FINAL CLINICAL STUDY REPORT

Study Title:	A Phase 2a, Double-blind, Randomized, Placebo-Controlled, Dose Escalation Study to Evaluate the Safety, Tolerability, Pharmacokinetics and Activity of GS-9450, a Caspase Inhibitor, in Subjects with Chronic Hepatitis C (GS-US-227-0102)
Name of Test Drug:	GS-9450
Dose and Formulation:	GS-9450 capsules of 5-, 10-, or 40-mg strength, providing daily doses of 5, 10, 40, or 80 mg.
Indication:	Liver inflammation
Sponsor:	Gilead Sciences, Inc. 4 University Place 4611 University Drive Durham, NC 27707, USA
Study No.:	GS-US-227-0102
Phase of Development:	Phase 2a
IND No.: **EudraCT No.:**	101,816 2007-001601-16
Study Start Date: **Study End Date**	12 June 2007 (First Subject Screened) 18 March 2009 (Last Subject Observation)
Principal or Coordinating Investigator:	Name: Michael P Manns (Prof) Affiliation: PPD
Gilead Responsible Medical Monitor:	Name: Ken Hirsch, MD Telephone: PPD Fax: PPD
Report Date:	02 June 2010

CONFIDENTIAL AND PROPRIETARY INFORMATION

This study was conducted in accordance with the guidelines of Good Clinical Practice, including archiving of essential documents.

STUDY SYNOPSIS
Study GS-US-227-0102:

Gilead Sciences, Inc.
4 University Place
4611 University Drive
Durham, NC 27707, USA

Title of Study: Study GS-US-227-0102: A Phase 2a, Double-blind, Randomized, Placebo-Controlled, Dose Escalation Study to Evaluate the Safety, Tolerability, Pharmacokinetics and Activity of GS-9450, a Caspase Inhibitor, in Subjects with Chronic Hepatitis C
Investigators: Multicenter (see Appendix 15.1.4 for complete list)
Study Centers: 10 study sites in the United States (6 sites), The Netherlands (1 site), and Germany (3 sites)
Publications: Manns MP, Lawitz E, Hoepelman AIM, Choi HJ, Lee JY, Cornpropst M, Liang W, King B, Hirsch KR, Oldach D, Rousseau FS. Short-term safety, tolerability, pharmacokinetics and preliminary activity of GS-9450, a selective caspase inhibitor, in patients with chronic HCV infection. In Program/Abstracts of the 45th Annual Meeting of the European Association for the Study of the Liver April 14-18, 2010, Vienna, Austria; abstract 474.
Study Period: 12 June 2007 (First Subject Screened) 18 March 2009 (Last Subject Observation)
Phase of Development: Phase 2a

Objectives:

The primary objective of this study was as follows:

- To investigate the safety and tolerability of multiple oral doses of GS-9450 in subjects with chronic hepatitis C (HCV).

The secondary objectives of this study were as follows:

- To investigate the pharmacokinetics (PK) of multiple oral doses of GS-9450 and its metabolites in subjects with chronic HCV
- To investigate the activity of multiple oral doses of GS-9450 in subjects with chronic HCV, as evidenced by: (1) change from baseline in alanine aminotransferase (ALT) levels (primary activity measure), (2) change in aspartate aminotransferase (AST) levels, and (3) change in noninvasive markers indicative of hepatic apoptosis, including cytokeratin 18 caspase-cleavage fragment [CK-18])
- To investigate the effects of GS-9450 on hepatitis C viral load.

