

The Nucleoside Inhibitors R1479, PSI-6130, and NM107 have a Higher Genetic Barrier to Resistance than the Non-Nucleoside Inhibitor HCV-796 and the Protease Inhibitor VX-950

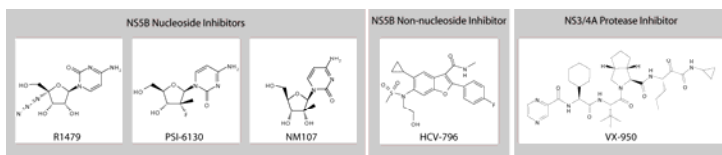
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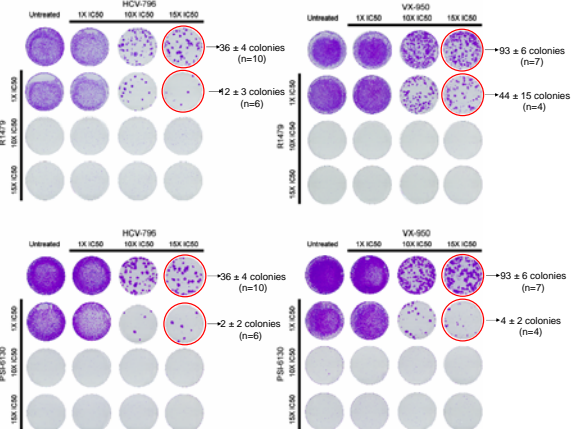
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Introduction

- Hepatitis C virus (HCV) is a positive-strand RNA virus of the Flaviviridae family
- The HCV RNA genome is 9.5 kb in length and is translated into 10 structural and non-structural viral proteins
- The current available therapy consists of pegylated-IFN in combination with ribavirin
- Approximately 50% of HCV Genotype 1 infected patients treated with pegylated-IFN/ribavirin achieve a sustained viral response
- New HCV specific antiviral compounds are needed to increase treatment efficacy
- Novel inhibitors of HCV replication include R1479 (NS5B nucleoside inhibitor), PSI-6130 (NS5B nucleoside inhibitor), NM107 (NS5B nucleoside inhibitor), HCV-796 (NS5B non-nucleoside inhibitor), and VX-950 (NS3/4A protease inhibitor)
- R1626 (prodrug of R1479), HCV-796, and VX-950 have all demonstrated efficacy in chronically infected HCV patients
- Given the HCV genetic heterogeneity, development of antiviral resistance is an issue that must be addressed
- In this study we analyzed the *in vitro* development of resistance to the NS5B nucleoside inhibitors R1479, PSI-6130, and NM107 in comparison to the NS5B non-nucleoside inhibitor HCV-796 and the NS3/4A protease inhibitor VX-950, both individually and in combination



Combination with a nucleoside NS5B polymerase inhibitor reduces the frequency of non-nucleoside and protease inhibitor drug resistant colonies



- The number of observed colonies was reduced with a combination of 1X R1479 or PSI-6130 and either HCV-796 or VX-950
- The replicon was cleared with a combination of 10X or 15X R1479 or PSI-6130 and either HCV-796 or VX-950

Methods

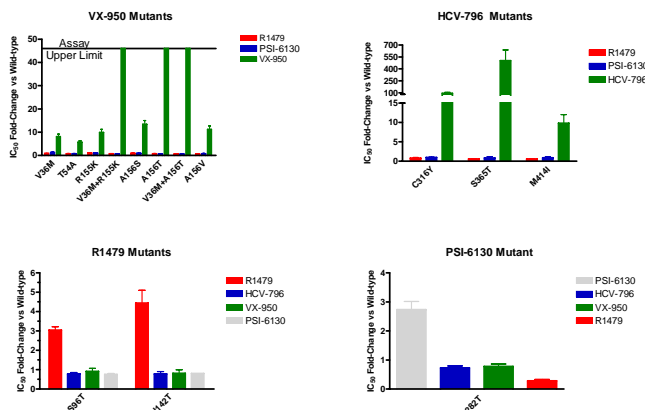
- The stable GT1b (Con1) subgenomic replicon cells were treated with a nucleoside inhibitor (R1479, PSI-6130, NM-107), a non-nucleoside inhibitor (HCV-796), or a protease inhibitor (VX-950) alone or in combination for three weeks at 1X, 10X, and 15X their respective IC₅₀'s. The cells were then either stained with Crystal Violet or the replicon RNA was extracted for sequencing of NS5B and NS3 coding regions.
- The potential for a mutation to confer cross-resistance among the inhibitors was examined by introducing the mutations observed after selection with each compound, as well as, previously reported mutations into a transient HCV replicon. The effect of the mutations on the potency of the inhibitors was determined.

