



Selection and Characterization of Hepatitis C Virus Replicons Using Combination of NS3 Protease and NS5B Non-nucleoside Inhibitors or Combination of NS5B Nucleotide Inhibitors

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Direct Acting Antivirals (DAA)

● Development of DAA.

- Targets: NS3 protease, NS4B, NS5A, NS5B (nucleosides/tides and non-nucleoside inhibitors).

● Promising data:

- single DAA plus SOC (*e.g.* VX-950, RG7128, PSI-7977).
- two DAA's without SOC (*e.g.* RG7227/RG7128).

● Challenge:

- Selection of resistant virus.

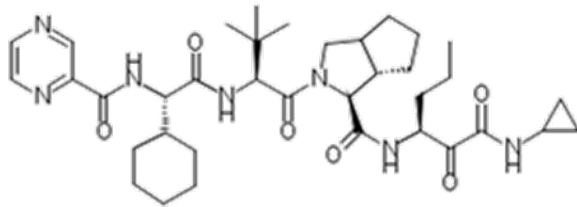


Study objective

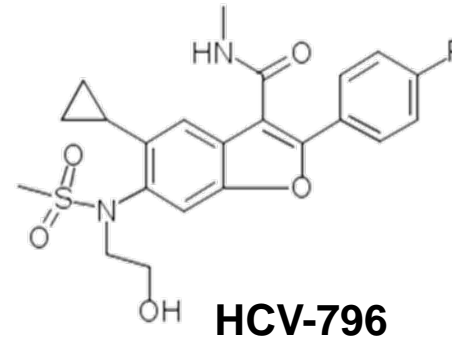
- **Evaluate antiviral compounds in combinations:**
 - Enhance inhibition of HCV replication.
 - Suppress emergence of resistance.
- **Combination sets:**
 - NS3 protease inhibitor + NS5B non-nucleoside inhibitor
 - Two NS5B nucleotide analogs



NS3 protease inhibitor and NS5B non-nucleoside inhibitor used in study



VX-950



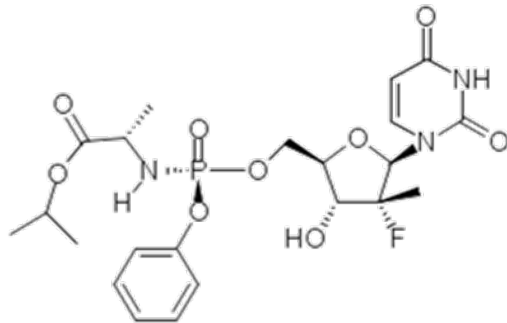
HCV-796

- **VX-950**: NS3 protease inhibitor.
- **HCV-796**: non-nucleoside NS5B inhibitor.
- Different viral targets/modes of action.
- Different resistance profiles.

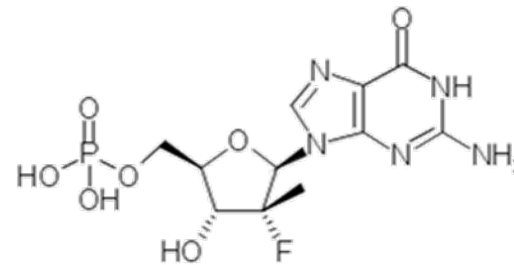


NS5B nucleotide inhibitors used in study

- **PSI-7977**, prodrug of 2'-F-2'-C-MeU-MP.
- **PSI-352938 (PSI-938)**, prodrug of 2'-F-2'-C-MeG-MP.



PSI-7977



**monophosphate
metabolite of PSI-352938**

- Same target: NS5B polymerase.
- Different metabolic pathways and resistance profiles.
 - **S282T: resistant to PSI-7977 but susceptible to PSI-352938**
- High barrier to resistance.
- Active across genotypes and subtypes.



Anti-HCV activity in replicon cells

Compounds	Target	EC ₅₀ (μM)		
		GT 1b	GT 1a	GT 2a
VX-950	NS3 protease	0.52	1.02	1.25
HCV-796	NS5B	0.007	0.004	0.25
PSI-7977	NS5B	0.092	0.20	0.24
PSI-352938	NS5B	0.13	0.20	0.14

GT 1b is derived from Con1, GT 1a is derived from H77, GT 2a is derived from J6/JFH-1.