Methodology: This pilot, phase 2a, randomized, double-blind, placebo-controlled study was designed to assess the safety, tolerability, PK, and preliminary activity of GS-9450 given in multiple, escalating doses to subjects infected with chronic HCV. Approximately 32 subjects were planned for randomization to receive once-daily GS-9450 or placebo for 14 consecutive days in one of four sequential cohorts (8 subjects in each cohort randomized 3:1 to receive GS-9450 [6 subjects] or placebo [2 subjects]):

Cohort 1: GS-9450 10 mg or placebo given daily for 14 days

Cohort 2: GS-9450 40 mg or placebo given daily for 14 days

Cohort 3: GS-9450 80 mg or placebo given daily for 14 days

Cohort 4: GS-9450 5 mg or placebo given daily for 14 days

Advancement to higher dose cohorts was dependent upon satisfactory safety and tolerability profiles of the preceding cohort as determined by Sponsor review (conducted in consultation with the Lead Investigator[s]).

The study consisted of a screening visit conducted within 45 days of the first dose, a check-in visit (on Day -1), a 14-day treatment period (beginning on Day 1 and comprising two separate sequestrations for single-dose and steady-state PK measurements), and a follow-up period (from Day 16 through at least Week 7 [Week 26 in France and Germany]). For all cohorts, subjects began their 14 days of consecutive treatment with a 3-day confinement, beginning with admission to the clinical research center on Day -1, followed by single doses of GS-9450 or placebo on Days 1 and 2 (approximately 24 hours after first dose). Subjects were thereafter discharged from the clinic, returning for outpatient visits on Days 3, 7, and 10 (where doses of GS-9450 or placebo were administered by clinic personnel), and again on Day 13 for the start of the second sequestration. GS-9450 or placebo was administered to confined subjects on Days 13 and 14 (last dose); subjects were discharged on Day 16, approximately 48 hours after the last dose. On Days 4–6, 8–9, and 11–12, subjects self-administered the cohort-directed number of study drug capsules. Outpatient follow-up visits were scheduled for all subjects on Days 17, 21, 28, 35, and once during Week 7, 5 weeks after ingesting the last dose of GS-9450 or placebo. Per protocol Amendments 1a and 1b, specific to France and Germany, respectively, additional follow-up visits were scheduled at Weeks 14 and 26; however, no subjects were enrolled in France.

For PK analyses of plasma GS-9450 and its metabolites, serial blood samples were collected beginning immediately prior to the GS-9450 dose on Day 1 and continuing at scheduled intervals for 24 hours postdose. After the last dose on Day 14, blood samples for the bioanalytical analysis were drawn from 0.5 to 72 hours postdose. Additional PK blood samples were collected prior to dosing on Days 3, 7, 10, 13, and 14.

Throughout the study, safety was monitored by adverse event (AE) reporting, and by treatment-emergent abnormalities in clinical laboratory tests (including ALT levels), vital signs, 12-lead electrocardiogram (ECG), and physical examination findings.

Number of Subjects (Planned and Analyzed):
Planned: 32 subjects
Evaluable: Pharmacokinetic, Day 1: 25
Pharmacokinetic, Day 14: 22
Safety: 33

Diagnosis and Main Criteria for Inclusion: Eligible subjects were men and nonpregnant, nonlactating women between the ages of 18 and 65 years (inclusive) with chronic HCV (any genotype, with detectable anti-HCV antibodies and HCV RNA levels at screening) previously treated with interferon (INF) or pegylated interferon (PEG) with or without RIBA who either did not achieve or maintain a sustained virologic response 6 months after cessation of INF/PEG, with or without RIBA; or did not tolerate RIBA and/or INF treatment. Eligible subjects were required to have screening ALT values ≥ 1.5 times the upper limit of the normal (ULN) laboratory range, but < 10 × ULN; a body mass index (BMI; calculated as kg/m^2) between 19 and 32 (inclusive); creatinine clearance (determined using the Cockcroft-Gault method) ≥ 70 mL/min; absolute neutrophil count ≥ $1000/mm^3$; hemoglobin ≥ 10 g/dL; and no clinical or laboratory evidence of hepatic decompensation (ie, platelets ≥ $75,000/mm^3$, total bilirubin ≤ 1.5 × ULN, prothrombin time ≤ 1.5 × ULN, and albumin ≥ 3.0 g/dL). Subjects with co-infection with hepatitis B virus (HBV) or human immunodeficiency virus (HIV), and those with pancreatitis or autoimmune disease were not eligible for study participation.

