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Safety, Pharmacokinetics, and Antiviral Activity of Single Oral Doses of the HCV NS3 Protease Inhibitor GS-9256

R Goldwater[1], M DeMicco[2], J Zong[3], G Chittick[3], G Yuen[3], S West[3], J Kagel[3], A Bae[3], H Mo[3], D Oldach[3], W Delaney[3], and J Findlay[3]

[1]PAREXEL International, Baltimore, MD, [2]Advanced Clinical Research Institute, Anaheim, CA, [3]Gilead Sciences, Foster City, CA.

Gilead Sciences, Inc.
333 Lakeside Drive
Foster City, CA 94404
Tel: (650)574-3000
Fax: (650)578-9264

Introduction

- GS-9256 is a potent and selective non-covalent HCV NS3 protease inhibitor
  - Genotype 1b K_i = 0.090 nM, replicon EC_{50} = 20 nM
  - Selectivity vs mammalian proteases of 1,000 to 28,500-fold
- *In vitro*, GS-9256 has additive antiviral activity with IFN-α, ribavirin, and certain NS5B inhibitors
- *In vitro* DMPK profile
  - No significant metabolism by CYP450 enzymes (for all tested CYP450s, GS-9256 metabolism was only 1-2% of controls)
  - Did not inhibit major CYP450 enzymes up to 25 µM
  - May be a substrate for efflux transporters (Caco-2)
  - A moderate inhibitor of P-gp and BSEP and a weak inhibitor of MRP1; not an inhibitor of MRP2 at clinically relevant concentrations

Objectives

- To evaluate the safety and tolerability of single doses of GS-9256 in healthy volunteers and genotype 1 (GT-1) HCV-infected subjects
- To characterize the plasma pharmacokinetics of GS-9256 after single doses in healthy volunteers and GT-1 HCV-infected subjects
- To evaluate the antiviral activity and characterize the HCV resistance of GS-9256 following single doses in GT-1 HCV-infected subjects

Methods

- **Study 1:** Phase 1a, randomized, double-blind, placebo controlled trial in healthy volunteers
  - Single ascending dose design
    - 150 mg, 300 mg, 600 mg or placebo under fasting conditions
    - N = 7/dose group (6:1, GS-9256:placebo)
  - Outcome measures
    - Safety & tolerability
    - Plasma pharmacokinetics
- **Study 2:** Phase 1b, randomized, double-blind, placebo controlled trial in GT-1 HCV-infected subjects
  - Single-dose, parallel-group design
    - 150 mg, 300 mg, 450 mg or placebo under fasting conditions
    - N = 8/dose group (including placebo)
  - Outcome measures
    - Safety & tolerability
    - Plasma pharmacokinetics
    - Antiviral efficacy

Results

Table 1. Subject Demography and Baseline Characteristics
A. Study 1 – Healthy Volunteers

	GS-9256 Regimen			
	150 mg (n = 6)	300 mg (n = 7)	600 mg (n = 6)	Placebo (n = 3)
Mean Age (yrs)	34	25	31	25
Sex (Male/Female)	3/3	5/2	3/3	1/2
Ethnicity (Hispanic/Non-Hispanic)	0/6	0/7	2/4	0/3
White	5	6	5	2
Black	1	0	1	1
Asian	0	1	0	0
Mean Body Mass Index (kg/m²)	25	24	28	24
Mean Weight (kg)	83	75	82	72

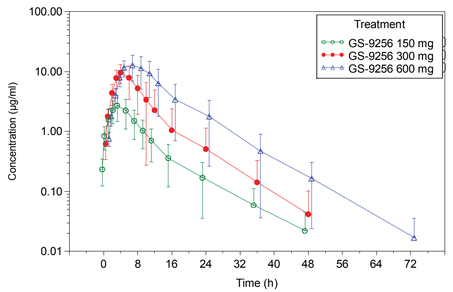
B. Study 2 – HCV-infected Subjects

	GS-9256 Regimen			
	150 mg (n = 8)	300 mg (n = 9)	450 mg (n = 8)	Placebo (n = 7)
Mean Age (yrs)	44	45	47	43
Sex (Male/Female)	5/3	7/2	7/1	6/1
Ethnicity (Hispanic/Non-Hispanic)	2/6	0/9	3/5	3/4
White	6	5	7	5
Black	2	4	1	0
other	0	0	0	2
Mean Body Mass Index (kg/m²)	27	26	26	26
Mean Weight (kg)	79	81	80	77
Median HCV RNA (log_{10} IU/mL)	6.37	6.29	6.41	6.20
HCV Genotype (1a/1b)	6/2	9/0	7/1	6/1