Inhibitory activity and cytotoxicity against HCV 1b replicon cells

Compound	GT 1b Con1	
	IC ₅₀ (μM) ^a	CC ₅₀ (μM) ^b
R1479	1.28 ± 0.08	>100
PSI-6130	0.61 ± 0.04	>100
NM107	1.13 ± 0.03	>100
HCV-796	0.017 ± 0.001	>100
VX-950	0.56 ± 0.05	26.7 ± 1.1

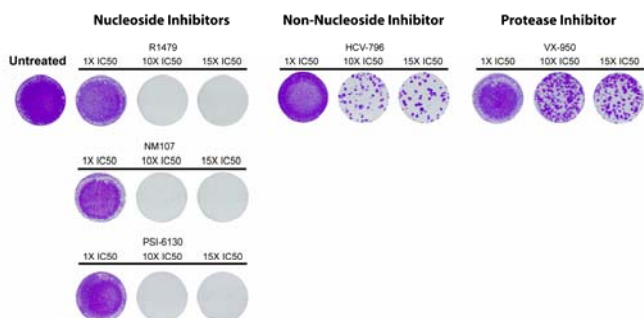
^a Inhibition of HCV replicon encoded renilla luciferase reporter activity after 3 day incubation
^b Cell viability determined by either MTT or WST-1 assay

Lack of cross resistance between different classes of inhibitors



- R1479 and PSI-6130 maintained potency against mutant replicons with reduced sensitivity to VX-950 or HCV-796
- HCV-796 and VX-950 maintained potency against mutant replicons with reduced sensitivity to R1479 or PSI-6130

Lack of resistant colonies after treatment with the NS5B nucleoside inhibitors NM107, R1479 or PSI-6130



- R1479, NM107, and PSI-6130 at 10X and 15x the IC₅₀ resulted in clearance of the replicon
- No mutations were identified in NS5B from 1X treated cells
- Distinct colonies were observed at 10X and 15x the IC₅₀ of HCV-796
- C316Y and S365S/A were identified in NS5B from 10X or 15X treated cells
- Distinct colonies were observed at 10X and 15x the IC₅₀ for VX-950
- A156T/S and T54T/A mutations were identified in NS3 from 10X or 15X treated cells

Conclusions

- The non-nucleoside inhibitor HCV-796 selected for the amino acid substitutions C316Y and S365S/A in the NS5B coding region (as previously reported) after a three week selection at 10X and 15x the IC₅₀
- The NS3/4A protease inhibitor VX-950 selected for the amino acid substitutions A156T/S and T54T/A in the NS3 coding region (as previously reported) after a three week selection at 10X and 15x the IC₅₀
- Under the same conditions, nucleoside inhibitors (R1479, PSI-6130, or NM107) did not select for resistance and the replicon was cleared
- This indicates that the *in vitro* genetic barrier to resistance may be higher for nucleoside inhibitors compared to non-nucleoside and protease inhibitors
- The *in vitro* selected amino acid substitutions that confer resistance to VX-950 (A156T/S or T54T/A) or HCV-796 (C316Y) correlate with the resistance mutations identified in clinical studies
- In combination with R1479 or PSI-6130 the number of VX-950 and HCV-796 resistant colonies was decreased
- This indicates that combination with a nucleoside inhibitor may decrease the frequency of drug resistance selection
- R1479 and PSI-6130 maintained potency against clinically relevant VX-950 and HCV-796 resistance mutations
- VX-950 and HCV-796 maintained potency against mutants with reduced sensitivity to R1479 and PSI-6130
- These data support further investigations into the potential clinical benefit of combination therapies