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Characterization of HCV Resistance from Single and Multiple Dose Clinical Trials of GS-9256, a Novel NS3 Protease Inhibitor

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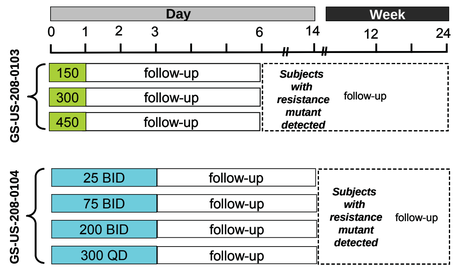
Background

- GS-9256 is a novel, rapidly reversible, non-covalent inhibitor of HCV NS3 serine protease
  - In NS3 enzymatic assays, GS-9256 K_i = 90 pM
  - In HCV 1b-con-1 replicon assays, GS-9256 EC_{50} = 15-20 nM
  - Pre-clinical resistance selections in HCV 1b-con-1 replicons identified A156T and D168A/G/E/N/V as primary GS-9256 resistance mutations
- Two GS-9256 clinical trials, GS-US-208-0103 (single ascending dose) and GS-US-208-0104 (multiple ascending doses) were conducted to evaluate:
  - The safety and tolerability of single and multiple ascending doses of GS-9256 in treatment naïve subjects with chronic Genotype 1 HCV infection
  - The HCV antiviral activity of single and multiple oral doses of GS-9256
  - The plasma pharmacokinetics of single and multiple doses of GS-9256

Figure 1. Single and Multiple Ascending Dose GS-9256 Clinical Trial Designs



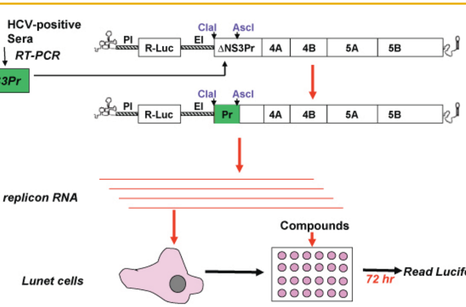
Objectives

- Identify mutations in the HCV NS3 genes from HCV patient isolates that are potentially associated with virologic resistance to GS-9256 during clinical studies GS-US-208-0103 and GS-US-208-0104
- Analyze the relationship between the selection of resistance mutations with the antiviral response to GS-9256 in patients
- Determine whether these mutations alter in vitro antiviral susceptibility to GS-9256 and evaluate the cross-resistance profile of these mutations
- Determine the persistence of resistance mutations following termination of treatment

Methods

- The complete NS3 protease domain from plasma of HCV subjects in GS-US-208-0103 and GS-US-208-0104 were PCR amplified and sequenced
  - The correlation of mutations to antiviral response was assessed
- The NS3 protease domains from baseline and treatment samples were cloned into a sub-genomic NS3 1b-con-1 replicon shuttle vector
- Replicons containing NS3 protease domains from clinical isolates were transiently transfected into Huh7-lunet cells and assayed for luciferase activity to determine:
  - Susceptibility to GS-9256
  - Cross-resistance to a panel of anti-HCV compounds

Figure 2. Cloning and Phenotyping Patient HCV NS3 Protease



Results

Figure 3. Antiviral Response to GS-9256 Single (A) and Multiple (B) Ascending Doses

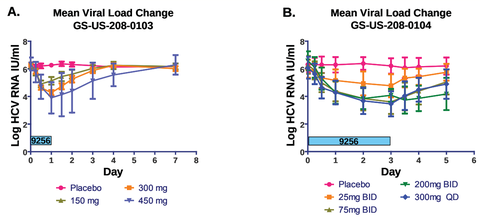
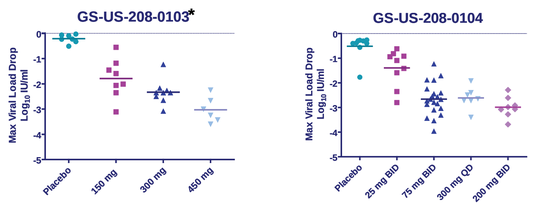