- **Study 1** – Healthy Volunteers
  - Single doses of GS-9256 were well-tolerated
    - No SAEs
    - Most frequently reported AEs (all mild) were diarrhea, nausea and headache
- No Grade 4 treatment emergent laboratory abnormalities; a single Grade 3 blood in urine considered secondary to menses
- Grade 1 increases in bilirubin (due to indirect bilirubin) observed in three subjects (one subject receiving 300 mg and two subjects receiving 600 mg); no ALT or AST elevations in any subjects
- **Study 2** – HCV-infected Subjects
  - Single doses of GS-9256 were well-tolerated
    - No drug-related SAEs
    - Most frequently reported AEs (mild/moderate) were nausea, diarrhea, dyspepsia, back pain, headache, and hyperhidrosis
  - Grade 3/4 treatment emergent laboratory abnormalities
    - Grade 3/4 glucose in urine (from one subject with known history of diabetes)
    - Grade 3 elevated amylase (n = 2) (not considered clinical pancreatitis)
  - No graded abnormalities in bilirubin

Figure 1. Mean (± SD) GS-9256 Plasma Concentration-Time Profile

A. Study 1 – Healthy Volunteers



B. Study 2 – HCV-infected Subjects

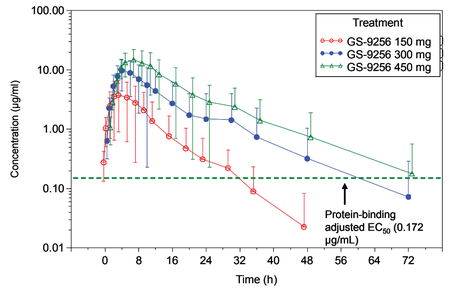


Table 2. Mean (CV%) GS-9256 Pharmacokinetic Parameters
A. Study 1 – Healthy Volunteers

GS-9256 PK Parameter	GS-9256 150 mg (n = 6)	GS-9256 300 mg (n = 6)	GS-9256 600 mg (n = 6)
C_{max} (µg/mL)	2.8 (46)	9.8 (36)	13.7 (38)
T_{max} (h)[a]	4 (3, 6)	4 (4, 6)	5 (4, 8)
$AUC_{0\text{-}inf}$ (µg•h/mL)	25.1 (50)	80.9 (68)	163.0 (54)
$T_{½}$ (h)[a]	7.7 (4.7, 9.4)	6.6 (5.0, 7.5)	7.6 (6.9, 8.3)
C_{12} (µg/mL)	0.7 (56)	2.3 (117)	6.4 (72)

a. Median (min, max)

Results (cont'd)

Table 2. Mean (CV%) GS-9256 Pharmacokinetic Parameters
B. Study 2 – HCV-infected Subjects

GS-9256 PK Parameter	GS-9256 150 mg (n = 7)	GS-9256 300 mg (n = 9)	GS-9256 450 mg (n = 7)
C_{max} (µg/mL)	3.8 (75)	10.1 (53)	15.7 (49)
T_{max} (h)[a]	4 (4, 6)	4 (4, 6)	6 (3, 10)
$AUC_{0\text{-}inf}$ (µg.h/mL)	42.7 (91)	132.8 (107)	241.4 (70)
$T_{½}$ (h)[a]	8.0 (7.0, 8.6)	8.5 (6.2, 13.0)	8.7 (6.9, 15.6)
C_{12} (µg/mL)	1.4 (109)	4.4 (125)	8.5 (74)

a. Median (min, max)

Figure 2. Median (Q1, Q3) Reduction from Baseline in HCV RNA (log_{10}IU/mL) after Single-Dose Administration of GS-9256 in HCV-infected Subjects

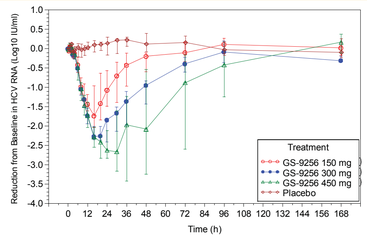
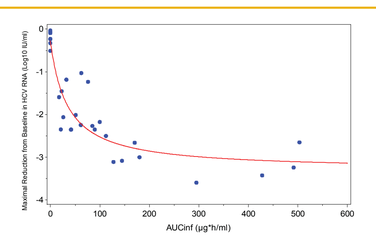


Figure 3. Exposure (AUC_{inf})-Response Relationship of Anti-HCV Activity of GS-9256 Following Single-Dose Administration



