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Enhanced *in Vitro* Antiviral Activity and Suppression of Resistance by Combining GS-9256, A Novel Protease Inhibitor, With GS-9190, a Non-Nucleoside NS5B Inhibitor

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Introduction

- Combination of multiple direct acting antivirals (DAAs) will be a critical approach to overcoming HCV resistance and enhancing SVR rates
- GS-9256, a novel HCV protease inhibitor (PI), and GS-9190, a non-nucleoside NS5B inhibitor (NNI), have demonstrated potent antiviral activity in genotype 1 HCV infected subjects during monotherapy studies.
- Previous *in vitro* studies indicated that the NS5B mutations C316Y, C445F, Y448H and Y452H were associated with reduced GS-9190 susceptibility. Y448H and Y452H mutations were observed in HCV GT-1 infected patients following GS-9190 treatment
- NS3 mutations at R155, D168 and A156 are associated with resistance to GS-9256 *in vitro* and in the clinic

Objectives

- To characterize the *in vitro* resistance and cross-resistance profiles of GS-9256 and GS-9190
- To determine the antiviral activity of GS-9256 and GS-9190 in short-term and long-term *in vitro* combination assays

Methods

- The susceptibility of HCV replicons to GS-9190 and GS-9256 was determined using luciferase or NS3 readouts
- GS-9256 and GS-9190 associated mutations were introduced into 1b replicons by site-directed mutagenesis and antiviral susceptibility was tested in a transient replication assay
- The antiviral activity of GS-9256 in combination with GS-9190 was monitored by reduction of luciferase signal after 3 days of treatment or by the suppression of HCV replicon RNA following long-term passage of the replicon in the presence of drugs
- The NS3/4A and/or NS5B genes were amplified from the HCV 1b-con-1 and 1a-H77 replicon cells passaged in the presence of GS-9256 or GS-9190 alone or in combination by RT-PCR, and were analyzed by population sequencing

Results

Table 1. Activity of GS-9256 against Genotype 1b Replicon (Huh-luc)

Compound	EC_{50} (nM)	CC_{50} (nM)	SI
GS-9256	20.0 ± 13	> 40,000	> 2000
VX-950	436 ± 136	66,000	151
BILN-2061	0.8 ± 0.5	35,000	43,750

Table 2. Activity of GS-9256 in Additional Replicon Cell Lines

Compound	Fold-change from Huh-luc GT 1b EC_{50}				
	GT 1b			GT 1a	GT 2a
	9-13	SL3	MH4	HSG-57	2aNeo-6
GS-9256	3.7	0.6	2.3	3.7	14.2
VX-950	0.8	0.7	1.4	2.3	0.9
BILN-2061	7.3	2.2	4.0	8.7	77.9

GT = genotype

Results (cont'd)

Table 3. Activity of GS-9256 and GS-9190 against NS5B Polymerase Mutations

Compound (Target)	EC_{50} (nM)	Fold Resistance*			
	WT	M423T	M414T	Y448H	C316Y/C445F/ Y452H
GS-9256 (NS3)	51	1.1	2.0	1.0	2.1
GS-9190 (NS5B)	1.5	0.8	0.7	38	> 343
2'-C-MeA (NS5B)	525	0.7	0.7	1.0	0.4
Benzothiadiazine (Abbott)	93	0.5	130	> 16	536
Thiophene (NS5B) (ViroChem)	337	28	1.8	0.8	0.5

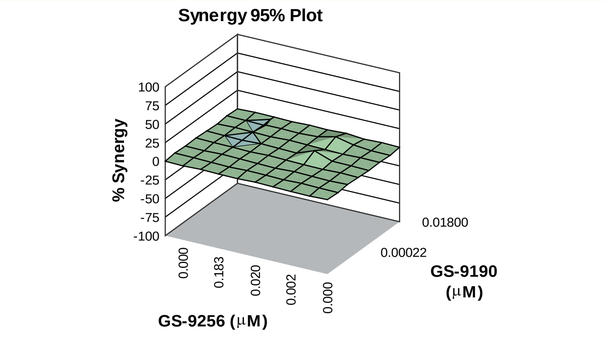
*Fold resistance = EC_{50} of the mutant / EC_{50} of 1b-con 1 WT
Values in red >4-fold; na, not available

