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Phenotypic Detection of HCV Polymerase Inhibitor Resistant Subpopulations is Dependent on Relative Resistance and Replication Capacity; IC_{95} Values Improve Detection

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BACKGROUND and AIMS

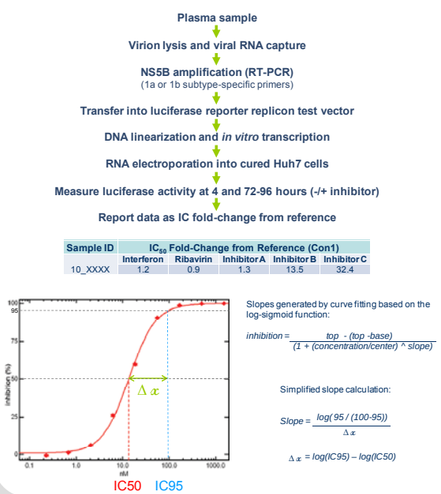
Numerous direct-acting antiviral (DAA) agents that target HCV polymerase are in clinical development. Amino acid substitutions within NS5B can confer resistance to these inhibitors. The ability to detect subpopulations of resistant variants was evaluated using a validated cell-based replicon assay that was developed to support clinical studies of investigational HCV polymerase inhibitors.

METHODS

• The reproducibility of the NS5B phenotyping assay was determined by evaluating inter-assay variation using samples from HCV infected patients.

• A panel of replicons containing polymerase inhibitor resistance mutations was constructed by the introduction of site directed mutations into a genotype 1b (GT1b) Con1 luciferase reporter replicon and a chimeric GT1b replicon containing the NS5B region of H77 (GT1a).

• Samples containing 0, 20, 40, 60, 80 and 100% mutant were prepared and used to evaluate replication capacity (RC; % of the Con1 reference replicon) and susceptibility (inhibitory concentration fold-change (FC) relative to Con1) to a panel of nucleoside and non-nucleoside polymerase inhibitors targeting sites A, B, C and D (NI, NNI-A, NNI-B, NNI-C, NNI-D, respectively), as well as the ability to detect sub-populations of resistant variants. The slopes of inhibitor susceptibility curves were also evaluated.



Sample ID	IC_{50} Fold-Change from Reference (Con1)				
	Interferon	Ribavirin	Inhibitor A	Inhibitor B	Inhibitor C
10_XXXX	1.2	0.9	1.3	13.5	32.4

Slopes generated by curve fitting based on the log-sigmoid function:

$$inhibition = \frac{top - (top - base)}{(1 + (concentration/center)^{\wedge} slope)}$$

Simplified slope calculation:

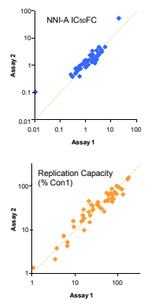
$$Slope = \frac{\log(95 / (100-95))}{\Delta x}$$

$$\Delta x = \log(IC95) - \log(IC50)$$

Figure 1: Reproducibility of the NS5B Phenotypic Assay

Assay Reproducibility		
IC_{50}	Number of pairwise comparisons:	285
	IC_{50}FC value capturing 95% of data:	1.75
IC_{95}	Number of pairwise comparisons:	260
	IC_{95}FC value capturing 95% of data:	1.70
RC	Number of pairwise comparisons:	55
	Log_{10}RC value capturing 95% of data:	0.27

Representative Reproducibility Data



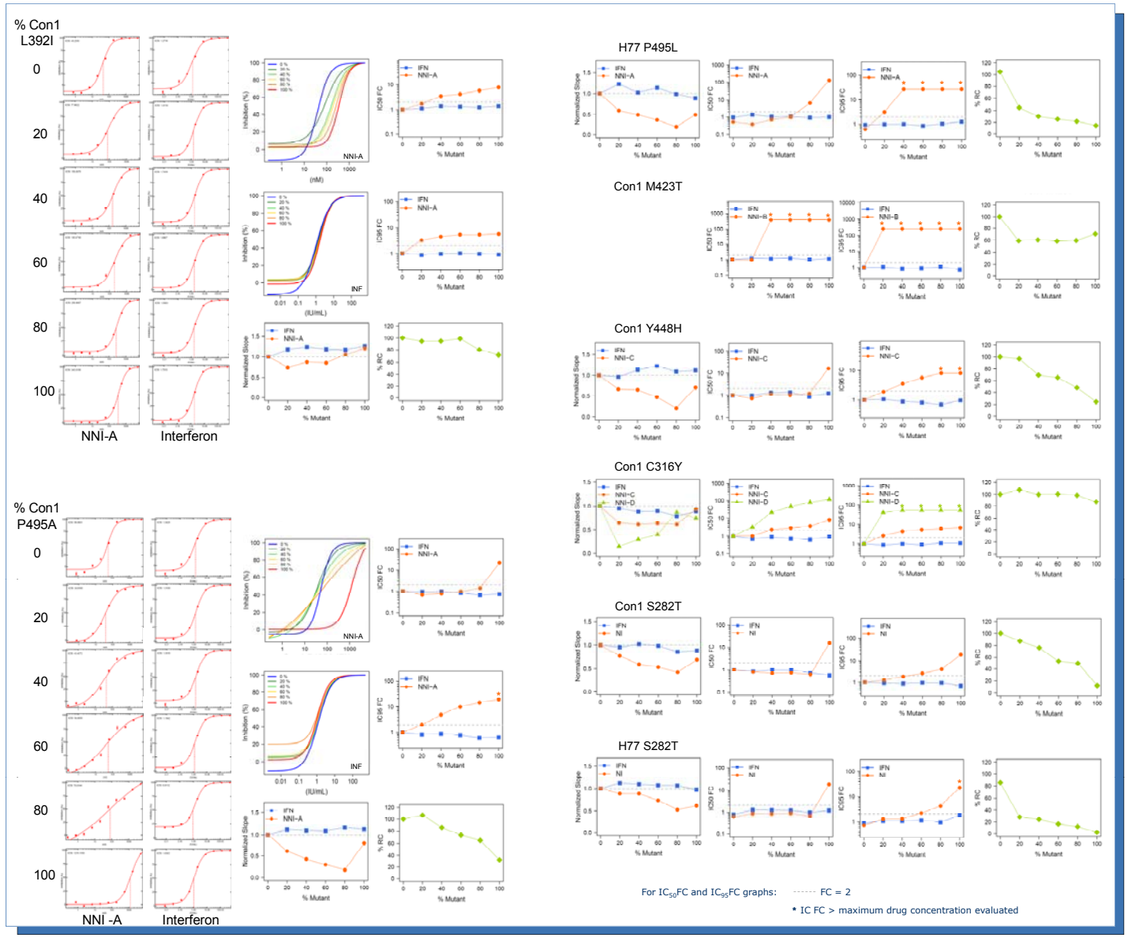
• The NS5B phenotyping assay can reproducibly detect 2-fold changes in inhibitor susceptibility.

