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Antiviral Response and Resistance Analysis of Treatment-Naïve HCV Infected Subjects Receiving Single and Multiple Doses of GS-9190

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Background

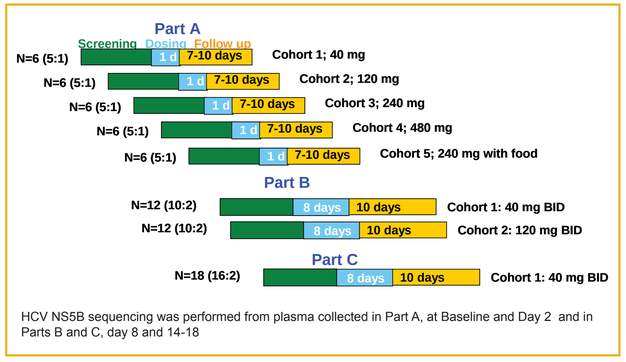
- GS-9190 is a novel non-nucleoside HCV NS5B polymerase inhibitor in Phase 2 studies
- *In vitro* resistance to GS-9190 includes the C316Y, C445F, Y448H and Y452H HCV NS5B mutants
- *In vitro*, mutations associated with NS3 protease inhibitors, nucleoside/non-nucleoside NS5B inhibitors remain fully susceptible to GS-9190
- GS-US-196-0101 was a Phase 1, randomized, double-blind, placebo controlled dose-escalation study in which treatment naïve HCV genotype 1 subjects received either single or multiple doses of GS-9190

Objectives

- Evaluate the antiviral response among HCV treatment naïve genotype 1-infected subjects receiving single or multiple doses of GS-9190 in Study GS-US-196-0101
- Identify resistance mutations in subjects receiving GS-9190
- Determine whether these mutations alter in vitro antiviral susceptibility to GS-9190 and to evaluate the cross-resistance profile of these mutations
- Determine the persistence of resistance mutations following termination of treatment

Methods

Figure 1. GS-196-0101 Study Design



HCV NS5B sequencing was performed from plasma collected in Part A, at Baseline and Day 2 and in Parts B and C, day 8 and 14-18

HCV RNA Quantitation

- Plasma HCV-RNA levels were measured using the Roche COBAS TaqMan assay (Roche Molecular System Inc., Branchburg, NJ)

Population Sequencing

- The NS5B polymerase was amplified patient plasma by RT-PCR and then sequenced. Through sequence alignments, amino acid changes between visits were identified for:
  - Part A, Baseline and Day 2
  - Parts B and C, Baseline and Day 8 or Day 14,follow up time points if available

Allele-Specific Real Time PCR

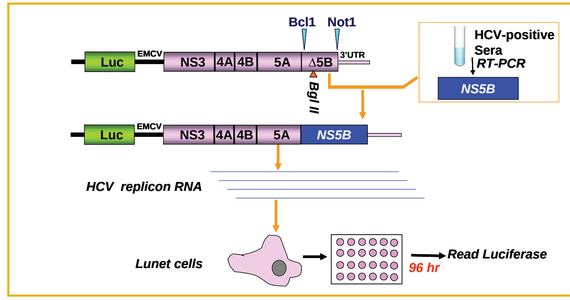
- For more sensitive detection of the Y448H quasispecies an AS-PCR assay using the MultiCode RTx technology was developed with Y448H detection limit of 0.5%
- Refer to poster 1897 "Sensitive Detection of Y448H NS5B Mutant Viruses in Patients Infected with Genotype 1a and 1b HCV" for assay conditions and procedures

Phenotype Assay (Figure 2)

- NS5B polymerase from baseline and on-treatment samples were cloned into an NS5B sub-genomic 1b-con-1 replicon shuttle vector
- Replicons containing NS5B polymerase from clinical isolates were transiently transfected into Huh7-lunet cells and assayed for luciferase activity to determine:
  - Susceptibility to GS-9190
  - Cross-resistance to a panel of anti-HCV compounds

Methods (cont'd)