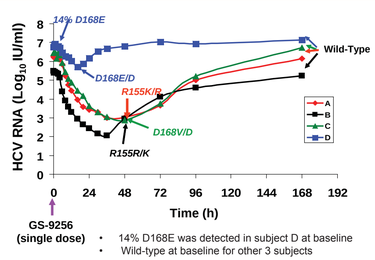
Data were analyzed by simple E_{max} model (WinNonlin Professional, v5.2): $E = E_0 + E_{max} \times AUC_{tau} \div (EC_{50} + AUC_{tau})$
Circle represents observed data and line represents predicted data.
Predicted parameters: E_{max} = -3.3 log_{10} IU/mL, E_0 = -0.3 log_{10} IU/mL, EC_{50} = 34.6 µg•h/mL, r = 0.91

Table 3. Selection of Resistance in GT-1 HCV-infected Subjects Who Received Single Doses of GS-9256

Mutation in NS3 Observed Post-Dosing	Number of positive subjects (subtype)			Fold Resistance to GS-9256
	GS-9256 150 mg (n = 8)[a]	GS-9256 300 mg (n = 9)[a]	GS-9256 450 mg (n = 8)	Site-Directed Mutants
R155R/K	0	0	2 (1a)	566
D168E/D	0	0	1 (1a)	120
D168D/V	0	0	1 (1b)	1832

a. The NS3/4A genes were successfully amplified in all patient samples at baseline, Days 2, 3 and 7; no protease resistance mutations were detected by population sequencing at any timepoint

Figure 4. Viral Load Reduction and Resistance Over Time from Subjects with Resistance Mutations Detected



- 14% D168E was detected in subject D at baseline
- Wild-type at baseline for other 3 subjects

Table 4. Susceptibility of GS-9256 Resistant Isolates to Other HCV Inhibitors

Compound (target)	Day 2 or 3 Isolate Fold Change in EC_{50} from Baseline		
	Subject A R155K/R	Subject D D168E/D	Subject C D168V/D
GS-9256 (NS3)	70	46	>252
GS-9451 (NS3)	27	38	>252
ITMN-191 (NS3)	9.2	52	>252
Telaprevir (NS3)	1.6	3.0	0.5
SCH-7 (NS3)	2.8	0.5	0.5
GS-9190 (NS5B)	0.6	2.4	0.9
IFN	0.7	2.9	1.2
RBV	0.9	1.3	1.0

Values in red > 4-fold

Table 5. Susceptibility of GS-9256 Resistance Mutations (SDM) to Other HCV Inhibitors

Compound (target)	Fold Change in EC_{50} from WT*		
	R155K	D168E	D168V
GS-9256 (NS3)	566	120	1832
GS-9451 (NS3)	>122	109	8321
TMC-435350 (NS3)	92.9	10.7	>182
Telaprevir (NS3)	7.6	0.6	0.4
GS-9190 (NS5B)	0.9	1.0	1.2

*Mutations were generated by site-directed mutagenesis
na, not available
Values in red > 4-fold

Results Summary

- Subject characteristics were generally well-balanced across regimens (Table 1)
- GS-9256 was well-tolerated, with no safety concerns
- GS-9256 plasma exposure was greater than dose proportional between 150 and 600 mg and 150 and 450 mg in healthy volunteers and HCV-infected subjects, respectively (Table 2), with mean concentration at 12-h post-dose C_{12} > 8-fold above the protein-binding adjusted EC_{50} for GT-1 for all doses tested in HCV-infected subjects (Figure 1)
- GS-9256 plasma exposures were slightly higher in HCV-infected subjects (~ 1-1.3 and 1.4-1.5-fold higher in C_{max} and $AUC_{0\text{-}inf}$, respectively) (Table 2).
- Median maximal HCV RNA declines were -1.8, -2.4 and -2.8 log_{10} IU/mL after single doses of 150, 300, and 450 mg, respectively, in GT-1 HCV-infected subjects (Figure 2)
- A strong correlation between plasma exposure of GS-9256 and HCV RNA suppression was observed following single dose administration (Figure 3)
- Consistent with the stronger antiviral activity, resistance mutations (R155K, D168E/V) were selected in 4/8 subjects who received single 450 mg doses of GS-9256, but not in any of the subjects who received lower doses; resistance mutations were no longer detectable seven days after dosing (Table 3 and Figure 4)
- GS-9256 resistant mutants remain susceptible to other classes of HCV inhibitors including GS-9190, IFN- α, and ribavirin (Tables 4 and 5)

Conclusions

- **The pharmacokinetic profile of GS-9256 following single-dose administration is similar between healthy volunteers and HCV-infected subjects with plasma concentrations well above the protein-adjusted EC_{50} values through 12 hours post-dose**
- **The potent antiviral activity observed following single-dose administration (-1.8 to -2.8 log_{10} IU/mL HCV RNA reductions) in HCV-infected subjects supports continued development of GS-9256 for the treatment of chronic HCV infection**
- **The lack of cross resistance between GS-9256 and GS-9190 supports the combination therapy with these agents in genotype 1a or 1b HCV-infected subjects**

Acknowledgements

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