Duration of Treatment: Subjects received 14 days of GS-9450 or placebo. Subjects were followed until at least Week 7 [Week 26 in France and Germany]).

Test Product, Dose, Mode of Administration, and Batch No.:
GS-9450 capsules of 5-mg (lot number: BF0802A1) or 40-mg strength (lot number: BF0804A1), administered orally

Reference Therapy, Dose, Mode of Administration, and Batch No.:
Placebo capsules to match GS-9450 capsules (lot number: BF0805A1)

Criteria for Evaluation:

Pharmacokinetics: For each subject, the following plasma PK parameters were calculated for GS-9450 and, where applicable, its metabolites (GS-9470, GS-9471, GS-9472, and GS-9473): the area under the concentration-time curve (AUC) extrapolated to infinite time (AUC_{inf}); the AUC from time 0 to the last quantifiable time point ($AUC_{0–last}$); the percent AUC extrapolated between $AUC_{0–last}$ and AUC_{inf} ($\%AUC_{exp}$); the maximum observed plasma concentration (C_{max}); the first observed time to reach C_{max} (T_{max}), the last observed quantifiable concentration of the drug (C_{last}) and the time of C_{last} (T_{last}); the estimate of the terminal elimination half-life of the drug ($T_{1/2}$), the terminal elimination rate constant (λ_z), the apparent oral clearance of the drug (CL/F), the apparent volume of distribution of the drug (V_z/F), and the area under the plasma concentration-versus-time curve over the dosing interval (tau) after multiple-dose administration (C_{tau}).

Safety: Safety was evaluated by adverse event (AE) monitoring, and by results of clinical laboratory tests, 12-lead electrocardiogram tracings, vital signs, physical examination findings, and assessment of concomitant medications.

Activity: Activity was assessed by observed change and percent change from baseline in ALT levels at Day 14.

Statistical Methods:

Pharmacokinetics: The PK analysis set included all subjects who had evaluable GS-9450 PK profiles. Dose proportionality information was obtained by comparing plasma levels of GS-9450 across all dose levels (5, 10, 40, and 80 mg) using a general formula $y = \beta_0 \times dose^{\beta_1}$ where y represents the dependent variables $AUC_{0\text{-last}}$, AUC_{inf}, AUC_{tau}, and C_{max}. β_1 was assessed by regressing the natural log-transformed PK parameter on the natural log-transformed dose (i.e., ln[dose]). The population mean slope (β_1) for ln(dose) was estimated along with the corresponding 90% confidence interval (CI). Due to the exploratory nature of this study, no dose-proportionality boundaries were defined.

Safety: The safety analysis set included all subjects who received at least one dose of study drug. Safety data were listed for all subjects with available data and summarized for subjects in the safety analysis set. Adverse events were coded using the Medical Dictionary for Regulatory Activities (MedDRA), Version 10.

Activity: The activity analysis set included all subjects who received at least one dose of study drug and had at least one postbaseline ALT assessment. Subjects in the activity analysis set were used for activity summaries and analyses. The primary activity endpoint was the change from baseline (both observed change and percent change) in ALT levels at Day 14. The observed change was analyzed by an analysis of covariance (ANCOVA) model with treatment and baseline as fixed effects. The percent change was analyzed by an analysis of variance (ANOVA) model with treatment as a fixed effect. An assessment of overall treatment effect was made, with pair-wise treatment comparison made subsequently between active dose group and pooled placebo.

