

# In Vitro Selection and Characterization of HCV Replicons with Reduced Sensitivity to PSI-6130



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## Introduction

- R7128 is a prodrug of PSI-6130 (β-D-deoxy-2'-fluoro-2'-C-methylcytidine), a potent and selective inhibitor of HCV NS5B that is in clinical development for the treatment of chronic hepatitis C.
- The primary goal of the study was to determine and characterize the *in vitro* selected mutations that confer reduced sensitivity to PSI-6130.

## Materials and Methods

**Characterization of PSI-6130 activity against clinical isolates:** NS5B coding sequences were cloned from GT-1a and GT-1b patients' serum into the respective genotype specific transient replicon cassette containing a Firefly luciferase reporter gene.

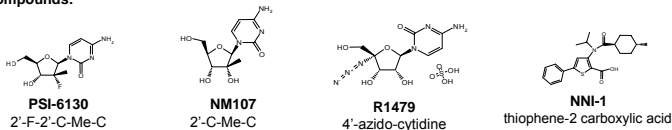
**Replicon clearance assay:** Huh-7 cells harboring a GT-1b stable replicon were treated for one month with a 10X EC<sub>50</sub> concentration of PSI-6130, NM107 or NNI-1 without G418 followed by 2 weeks treatment with G418 without the compounds. Replicon levels were determined by quantitative kinetic PCR.

**Selection of replicons in PSI-6130 and R7128:** Huh-7 cells harboring the GT-1b Con1 replicon were cultured for 6-8 months in the presence of PSI-6130 (set #1 and set #2) or R7128 (set #3) in the presence of G418. The activity of PSI-6130 was determined using quantitative kinetic PCR and the presence of amino acid substitutions was determined by sequencing the NS5B coding regions.

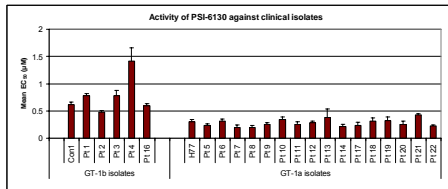
**Characterization of amino acid substitutions in NS5B:** All the amino acid substitutions detected in the selection of replicon cells with PSI-6130 were introduced by site-directed-mutagenesis and examined for activity in the transient replicon system. Replication capacity was determined by normalizing the 96h luciferase signal to the 4h input signal and in turn compared to the normalized WT replicon signal.

**Characterization of the S282T substitution:** The S282T amino acid substitution was characterized using the stable and transient replicon systems as well as in the recombinant NS5B assay.

**Compounds:**

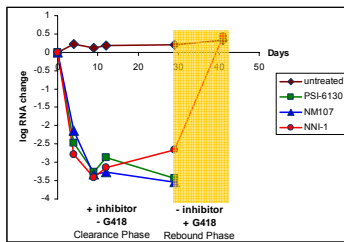


## PSI-6130 exhibits potent activity against GT-1a and GT-1b clinical isolates



- PSI-6130 demonstrates potent and highly consistent activity against HCV replicons containing NS5B genes derived from multiple genotype 1a and 1b clinical isolates.

## Treatment with PSI-6130 results in clearance of the replicon



- Treatment of cells harboring a subgenomic replicon for one month with a 10 X EC<sub>50</sub> concentration of PSI-6130 cleared the viral RNA from the cells without selecting resistant variants (N=3).

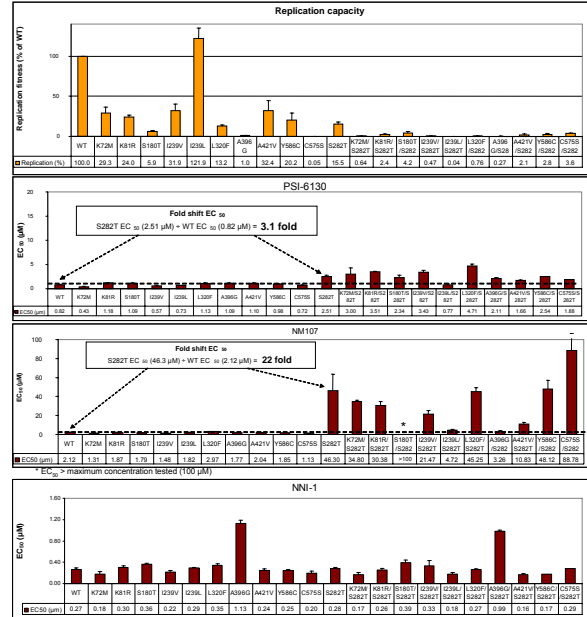
## Selection of replicon-containing cells with reduced sensitivity to PSI-6130