Figure 4. Maximum Viral Load Reduction by Dose Group



*One subject excluded from analysis due to 14% D168E detected at baseline

Table 1. GS-US-208-0103: Single Dose Resistance Mutation Detection

	GS-9256 Dose (number of subjects)			
Mutation	**Placebo (n=7)**	**150 mg (n=8)**	**300 mg (n=9)**	**450 mg (n=8)**
R155R/K	-	-	-	2
D168D/E	-	-	-	1
D168D/V	-	-	-	1
Total (%)	-	-	-	4 (50%)

Figure 5. GS-US-208-0103: Viral Load and NS3 Sequencing in Subjects with Resistance Mutations Detected (GS-9256: 450 mg single dose)

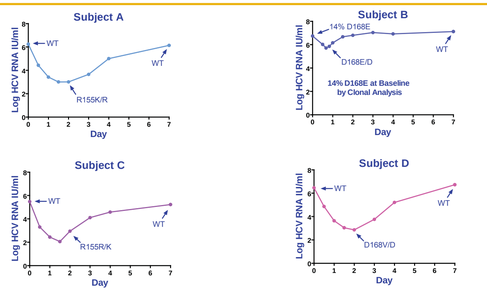


Table 2. GS-US-208-0104: Multiple Dose Resistance Mutation Detection

	GS-9256 Dose (number of subjects)				
Mutation	**Placebo n=9**	**25 mg BID n=9**	**75 mg BID n=21**	**200 mg BID n=8**	**300 mg QD n=7**
R155R/K	-	-	3	1	2
R155K	-	-	-	4	-
A156A/V	-	-	1	-	-
D168D/E/V	-	-	-	-	1
D168D/V	-	-	-	1	-
D168G/V	-	-	1	-	-
D168V	-	-	1	-	-
D168G	-	-	-	1	-
R155K/R, D168D/V	-	-	1	-	-
R155K/R, D168D/G	-	-	1	1	-
A156A/V, D168D/N	-	-	1	-	-
Total (%)	-	-	9 (43%)	8 (100%)	3 (43%)

Figure 6. GS-US-208-0104: Viral Load and NS3 Sequencing from Four Subjects

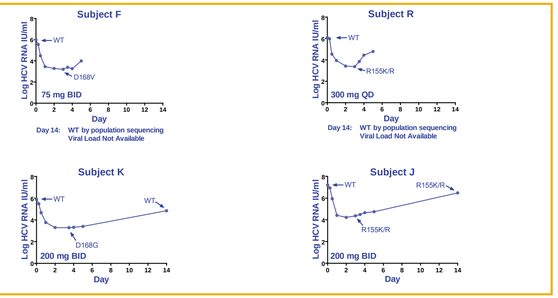
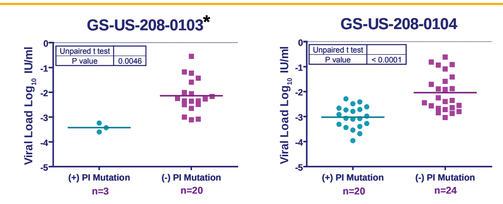


Figure 7. Maximum Viral Load Reduction Among Subjects with and without PI Resistance Mutations Detected



*One subject excluded from analysis due to 14% D168E detected at baseline

Table 3. Phenotypic Analysis Summary for GS-US-208-0103

Subject	GT	Dose (mg)	Time	NS3 AA Change from Baseline	GS-9256 EC_{50} Fold Change from Baseline
A	1a	450	Day 3	R155K/R*	70
B	1a	450	Day 2	D168E/D	46
C	1a	450	Day 3	R155R/K^	2.2
D	1b	450	Day 3	D168V/D	>252

* The majority of this mixed population of virus at position 155 is mutated to K
^ The majority of this mixed population of virus at position 155 is wild-type R

Table 4. Cross Resistance Analysis Summary for GS-US-208-0103

	EC_{50} Fold Change from Baseline*		
	Subject A	**Subject B**	**Subject D**
	R155K/R	D168E/D	D168V/D
GS-9256	70	46	>252
GS-9451	27	38	>252
ITMN-191	9.2	52	>252
SCH-7	2.8	0.5	0.5
VX-950	1.6	3.0	0.5
GS-9190	0.6	2.4	0.9
IFN-α	0.7	2.9	1.2
RBV	0.9	1.3	1.0