SUMMARY – RESULTS:

Subject Disposition and Demographics: Thirty-three subjects (mean age of 49 years), including 22 male and 11 female subjects, were sequentially enrolled and treated in the study: 8 subjects into Cohort 1 (6 subjects to GS-9450 10 mg, 2 subjects to placebo); 8 subjects into Cohort 2 (6 subjects to GS-9450 40 mg, 2 subjects placebo); 9 subjects into Cohort 3 (7 subjects to GS-9450 80 mg, 2 subjects to placebo); and 8 subjects into Cohort 4 (6 subjects to GS-9450 5 mg, 2 subjects to placebo). One subject who was randomized to receive placebo in Cohort 3, was administered 1 dose of study medication on Day 1, and was withdrawn from the study after having tested positive for cocaine. Thirty-two (97%) subjects completed the study.

Activity Results: Results for the primary activity endpoint, absolute change in ALT levels from baseline (last available measurement obtained prior to dosing) to Day 14, showed that each GS-9450 dose group produced median improvements in ALT values over those observed at baseline. Thereafter, off-treatment follow-up ALT values generally returned to predose levels.

While on-treatment reductions in ALT values among subjects treated with GS-9450 at the 5-mg (-28.0 U/L), 10-mg (-55.5 U/L), 40-mg (-16.5 U/L), and 80-mg (-34.5 U/L) doses were numerically greater than those observed among subjects treated with placebo (-12.0 U/L), none of the reductions associated with GS-9450 differed significantly from placebo. The greatest percent change in ALT at the completion of 14 days of treatment occurred among subjects who received GS-9450 at the 80-mg dose (-43.6%), compared with subjects who received the 5-mg (-26.9%), 10-mg (-38.1%), 40-mg (-18.9%), or placebo (-17.1%) doses. Only in the 80-mg GS-9450 group did more than 2 subjects have ALT values that fell to within the normal laboratory reference range at any point during the study (4 subjects on Days 10, 13, 14, and 15; 3 subjects on Day 16).

The overall treatment effect on ALT change from baseline at Day 14 was not statistically significant (alpha=0.05) using either the ANCOVA model for observed change (P=0.1529), or the ANOVA model for percent change from baseline (P=0.1163); results of nonparametric testing (Kruskal-Wallis test) corroborated these findings, indicating no difference among the treatments (pooled placebo and active dose groups).

	Placebo n = 8[a]	GS-9450			
		5 mg (Cohort 4) n = 6[b]	10 mg (Cohort 1) n = 6	40 mg (Cohort 2) n = 6	80 mg (Cohort 3) n = 7
Baseline[c] ALT level – U/L					
Mean (SD)	86.6 (41.0)	112.3 (44.1)	128.0 (52.4)	87.0 (54.6)	88.6 (50.4)
Median	76.0	110.5	112.0	71.0	65.0
Q1, Q3	65.5, 95.5	75.0, 138.0	99.0, 171.0	52.0, 110.0	61.0, 127.0
Day 14 ALT level – U/L					
Mean (SD)	62.1 (18.2)	81.6 (31.4)	76.8 (37.9)	69.5 (50.5)	38.7 (18.6)
Median	56.0	76.0	64.0	43.5	35.5
Q1, Q3	52.0, 78.0	72.0, 107.0	45.0, 102.0	37.0, 106.0	26.0, 36.0
Change from baseline in ALT – U/L					
Mean (SD)	-26.4 (35.9)	-29.8 (22.5)	-51.2 (18.8)	-17.5 (16.4)	-33.8 (19.0)
Median	-12.0	-28.0	-55.5	-16.5	-34.5
Q1, Q3	-42.0, -7.0	-31.0, -22.0	-68.0, -39.0	-27.0, -4.0	-52.0, -25.0
% change from baseline in ALT – U/L					
Mean (SD)	-23.2 (22.3)	-25.3 (13.4)	-40.6 (9.6)	-20.7 (20.7)	-44.2 (21.1)
Median	-17.1	-26.9	-38.1	-18.9	-43.6
Q1, Q3	-44.2, -12.5	-35.9, -22.5	-49.6, -32.5	-40.4, -3.6	-60.0, -40.9
Subjects with normal ALT at Baseline					
Number of subjects (%)	1/8 (12.5)	0/6 (0.0)	0/6 (0.0)	0/6 (0.0)	0/7 (0.0)
Subjects with normal ALT at Day 14					
Number of subjects (%)	1/7 (14.3)	1/5 (20.0)	2/6 (33.3)	1/6 (16.7)	4/6 (66.7)

