ABBREVIATED CLINICAL STUDY REPORT

Study Title:	A Phase 2b, Double-Blind, Randomized, Parallel-Group, Placebo-Controlled Study to Evaluate the Safety, Tolerability, and Efficacy of GS-9450 in Adults with Chronic Hepatitis C Virus Infection
Name of Test Drug:	GS-9450
Dose and Formulation:	10- and 40-mg capsules
Indication:	Liver Inflammation
Sponsor:	Gilead Sciences, Inc. 4 University Place 4611 University Drive Durham, NC 27707
Study No.:	GS-US-227-0106
Phase of Development:	Phase 2b
IND No.:	101,816
EudraCT No.:	2008-007456-96
Study Start Date:	26 May 2009 (First Subject Screened)
Study End Date	20 July 2010 (Last Subject Observation)
Principal or Coordinating Investigator:	Name: Michael Manns, Prof. Dr. med Affiliation: [redacted]
Gilead Responsible Medical Monitor:	Name: Mani Subramanian, MD Telephone: [redacted] Fax: [redacted]
Gilead Clinical Research Lead:	Name: [redacted] Telephone: [redacted] Fax: [redacted]
Report Date:	13 September 2011

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-227-0106
Gilead Sciences, Inc.
4 University Place
4611 University Drive
Durham, NC 27707

Title of Study: Study GS-US-227-0106: A Phase 2b, Double-Blind, Randomized, Parallel-Group, Placebo-Controlled Study to Evaluate the Safety, Tolerability, and Efficacy of GS-9450 in Adults with Chronic Hepatitis C Virus Infection
Investigators: Multicenter
Study Centers: There were 66 enrolling study sites in 5 countries, including the United States (49 sites), Germany (4 sites), Poland (6 sites), Canada (5 sites), and the United Kingdom (2 sites).
Publications: Manns M, Palmer M, Flisiak R, DeJesus E, Hazan L, Liang W, et al. A Phase-2B Trial to Evaluate the Safety, Tolerability, and Efficacy of a Caspase Inhibitor, GS-9450, in Adults Failing PEG/RBV Therapy for Chronic HCV Infection. [Abstract 126]. J Hepatol 2011; 54(1):S55-56.
Study Period: 26 May 2009 (First subject screened) 20 July 2010 (Last subject observation)
Phase of Development: Phase 2b

Objectives:

The primary objective of this study was as follows:

- To compare the efficacy of 2 oral doses of GS-9450 to placebo in subjects with chronic hepatitis C virus (HCV) infection as evidenced by histologic response (≥ 2-point decrease in Knodell necroinflammatory score with no concurrent worsening in the Knodell fibrosis score) at Week 24

The secondary objectives of this study were as follows:

- To compare the safety and tolerability of 2 oral doses of GS-9450 to placebo in subjects with chronic HCV
- To compare the efficacy of 2 oral doses of GS-9450 to placebo in subjects with chronic HCV, as evidenced by:
  - change from baseline in the Knodell necroinflammatory score
  - change from pretreatment in alanine aminotransferase (ALT) levels
  - change from pretreatment in aspartate aminotransferase (AST) levels
  - change from baseline in cytokeratin-18 (CK–18) caspase-cleavage fragments
  - change from baseline in hepatic collagen content as measured by morphometry of liver biopsy specimens
  - evaluation of Ishak scoring relative to baseline
  - change from baseline in hepatic apoptosis as measured by terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) staining of liver biopsy specimens
  - change from baseline in hepatic CK–18 neoantigen expression as measured by immunohistochemical staining of liver biopsy specimens

Methodology: This was a Phase 2b, randomized, double-blind, parallel-group, placebo-controlled, multicenter study to investigate the safety, tolerability, and efficacy of 2 oral doses of GS-9450 in adults with chronic HCV infection. For the purposes of this study, chronic HCV infection was defined as having documented HCV infection (antibody or ribonucleic acid [RNA] positivity) at least 6 months prior to baseline (Day 1), with HCV viremia at screening. Eligible subjects must have previously failed pegylated interferon (PEG)/ribavirin (RBV) therapy. Subjects could also have been eligible if they were unable to tolerate or had contraindications to receiving interferon (IFN) or RBV therapy. Approximately 330 subjects (18 to 65 years of age) who met the study entry criteria, including having a Knodell necroinflammatory score ≥ 3 (as determined by local pathologist evaluation of liver biopsy specimen) anytime during the 45-day screening period and an ALT value at the screening visit that was > upper limit of the normal range (ULN) but < 10 × ULN, were planned for randomization (1:1:1) to 1 of 3 parallel treatment groups (GS-9450 10 mg, GS-9450 40 mg, and matching placebo).