Table 1: Inhibitor Susceptibility, Replication Capacity and Detection of Subpopulations of Resistant Variants

Mutant	Inhibitor	Phenotypic Data			% mutant detected*	
		IC_{50}FC	IC_{95}FC	%RC	IC_{50}FC	IC_{95}FC
Con1 L392I	NNI-A	7.60	5.82	71	40	20
Con1 P495A	NNI-A	21.67	>19	33	100	40
H77 P495L	NNI-A	128.95	>27	15	80	20
Con1 M423T	NNI-B	>410	>51	71	40	20
Con1 Y448H	NNI-C	16.39	>8	25	100	40
Con1 C316Y	NNI-C	8.15	6.61	87	40	20
	NNI-D	116.91	>53		20	20
Con1 S282T	NI	15.64	19.36	12	100	60
H77 S282T	NI	18.27	>22	2	100	60

*IC FC value ≥2. Analysis of samples containing 20, 40, 60, 80 and 100% mutant.

Figure 2: Detection of Subpopulations of HCV Polymerase Inhibitor Resistant Variants



RESULTS

• **Figure 1.** ***Assay Reproducibility (inter-assay variation):***
  • 95% of replicate IC_{50}FC and IC_{95}FC values were within 1.75 and 1.7-fold based on 285 and 260 pairwise comparisons, respectively. Therefore, the assay can reproducibly detect 2-fold changes in inhibitor susceptibility.
  • 95% of replicate RC values varied by ≤ 0.27 $log_{10,}$ based on 55 pairwise comparisons.

• **Table 1.** ***RC and HCV polymerase inhibitor susceptibility of replicons containing resistance mutations:***
  • Con1 L392I, P495A mutations and H77 P495L mutations reduced NNI-A susceptibility (IC_{50}) ~8, 22 and 129-fold and reduced RC to 71, 33 and 15%, respectively.
  • Con1 M423T mutations conferred a > 410-fold reduction in NNI-B susceptibility and reduced RC to 71%.
  • Con1 Y448H mutations reduced NNI-C susceptibility ~16-fold and reduced RC to 25%.
  • Con1 C316Y mutations variably reduced NNI-C and NNI-D inhibitor susceptibility to ~8 and 117-fold, respectively, and conferred a small reduction in RC (87%).
  • Con1 S282T and H77 S282T mutations reduced NI susceptibility ~16- and 18-fold and reduced RC to 12 and 2%, respectively.

• **Figure 2 and Table 1.** ***Sensitivity to detect subpopulations of HCV polymerase inhibitor resistant variants:*** The ability to detect subpopulations of resistance variants was defined as the lowest mixture that produced a ≥ 2-fold difference in IC_{50}FC or IC_{95}FC values.
  • Subpopulations of up to 80% of S282T mutants were not detected using IC_{50} values, consistent with prior reports, however we found that IC_{95} values allowed for improved detection with 60% mixtures discernable.
  • 20% mixtures of C316Y, L392I, M423T and P495L were detected from IC_{95} values, compared to 20% for C316Y with NNI-D, 40% for C316Y with NNI-C, L392I and M423T and 80% for P495L from IC_{50} values.
  • 40% subpopulations of Y448H and P495A were detected from IC_{95} values, whereas subpopulations of up to 80% were not discernable from IC_{50} values.
  • Overall, the detection of subpopulations of polymerase inhibitor resistant mutants correlated with RC, inhibitor susceptibility and the slope of susceptibility curves. RC was the most significant covariate for the detection of the subpopulations.

SUMMARY & CONCLUSIONS

• Replicons containing polymerase inhibitor resistance mutations exhibited variable reductions in replication capacity and susceptibility to polymerase inhibitors.

• The detection of resistant subpopulations varied from 20-60% depending on the mutation and inhibitor evaluated, reflecting the degree of reduced inhibitor susceptibility, the slope of susceptibility curves and the reduction in RC conferred by the specific mutation.

• IC_{95} values often improve the detection of NS5B inhibitor resistant subpopulations compared to IC_{50} values, and likely reflect the increased contribution of the mutant to RC at increased drug concentrations.