*Mixtures of mutant and wild-type within the clinical sample may limit cross-resistance detection

Table 5. Phenotypic Analysis Summary for GS-US-208-0104

Subject	GT	Dose (mg)	Time	NS3 AA Change from Baseline	GS-9256 EC_{50} Fold Change from Baseline
E	1b	75 BID	Day 4	D168G/V	>201
F	1b	75 BID	Day 4	D168V	>670
G	1b	75 BID	Day 4	A156A/V	0.98*
H	1a	200 BID	Day 4	R155K/R	>264
I	1a	200 BID	Day 4	R155K/R, D168D/G	>164
J	1a	200 BID	Day 4	R155K/R	>278
L	1a	200 BID	Day 14	R155K	>354
M	1a	200 BID	Day 4	R155K/R	>125
N	1a	200 BID	Day 14	R155K	>268
O	1b	200 BID	Day 14	D168D/V	>859
P	1a	300 QD	Day 4	R155K/R	>849
Q	1b	300 QD	Day 14	D168D/E/V	>122

* Low replication capacity

Table 6. Cross Resistance Analysis Summary for GS-US-208-0104

	EC_{50} Fold Change from Corresponding Baseline Sample*							
	E	**F**	**Q**	**O**	**L**	**H**	**J**	**I**
	D168G/V	**D168V**	**D168D/E/V**	**D168D/V**	**R155K**	**R155K/R**	**R155K/R**	**R155KR, D168D/G**
GS-9256	>201	>670	>122	>859	>354	>264	>278	>164
GS-9451	>2049	>670	>460	>3106	>1524	>1149	>1344	>825
ITMN-191	2.0	21.7	2.2	0.4	144.7	129	436	288
TMC-435350	>1409	>474	2.7	0.5	>3267	473	>6411	>715
VX-950	0.3	1.2	1.1	1.5	19.5	5.7	0.9	7.8
GS-9190 (NS5B)	0.9	0.8	0.2	0.8	2.7	1.8	3.4	4.6
IFN-α	0.7	0.2	0.3	0.4	2.2	0.9	0.6	1.7
RBV	0.3	0.8	0.2	0.2	2.1	2.0	0.7	0.1

*Mixtures of mutant and wild-type within the clinical sample may limit cross-resistance detection

Table 7. Frequency of Resistance Mutations by Genotype Subtype (GS-US-208-0103 and GS-US-208-0104 Combined)

	Number of Subjects with a Mutation at Position(s)				
GT	**R155**	**D168**	**A156**	**R155 + D168**	**D168 +A156**
1a	12	1	0	3	0
1b	0	6	1	0	1

- An NS3 protease resistance mutation was detected by population sequencing in 24/70 GS-9256 treated subjects from both trials
  - R155 mutations were only identified in GT1a isolates
  - D168 mutations were more frequent in GT1b isolates than GT1a
  - A156 mutations were only detected in GT1b

Summary

- Subjects with a PI mutation detected had an average maximal viral load reduction of >3 log_{10} IU/mL whereas those without averaged 1 log_{10} IU/mL less maximal viral load reduction
- Maximum viral load reductions were observed at 200 mg BID
  - Subject B in GS-US-208-0103 had ~14% D168E at baseline accounting for the 1 log_{10}IU/mL maximal viral load reduction compared to the>3 log_{10}IU/mL average reduction for the remainder of the cohort
- Phenotypic analysis of clinical isolates with mutations at positions R155 or D168 showed reduced susceptibility to GS-9256 and some other PIs
  - R155K showed the broadest cross-resistance among PIs
- GS-9256 resistance mutations are fully susceptible to other HCV inhibitor classes, including GS-9190 (NS5B), interferon-α, and ribavirin

Conclusions

- **PI resistant mutant viruses likely pre-exist at low levels among the HCV quasispecies and were readily detected after potent inhibition of wild-type viruses by GS-9256**
- **The lack of cross-resistance between GS-9256 and GS-9190, as well as IFN and RBV supports the combination of GS-9256 with these HCV inhibitors**

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