Following randomization, subjects were required to return to the study center within 7 business days for a baseline visit (Day 1). At this baseline visit, study medication was dispensed and subjects entered a 26-week Treatment Phase (on-treatment period). During the Treatment Phase, subjects were to receive study drug once daily by mouth for 24 weeks and then taper-off of the study drug over the following 2 weeks by receiving study drug once every other day (every 48 hours) for 1 week, and then every 3 days (Q 72 hours) for 1 week. Following completion of the Treatment Phase (inclusive of the 2-week taper), subjects were to enter a 4-week off-treatment follow-up period.

All available safety and tolerability data was to be reviewed by an independent data monitoring committee (DMC) after 75 subjects had been randomized, and after at least 60 subjects had completed 12 weeks of treatment. The DMC met 3 times and provided recommendations as necessary to Gilead Sciences, Inc. (GSI) regarding study conduct and subject management.

In order to ensure subject safety, the study was terminated early (20 April 2010) following episodes of transaminase elevations in some cases with associated bilirubin increases suggestive of idiosyncratic hepatotoxicity or drug induced liver injury (DILI). To maximize patient safety, GSI requested that study drug dosing be discontinued immediately without a taper in any patients who were still undergoing study treatment.

Number of Subjects (Planned and Analyzed):
Planned: Approximately 330 subjects were planned for randomization into 1 of 3 treatment groups (110 subjects per treatment group)
Analyzed:
Safety analysis set: 307 subjects (103 subjects each in the GS-9450 40-mg and placebo groups; 101 subjects in the GS-9450 10-mg group)

Diagnosis and Main Criteria for Inclusion: Adult subjects, 18 to 65 years of age, with chronic hepatitis C virus (HCV) infection, who had a screening Knodell necroinflammatory score ≥ 3 based on liver biopsy evaluation (as determined by local pathologist) conducted anytime during the 45 day screening period and elevated ALT (> ULN but < 10 × ULN) at the screening visit were eligible for enrollment. For the purpose of this study, chronic HCV infection was defined as having documented HCV infection (antibody or RNA positivity) at least 6 months prior to Baseline (Day 1), with HCV viremia at screening. Eligible subjects must have previously failed PEG/RBV therapy. Subjects could also have been eligible if they were unable to tolerate or had contraindications to receiving IFN or RBV therapy. Potential subjects were required to have a body mass index (BMI) between 19 and 36 kg/m^2 (inclusive); creatinine clearance ≥ 70 mL/min; absolute neutrophil count ≥ $1000/mm^3$; hemoglobin ≥ 10 g/dL; and have had no clinical or laboratory evidence of hepatic decompensation (ie, subjects were required to have platelets ≥ $75,000/mm^3$, total bilirubin ≤ 1.5 × ULN, prothrombin time ≤ 1.5 × ULN, and albumin ≥ 3.0 g/dL). Subjects with any of the following were not eligible for study participation: decompensated liver disease; Child-Pugh Grade B or C cirrhosis; evidence of hepatocellular carcinoma (e.g., α-fetoprotein > 50 ng/mL or as indicated by recent imaging performed within 3 months prior to screening; if recent imaging had not been performed, presence of a hepatic nodule on right upper quadrant ultrasound that was consistent with a diagnosis of HCC); positive urine drug screen for cocaine or amphetamines; infection with HCV genotype 3; coinfection with hepatitis B virus (HBV) or human immunodeficiency virus (HIV); pancreatitis; recent significant infection; autoimmune disorders; any history of seizure; was an operator of public means of transportation or heavy construction machinery; solid organ transplantation; history of malignancy; current excessive alcohol ingestion, averaging > 3 drinks/day for females and > 4 drinks/day for males; or a history of or current binge drinking.