Table 4. Activity of GS-9190 and GS-9256 against NS3 Protease Mutations

Compounds (Target)	EC_{50} (nM)	Fold Resistance*					
	WT	V36M	T54A	R155K	A156T	D168E	D168V
GS-9190 (NS5B)	0.8	1.1	1.0	0.9	0.6	1.0	1.2
GS-9256 (NS3)	21	3.0	0.6	566	1882	120	1832
BILN-2061 (NS3)	1.0	1.7	0.8	553	1123	62	4582
VX-950 (NS3)	371	6.5	2.4	7.6	29	0.5	0.5

*Fold resistance = EC_{50} of the mutant / EC_{50} of 1b-con 1 WT
Values in red >4-fold; na, not available

Figure 1. Combinations of GS-9256 with GS-9190 in a 3-day Antiviral Assay



- Additive anti-HCV activity observed; no cytotoxicity was detected at the highest concentrations tested in combination

Figure 2. Long-term Antiviral Activity of GS-9190 and GS-9256 Combinations in GT 1a H77 Replicon

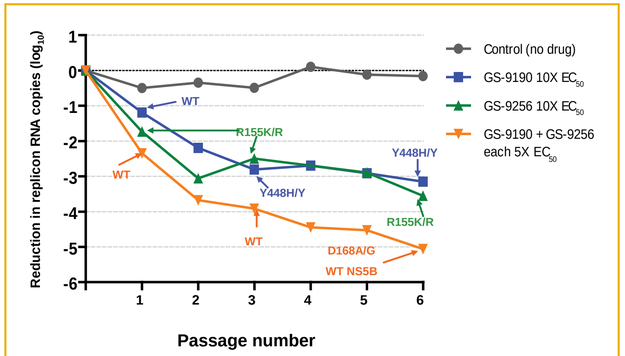
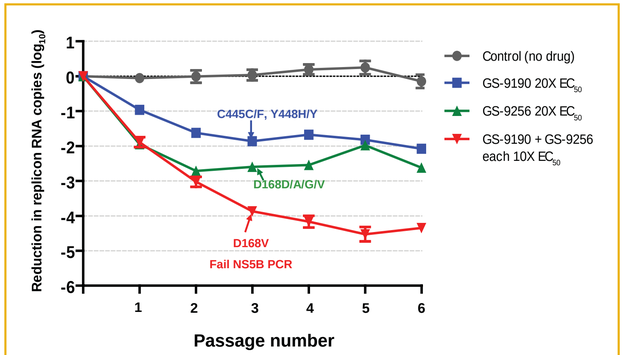


Figure 3. Long-term Antiviral Activity of GS-9190 and GS-9256 Combinations in GT 1b con-1 (Huh-luc) Replicon



Results Summary

- GS-9256 is a potent HCV inhibitor with an EC_{50} value of ~20 nM against the HCV 1b replicon and a selectivity index of > 2000
- Antiviral activity was similar across a number of additional genotype 1b and 1a replicon cell lines (Tables 1 and 2)
- GS-9190 retained wild-type potency against mutations that confer resistance to GS-9256 or other HCV PIs (Table 3)
- GS-9256 retained wild-type potency against mutations that confer resistance to GS-9190 and other classes of HCV NNIs (Table 4)
- The combination of GS-9256 with GS-9190 produced additive antiviral activity without detectable cytotoxicity in 3-day replicon assays (Figure 1).
- Long-term treatment of HCV replicons with combinations of GS-9256 and GS-9190 resulted in substantial reductions in HCV RNA that were significantly greater than those observed with treatment with either compound alone (Figures 2 and 3).
- Amino acid substitutions of R155K or D168A/G/V in NS3 or C445F or Y448H mutations in NS5B were detected in replicon cells treated with GS-9256 or GS-9190 alone, respectively (Figures 2 and 3)
- The combination of GS-9190 and GS-9256 delayed and reduced the frequency of resistance mutation selection compared to single drug treatments (Figures 2 and 3)

Conclusions

- **These findings support the clinical strategy of combining GS-9256 with GS-9190 to more effectively suppress HCV replication and reduce resistance development**
- **Accordingly, the combination of GS-9256 and GS-9190 is in Phase 2 clinical development**
  - **Late breaker oral presentation by Zeuzem at al, Short-term Combination Protease Inhibitor (GS-9256) and Polymerase Inhibitor (GS-9190) Therapy Alone, in Combination with Ribavirin (RBV), and in Combination with Pegylated Interferon (PEG)/RBV for up to 28 days in Treatment Naïve, Genotype 1 HCV Subjects**

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