Figure 2. Cloning and Phenotyping Patient HCV NS5B Polymerase

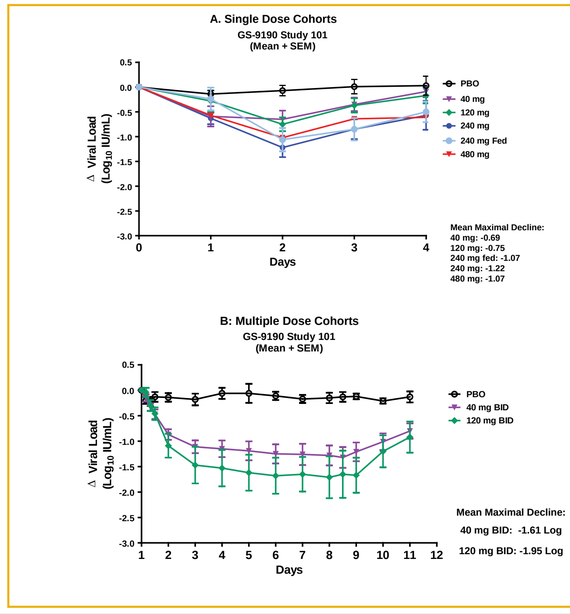


Results

Table 1. Baseline Patient Characteristics- Cohorts A,B,C

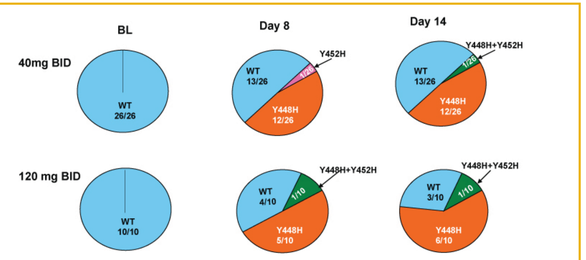
	Single Dose						Multiple Dose		
	40 mg	120 mg	240 mg	240 mg (fed)	480 mg	Placebo	40 mg	120 mg	Placebo
N	5	6	5	5	5	5	26	11	8
Mean Age, Years	35.8	47.0	36.2	45.8	49.2	47.4	44.6	45.5	42.9
Sex (M/F)	2/3	4/2	4/1	4/1	4/1	2/3	20/6	8/3	7/1
Caucasian	4	4	5	4	3	4	16	10	7
African AM	1	0	0	0	2	1	7	1	1
Native Am	1	1	0	1	0	0	0	0	0
Other	0	1	0	0	0	1	3	0	0
Weight (kg)	76.1	82.4	84.1	81.7	81.9	71.7	79.0	73.5	78.0
BMI (kg/m2)	26.8	27.5	26.8	27.6	26.7	24.2	26.5	25.4	25.7
HCV GT 1a/1b	4/1	5/1	4/1	4/1	3/1	4/1	14/12	8/3	3/5
Median HCV Viral load, log_{10} IU/mL	6.87	6.97	6.50	6.48	6.40	5.85	6.48	6.54	6.39

Figure 3. Antiviral Response in Single and Multiple Dose Cohorts



Results (cont'd)

Figure 4. Resistance Identified by Population Sequencing



No resistance mutations were observed in any subjects at Baseline in the single and multiple dose cohorts; No mutations were observed at Day 2 of the Single Dose cohorts

Table 2. Detection of Low Level Y448H by AS-PCR in the Single Dose Cohort

Subjects Receiving Single Dose GS-9190 (n)	(n >0.5% Y448H) at Baseline	(n >0.5% Y448H) at Day 2
25	2/25 (8%)	5/24 (21%)

Subjects with >0.5% Y448H Detected at Baseline and/ or Day 2

Subject ID	Genotype	GS-9190 Dose	% of Y448H Baseline*	% of Y448H Day 2*
A	1a	40 mg, fasting	no RNA	0.8%
B	1a	40 mg, fasting	1.0%	1.3%
C	1a	120 mg, fasting	< 0.5%	2.0%
D	1a	240 mg, fasting	3.0%	1.4%
E	1a	480 mg, fasting	< 0.5%	2.8%

*By population sequencing, the mutant Y448H was not detected at Baseline and Day 2

Table 3. Summary of % Y448H Detected by AS-PCR in Multiple Dose Cohorts

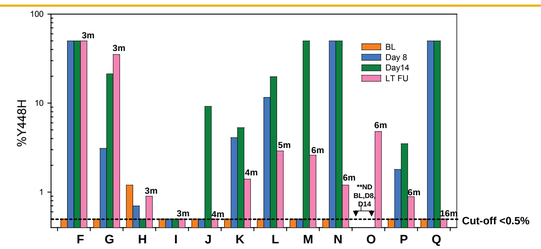
A. % Y448H detected in subjects (n=15) with wild-type sequence by population sequencing at Days 8 or 14