Combination studies in GT 1b replicon cells

Ratio	Combination Index (CI)		
HCV-796 : VX-950	EC ₅₀	EC ₇₅	EC ₉₀
1 : 1600	0.70	0.70	0.72
1 : 800	0.77	0.73	0.72
1 : 400	0.80	0.70	0.69
1 : 200	0.91	0.74	0.71

Ratio	Combination Index (CI)		
7977 : 352938	EC ₅₀	EC ₇₅	EC ₉₀
1 : 8	1.01	0.97	0.92
1 : 4	1.01	1.05	1.10
1 : 2	1.04	1.07	1.09
1 : 1	1.05	1.05	1.05

CI <1: synergy, CI ~1: additive, CI >1: antagonism.



Resistance selection by combining VX-950 and HCV-796

Selection conditions		VX-950: EC ₅₀ (μM)			HCV-796: EC ₅₀ (μM)		
Days under selection	VX-950/HCV-796 [μM/μM]	Control cells	Compounds-selected cells	EC ₅₀ fold change	Control cells	Compounds-selected cells	EC ₅₀ fold change
21	2.0/0.010	0.62	2.39	3.8	0.009	0.025	2.9
46	3.0/0.015	0.70	7.95	11.4	0.002	0.032	16.0
56	4.0/0.020	0.35	>10	>30	0.002	0.045	22.5

Selection was performed using GT 1b replicon cells starting at 1 μM VX-950 and 0.005 μM HCV-796



Selection with PSI-7977 and PSI-352938

Selection conditions		PSI-7977: EC ₅₀ (μM)			PSI-352938: EC ₅₀ (μM)		
Days under selection	PSI-7977/PSI-352938 [μM/μM]	Control cells	Compounds-selected cells	EC ₅₀ fold change	Control cells	Compounds-selected cells	EC ₅₀ fold change
21	0.12/0.40	0.15	0.12	0.8	0.18	0.11	0.6
46	0.15/0.50	0.066	0.099	1.5	0.15	0.21	1.5
77	0.18/0.60	0.047	0.10	2.2	0.020	0.11	5.7
92	0.18/0.60	0.10	0.18	1.8	0.054	0.12	2.3
120	0.30/1.00	0.044	0.12	2.7	0.034	0.11	3.3

Selection was performed using GT 1b replicon cells starting at 0.03 μM PSI-7977 and 0.1 μM PSI-352938.

No significant change in susceptibility of selected replicon cells was observed for each compound over 120 days.



Identification of resistance mutations

Resistance mutations	VX-950 & HCV-796	VX-950	HCV-796
NS3 F43S	1/5		
NS3 A156S	1/5	1/5	
NS3 A156T	1/5	2/5	
NS3 A156V		2/5	
NS3 V170A	2/5	1/5	
NS5B S282T			
NS5B C316Y			5/7
NS5B C445F	5/5		3/7



Identification of resistance mutations

Resistance mutations	VX-950 & HCV-796	VX-950	HCV-796	PSI-7977 & PSI-352938 ^a	PSI-7851 ^b
NS3 F43S	1/5				
NS3 A156S	1/5	1/5			
NS3 A156T	1/5	2/5			
NS3 A156V		2/5			
NS3 V170A	2/5	1/5			
NS5B S282T				0/10	2/6
NS5B C316Y			5/7		
NS5B C445F	5/5		3/7		

^a No amino acid changes in the NS5B polymerase have been identified that conferred resistance to PSI-352938.

^b PSI-7977 is a stereoisomer of PSI-7851.



Conclusions

- Enhanced inhibition with combinations:
 - **VX-950 and HCV-796: synergistic effect**
 - **PSI-7977 and PSI-352938: additive effect**
- Selection with VX-950 and HCV-796 produced dually resistant HCV replicon cells within 46 days.
- Selection with PSI-7977 and PSI-352938 produced no resistant HCV replicons over 120 days.
- Combination therapies using complementary NS5B nucleotide analogs could provide:
 - a significant barrier to resistance that might not be seen with a combination of a protease inhibitor and a non-nucleoside inhibitor.

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