ALT= alanine aminotransferase; SD=standard deviation; Q=quartile
a n=7 for placebo at Day 14
b n=5 for GS-9450 Cohort 4 at Day 14
c Last available measurement obtained prior to dosing

For AST, the largest median percent reductions from baseline to Days 3, 7, and 14 (i.e., treatment period) were generally associated with the 10 mg (-21.8, -33.5, and -35.5% U/L, respectively) and 80 mg (-27.6, -29.6, and -40.2% U/L) GS-9450 dose groups. Similarly, GS-9450 at the 80-mg dose strength produced the greatest inhibition of CK-18 fragments among all tested treatments, with median percent reductions from baseline to Days 3, 7, 10, and 14 being -27.4, -31.2, -29.8, and -31.6% U/L, respectively (for subjects in the pooled placebo group, changes ranged from -3.4 to +9.7% U/L across these time points).

Across all dose groups, the log_{10} of HCV RNA remained relatively unchanged from baseline after 14 days of treatment, regardless of dose.

Pharmacokinetic Results: Key pharmacokinetic parameters obtained following GS-9450 dosing on Days 1 and 14 are summarized in the table below.

Cohort (GS-9450 Daily Dose)	**n**	**Day**	**T_{max}** (hr) Median (min, max)	**C_{max}** (ng/mL) Mean ± SD	**AUC_{inf}**[a] (hr*ng/mL) Mean ± SD	**$T_{1/2}$** (hr) Median (min, max)
5 mg (Cohort 4)	6	1	**1.50** (1.50, 1.50)	**10.94** ± 4.306	**92.65** ± 14.771	**13.87** (7.08, 6.05)
	6	14	**2.00** (1.00, 3.00)	**12.56** ± 4.905	**100.46** ± 26.158	**20.14** (5.66, 60.05)
10 mg (Cohort 1)	6	1	**1.75** (1.00, 3.00)	**14.88** ± 5.529	**110.64** ± 30.316	**10.33** (8.63, 20.95)
	6	14	**2.00** (1.00, 3.98)	**13.77** ± 3.538	**111.87** ± 35.742	**19.54** (9.91, 34.53)
40 mg (Cohort 2)	6	1	**1.75** (0.50, 3.05)	**100.21** ± 33.652	**605.85** ± 138.852	**12.85** (8.95, 20.93)
	4	14	**1.24** (1.00, 4.00)	**89.07** ± 27.793	**529.07** ± 62.629	**22.24** (12.08, 31.65)
80 mg (Cohort 3)	7	1	**1.00** (1.00, 4.00)	**201.03** ± 112.635	**1080.38** ± 394.531	**12.15** (8.60, 22.52)
	6	14	**1.50** (0.60, 3.00)	**216.54** ± 102.150	**1111.16** ± 396.621	**20.80** (13.79, 31.91)

a: Day 14 values are AUC_{tau}

After administration of an oral GS-9450 dose under fasting conditions, GS-9450 C_{max} typically occurred between 1 and 2 hours (median T_{max}). Multiple-dose $T_{1/2}$ values ranged between 20-22 hours (median values). Exposures were dose-proportional over the dose range tested, and were not found to be time-dependent. There was modest GS-9450 accumulation (15-42%) after 14 days of treatment, which was generally predictable from single-dose $T_{1/2}$ and a daily dosing frequency. GS-9473 and GS-9471 were the major metabolites detected in human plasma. The time-weighted average change from baseline through Day 14 (DAVG14) in CK-18 response was linear with increasing dose and exposure; the DAVG14 in ALT response was undifferentiated by dose and exposure.