Multiple Dose Cohort	40 mg BID	120 mg BID
Number of subjects by Cohort/ (Failed AS-PCR)	12 / (0)	3 / (0)
Number with % Y448H >0.5%	11/12 (92%)	3 (100%)
Level of %Y448H detected by cohort (Range)	1.3 - 9.7%	2.0 - 3.0%

B. % Y448H detected in subjects (n=21) with Y448H by population sequencing at Days 8 or 14

Multiple Dose Cohort	40 mg BID	120 mg BID
Number of subjects by Cohort/ (Failed AS-PCR)	14 / (1)	7 / (1)
Number with % Y448H >0.5%	13 (100%)	6 (100%)
Level of %Y448H detected by cohort (Range)	2.7 - >50%	7.7- 50%

Figure 5. %Y448H Through Long-Term Follow Up (3-16 months) Detected by AS-PCR in Subjects Who Developed Y448H in the Multiple Dose Cohort*



*21/36 subjects receiving multiple dosing developed Y448H or H/Y by population sequencing; Only 12 subjects had follow up visits for Y448H persistence monitoring
**AS-PCR failed for BL, Day 8 and Day 14

Table 4. Summary of Phenotypic Susceptibility of Patient Isolates* from GS-9190 Recipients in Parts B and C of GS-US-196-0101

Number of Subjects Tested / Total	Amino Acid Change at Day 8 from Baseline in NS5B	Mean (range) GS-9190 EC_{50} Fold Change from Baseline
6/9	No change	1.12 ± 0.53 (0.33–1.79)
4/4	Mutations at polymorphic sites	1.83 ± 0.95 (0.65–2.75)
10/17	Y448H/Y mixture with/without mutations at polymorphic residues	1.48 ± 0.93 (0.42–3.3)
1/1	Y452H/Y mixture with mutations at polymorphic residues	1.95
1/2	Y448H with mutations at polymorphic residues	27.1**
1/1	Y448H + Y452H/Y	78.5**

*HCV NS5B were amplified from patients and cloned into the NS5B shuttle vector. EC_{50} values were determined using a transient replication assay
**EC_{50} change from baseline >4-fold

Table 5. Susceptibility of GS-9190 Resistant Isolates to Other HCV Inhibitors

Compound	1b-Con1 Wild-Type EC_{50} (nM)	40mg bid Subject >3log_{10} decline in HCV RNA Y448H at Day 8 Day 8 Fold Change from Baseline	120 mg bid Subject >3log_{10} decline in HCV RNA Y448H at Day 6 Day 6 Fold Change from Baseline
GS-9190	1.65	27.1*	78.5*
IFN α (U/mL)	1.05	0.9	1.1
RBV	17023	1.1	1.2
VX-950	307.3	0.9	1.6
SCH-503034	151.6	0.6	1.1
R1626	10654	1.0	1.0

*EC_{50} change from baseline >4-fold

Results Summary

- Single and multiple doses of GS-9190 resulted in mean maximal HCV RNA reductions ranging from -1.22 and -1.95
- No NS5B polymerase mutations were observed by population sequencing in the single dose cohorts
- Sequence analysis showed the Y448H NS5B polymerase mutation in 58% of subjects in the multidose cohorts
- Y448H+Y452H was only observed in 1b subjects (2/36)
- In subjects receiving GS-9190, AS-PCR detected pre-existing Y448H at baseline in 8% of subjects. At the end of dosing, low levels were detected in 21% of subjects in the single dose cohort and 92% of subjects in the multidose cohorts
- Levels of Y448H declined after therapy was stopped suggesting impaired replication of this mutant
- Phenotypic analysis of clinical isolates with the Y448H alone or in combination with Y452H showed reduced susceptibility to GS-9190
- Clinical isolates with GS-9190 resistance mutations remained sensitive to Interferon-α, Ribavirin as well as HCV NS3 protease and NS5B polymerase inhibitors

Conclusions

- **Significant reductions in HCV RNA were observed in genotype 1 HCV-infected subjects receiving single or multiple doses of GS-9190**
- **HCV resistant variants are pre-existing and can be detected when wild-type virus is inhibited by treatment with GS-9190**
- **The lack of cross-resistance of GS-9190-resistant isolates to VX-950, SCH-503034, IFN and RBV supports the use of GS-9190 in combination with these anti-HCV agents.**