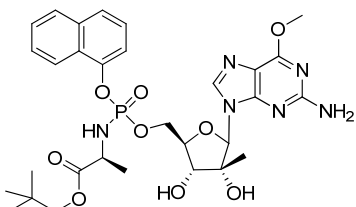


Figure 2: Inhibition of NS5B Mutants in Transient Replicon

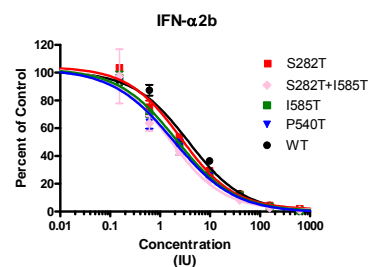
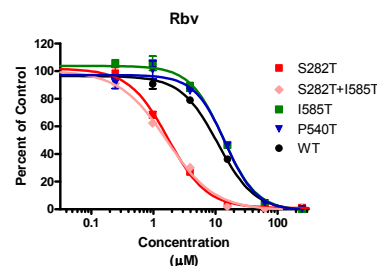
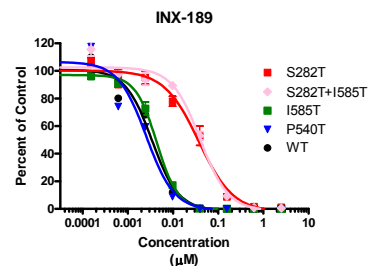


Table 2: Lack of Cross-Resistance

NS5B Sequence	EC ₅₀ (µM)	Fold Change
Con1 WT	0.006 ± 0.003	1.0
S96T / N142T	0.006 ± 0.001	1.0
M243L	0.004 ± 0.000	0.7
C316Y	0.003 ± 0.000	0.5

Table 1: NS5B Mutant Effects on Replication Fitness and Inhibitor Potency

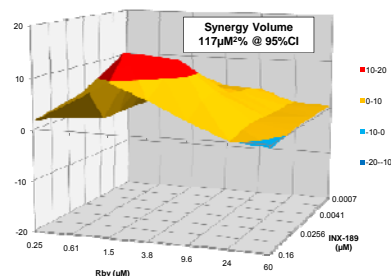
NS5B Sequence	Replication Efficiency ^a (%)	INX-189		Rbv		IFN-α	
		EC ₅₀ (µM) ^b	Fold Change	EC ₅₀ (µM) ^b	Fold Change	EC ₅₀ (µM) ^b	Fold Change
Con1 WT	100	0.006 ± 0.003	1.0	12.157 ± 1.753	1.0	4.974 ± 1.511	1.0
S282T	4	0.074 ± 0.026	12.3	1.728 ± 0.016	0.1	3.116 ± 0.439	0.6
I585T	165	0.005 ± 0.001	0.8	15.247 ± 1.639	1.3	3.380 ± 1.383	0.7
A540T ^c	86	0.004 ± 0.001	0.7	17.250 ± 3.235	1.4	3.294 ± 1.251	0.7
S282T / I585T	8	0.055 ± 0.018	9.2	1.736 ± 0.110	0.1	1.937 ± 0.662	0.4

^a Percent luciferase activity compared to wild-type replicon.

^b All data are averages ± standard deviations of at least three independent experiments.

^c AA position 540 encodes a proline in genotype 1b strain Con 1 and an alanine in genotype 1a strain H77.

Figure 3: INX-189 and Rbv are Synergistic Inhibitors of S282T Replicon



Conclusions

INX-189 potency against S282T mutant replicons was reduced approximately 13-fold, while INX-189 potency against all other NS5B mutants was unaffected. Average EC₉₀ values against transiently expressed S282T replicons were determined to be less than 0.4 µM. Ribavirin had approximately 6 fold greater potency against S282T mutants while the potency of IFN-α2b was unaffected.

Cross-resistance was not observed with INX-189 in replicons expressing NS5B mutations known to confer resistance against other nucleoside and non-nucleoside NS5B inhibitors in these experiments.

Combinations of INX-189 and Rbv demonstrated a synergistic inhibitory effect against S282T mutant replicons.

INX-189 was able to completely clear the S282T mutant replicon *in vitro* after 14 days of culture at a concentration of 0.640 µM whereas the wild-type replicon was cleared after 14 days in the presence of only 0.020 µM INX-189.

Figure 4: Inhibition of Stable HCV 1b S282T Replicon

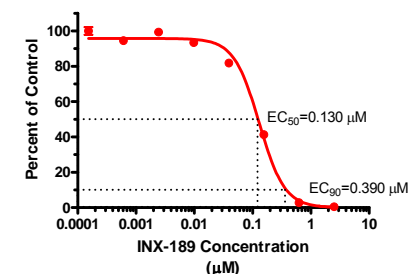


Figure 5: Clearance of HCV 1b S282T Stable Replicon with INX-189