Selection Set	Passage number	PSI-6130 concentration (µM)	Fold shift EC <sub>50</sub> *	Amino acid substitutions in NS5B
Untreated	35	0	No shift	No substitutions
Untreated	56	0	No shift	No substitutions
#1	6	5	7	Y586C
#1	28	50	32	S282T, C575S, Y586C
#1	53	100	51	S282T, K81R, I239L, L320F, A421V, Y586C
#2	25	50	ND	S282T, I239V, A396G
#2	27	25	25	S282T
#2	28	25	ND	S282T, I239L
#2	32	25	13	S282T
#3	21	30	120	K72M, S282T
#3	26	30	107	K72M, S282T

\*EC<sub>50</sub> shift represents the combined effects from cellular and viral determinants.

- Long-term (6-8 months) sequential passage of cells harboring the GT-1b wild-type replicon with PSI-6130 or R7128 resulted in the selection of a number of amino acid substitutions.

## Characterization of NS5B substitutions observed after PSI-6130 selection



- All amino acid substitutions reduced replication capacity with the exception of I239L.
- The S282T substitution conferred 3.1 fold reduced sensitivity to PSI-6130 alone and 3-8 fold reduced sensitivity in combination with other selected amino acid substitutions.
- S282T conferred 22 fold reduced sensitivity to NM107

## S282T confers a moderate reduction in sensitivity of the replicon and the recombinant NS5B to PSI-6130

Compound	Transient replicons EC <sub>50</sub> (µM)			Stable replicons EC <sub>50</sub> (µM)		
	Wild type	S282T	Fold shift	Wild type	S282T	EC <sub>50</sub> fold shift
PSI-6130	0.82 ± 0.04	2.51 ± 0.29	3.1	0.31 ± 0.08	0.75 ± 0.22	2.4
R7128	0.89 ± 0.07	4.68 ± 0.91	5.3	0.32 ± 0.09	1.08 ± 0.03	3.4
NM107	2.12 ± 0.19	46.30 ± 17.17	21.8	1.55 ± 0.31	26.31 ± 3.77	17.0

Compound	WT NS5B IC <sub>50</sub> (µM)	S282T NS5B IC <sub>50</sub> (µM)	IC <sub>50</sub> Fold Shift
PSI-6130-TP	0.13 ± 0.01	0.70 ± 0.12	5.9
R1479-TP	0.32 ± 0.14	0.30 ± 0.13	1.1
NM107-TP	0.09 ± 0.02	10 ± 0.6	111.4

- The S282T substitution conferred a 2-5 fold reduced sensitivity of the replicon to PSI-6130 compared to a 17-22 fold loss to NM107 (in transient and stable replicon systems, respectively), and no loss in sensitivity to R1479.
- The S282T substitution reduces the sensitivity of recombinant NS5B by 5.9 fold to PSI-6130 compared to 111.4 fold to NM107

## PSI-6130 and R1479 select for different mutations in NS5B

- Long-term culture of replicon cells containing the S96T/N142T substitutions in NS5B, known to confer reduced sensitivity to R1479 (4'-azido-cytidine), in the presence of PSI-6130 resulted in the selection of the S282T substitution and the reversion of the threonine 96 to wild-type serine.
- Long-term culture of the replicon cells containing the S282T substitution in NS5B in the presence of R1479 resulted in the selection of S96T and reversion of S282T to wild-type serine.
- R1479 demonstrates similar activity against the replicons containing the S282T mutation as WT replicon. PSI-6130 shows equivalent activity against the replicon with the S96T substitution (data not shown).

## Conclusions

- Selection of Huh-7 replicon cells with PSI-6130 resulted in the selection of the S282T substitution within 6-8 months.
- The S282T substitution, either alone or in combination with other observed substitutions, conferred a 3-8 fold reduced sensitivity of the replicon to PSI-6130.
- PSI-6130 demonstrated potent and highly consistent activity against GT-1a and GT-1b clinical isolates
- In the replicon clearance assay, treatment with 10X EC<sub>50</sub> concentration of PSI-6130 resulted in clearance of the replicon within one month.
- PSI-6130 and R1479 select for different mutations in NS5B.