Duration of Treatment: The screening period was up to 45 days. Within 7 business days after randomization, subjects entered a 26-week treatment period, followed by a 4-week off-treatment follow-up period. Each subject's total participation in the study was planned to be up to 9 months.

Test Product, Dose, Mode of Administration, and Batch No.:

GS-9450 10-mg capsule (Lot No. BF0803A2, BF0810A1, BF0903A1)
GS-9450 40-mg capsule (Lot No. BF0804A2, BF0901A1)

Study treatments were administered by mouth once daily without regard to food.

Reference Therapy, Dose, Mode of Administration, and Batch No.:

Placebo capsule (Lot No. BF0806A2); administered by mouth once daily without regard to food

Criteria for Evaluation:

Efficacy: The primary efficacy endpoint was the histologic response (≥ 2-point decrease in Knodell necroinflammatory score, with no concurrent worsening in the Knodell fibrosis score) at Week 24.

The secondary efficacy endpoints at Week 24 were as follows:

- Change (absolute, percent) from baseline in the Knodell necroinflammatory score
- Change (absolute, percent) from pretreatment in ALT levels
- Percentage of subjects achieving normal ALT
- Change (absolute, percent) from pretreatment in AST levels
- Percentage of subjects achieving normal AST
- Change (absolute, percent) from baseline in CK-18 caspase-cleavage fragment levels
- Change from baseline in collagen content at Week 24, as measured by morphometry of liver biopsy specimens
- Evaluation of Ishak scoring relative to baseline
- Change from baseline in the percent of apoptotic cells (TUNEL-positive), as measured by TUNEL staining of liver biopsy specimens
- Change from baseline in CK-18 neoantigen expression (percent of anti-M30 monoclonal antibody positive hepatocytes), as measured by immunohistochemical staining of liver biopsy specimens

Liver biopsies (screening and on-treatment) were to be reviewed and evaluated (Knodell scoring) by a central pathologist (blinded to treatment arm and timepoint when reading the biopsies). Liver biopsies (screening and on-treatment) were also to be reviewed and evaluated for Knodell and Ishak scoring, morphometry, TUNEL staining, and immunohistochemical staining for CK-18 neoantigen expression by a central pathologist.

Pharmacokinetics: Blood samples were taken at random postdose times at each treatment phase visit for pharmacokinetic analysis. Plasma concentrations of GS-9450 were determined in plasma samples from subjects collected during study visits using a validated bioanalytical assay.

Safety: Safety was to be evaluated by assessment of clinical laboratory tests, physical examinations, vital signs measurements, and adverse events (AEs). Concomitant medication intake was also recorded.

Statistical Methods:

Efficacy: Following the early termination (20 April 2010) of the study due to unexpected safety outcomes, the protocol-specified comparative efficacy analysis was no longer deemed feasible. For the primary endpoint, there were only 87 subjects with evaluable paired biopsies for Knodell scoring at both screening and on-treatment. Biopsies were not further evaluated for the following planned secondary endpoints: Ishak scoring, morphometry, TUNEL staining, and immunohistochemical staining for CK-18 neoantigen expression. Additionally, planned plasma CK-18 cleavage fragment analyses were not performed.

No protocol-specified efficacy analyses were conducted; instead, all available data for the primary endpoint (ie, Knodell scoring) and secondary endpoints (ie, ALT, AST, and bilirubin) were listed by subject and time point. Summaries of the change from screening in the Knodell necroinflammatory score and the Knodell fibrosis score, and summaries of the number and proportion of subjects meeting the primary endpoint, standard error (SE), and 95% confidence intervals (CIs) were presented by treatment and overall for all subjects in the efficacy-evaluable analysis set and for the efficacy-evaluable subjects who completed 23 weeks of dosing by the time of the on-treatment biopsy per protocol. Listings were provided for all available ALT, AST, and bilirubin data by subject and time point for the safety analysis set. In addition, the proportion of subjects with normalized ALT by study treatment and time point were summarized.