Safety Results: The safety results show that administration of GS-9450 at dose strengths ranging from 5 mg to 80 mg was generally well tolerated. While most (76%; 25/33) subjects experienced at least 1 treatment-emergent AE during the study, the frequency of AEs occurrence did not appear to be dependent on GS-9450 dose; AEs were most commonly observed in the presence of GS-9450 10 mg (6/6 subjects; 100%), GS-9450 80 mg (6/7 subjects; 86%), or placebo (6/8 subjects; 75%). The greatest number of AEs occurred in subjects treated with GS-9450 10 mg (28 events) or placebo (26 events). The most common AE was headache, experienced by 3 subjects each in the GS-9450 10-mg and placebo groups, 2 subjects each in the GS-9450 40-mg and 80-mg groups, and 1 subject in the GS-9450 5-mg group. Other commonly experienced AEs (≥ 3 subjects overall) were nausea (5 subjects); fatigue (4 subjects); affect lability, back pain, and skin laceration (3 subjects each); the events of fatigue and affect lability were only noted in the GS-9450 10-mg and placebo groups.

Of the 33 subjects who received study drug or placebo, 11 had at least 1 AE considered by the investigator to be related to treatment (ranging from 1 subject in the GS-9450 40-mg group to 3 subjects in the GS-9450 10-mg and 80-mg groups). The most commonly experienced treatment-related AE was headache (2 subjects in the GS-9450 80-mg group, one subject each in the remaining treatment groups).

Most treatment-emergent AEs were considered by the investigator to be of mild or moderate intensity. Two subjects experienced serious adverse events (SAEs): 1 subject in the GS-9450 40-mg group had acute cholecystitis (onset: Day 22), and 1 subject in the GS-9450 5-mg group had a facial bone fracture (onset: Day 171); neither SAE was considered by the investigator to be related to GS-9450. No subject died, and no subject experienced an AE that resulted in treatment discontinuation or withdrawal from the study.

One event of drug-related hypertension (systolic/diastolic blood pressures: 137/97 mmHg) was noted in the presence of GS-9450 80 mg; no other clinically important changes in laboratory values, vital signs, physical examination, or ECG findings were reported.

CONCLUSIONS:

- After administration of an oral GS-9450 dose under fasting conditions, GS-9450 C_{max} typically occurred between 1 and 2 hours (median T_{max}). Multiple-dose $T_{1/2}$ values ranged between 20–22 hours (median values). Exposures were dose-proportional over the dose range tested, and were not found to be time-dependent. There was modest GS-9450 accumulation (15-42%) after 14 days of treatment, which was generally predictable from single-dose $T_{1/2}$ and a once-daily dosing frequency. GS-9473 and GS-9471 were the major metabolites in human plasma.
- After 14 days of once-daily treatment, GS-9450 produced improvements in ALT values over those observed at baseline, most notably with GS-9450 80 mg; reductions in ALT, however, did not appear to be dependent on dose strength. The DAVG14 in ALT response was undifferentiated by dose and exposure.
- Subjects in the GS-9450 10 mg and 80 mg groups showed the greatest median percent reductions in AST from baseline to Day 14.
- Treatment with GS-9450 at doses of 40 mg and 80 mg produced rapid and marked inhibition of CK-18 during the treatment period; DAVG14 in CK-18 response was linear with increasing dose and exposure.
- The log10 of HCV RNA remained relatively unchanged from baseline after 14 days of GS-9450 treatment at any dose strength.
- Administration of GS-9450 at any dose was considered generally well tolerated in subjects with chronic HCV infection.
- GS-9450 reduces ALT levels and CK-18 fragments, most likely through a reduction of apoptosis in the liver.