Several ad hoc summaries and analyses were conducted. The changes from screening in the histologic response in the efficacy analysis sets, excluding subjects with potential DILI, were summarized. Changes from baseline for ALT were compared between treatment groups using a t-test for the difference in mean values and a Wilcoxon-rank sum test for the difference in median values for the safety analysis set, with and without exclusion of subjects with potential DILI. The percent of subjects with normalized ALT was compared between treatment groups using a Fisher exact test. Correlations between the change in ALT and histologic response were computed using Spearman correlation analyses. Changes from baseline for HCV RNA in subjects with treatment-emergent Grade 4 ALT elevation, Grade 3/4 ALT elevation with treatment-emergent Grade 1 or above total bilirubin elevation, or who were in the efficacy-evaluable analysis set were summarized. In addition, shifts from baseline to the highest post-treatment graded ALT were also summarized.

Pharmacokinetics: No protocol-specified pharmacokinetic analyses were conducted; rather, a by-subject listing of the GS-9450 plasma concentration data for the 1080 analyzed samples was prepared for the safety analysis set. Following early termination of the study, the remainder of the 300 samples collected were not analyzed by the bioanalytical lab and therefore results are not reported. No pharmacokinetic analysis set was defined per the protocol planned analysis since not all available pharmacokinetic samples were analyzed for plasma concentration levels and no pharmacokinetic parameter calculations were performed.

Safety: The safety analysis set included all subjects who received at least one dose of study drug. Safety data (including AEs, physical examination findings, vital signs, and laboratory data) were listed and summarized for all subjects in the safety analysis set.

Additional assessments of available laboratory results were also made to investigate possible cases of drug induced liver injury (DILI). Listings of aminotransferase (aspartate aminotransferae [AST)] and alanine aminotransferase [ALT]) elevations to > 3 x ULN, > 5 x ULN (ie, Grade 3 or 4), > 10 x ULN (ie, Grade 4), and > 20 x ULN are provided. Summaries (on-treatment, off-treatment, and overall) for treatment-emergent ALT/AST elevations, with the categories of > 3 x ULN, > 5 x ULN, > 10 x ULN, and > 20 x ULN are also provided by treatment group. Treatment-emergent ALT/AST elevations were defined as ALT/AST values that were greater than baseline. As a general screen for possible DILI, a listing and summary of the frequency of any treatment emergent Grade 3 or Grade 4 aminotransferase elevation that occurred simultaneously with any treatment emergent graded bilirubin elevation (Grade 1 or higher) is provided. In addition, a listing of possible Hy's Law cases (ie, subjects with any elevated ALT or AST of > 3x ULN and alkaline phosphatase (ALP) < 2 x ULN that were associated with an increase in bilirubin ≥ 2 x ULN) is presented.

SUMMARY – RESULTS:

Subject Disposition and Demographics: Of the 307 subjects treated in the study, 51 subjects (16.6%) completed the study as planned. A total of 256 subjects (83.4%) discontinued from the study prematurely, and the majority of these subjects were discontinued due to early termination of the study by the sponsor (219/307 subjects [71.3%]).

Subject Disposition[a]	GS-9450 10 mg (N=101) n (%)	GS-9450 40 mg (N=103) n (%)	Placebo (N=103) n (%)	Overall (N=307) n (%)
Completed Study	18 (17.8%)	17 (16.5%)	16 (15.5%)	51 (16.6%)
Discontinued Study Drug Prematurely	83 (82.2%)	86 (83.5%)	87 (84.5%)	256 (83.4%)
Study Discontinued by Sponsor	71 (70.3%)	74 (71.8%)	74 (71.8%)	219 (71.3%)
Safety, Tolerability, or Efficacy Reasons	5 (5.0%)	5 (4.9%)	3 (2.9%)	13 (4.2%)
Consent Withdrawal	2 (2.0%)	5 (4.9%)	3 (2.9%)	10 (3.3%)
Lost to Follow-up	2 (2.0%)	1 (1.0%)	4 (3.9%)	7 (2.3%)
Protocol Violation	2 (2.0%)	1 (1.0%)	1 (1.0%)	4 (1.3%)
Investigator's Discretion	1 (1.0%)	0	2 (1.9%)	3 (1.0%)

a Safety analysis set includes all randomized subjects who received at least 1 dose of study drug.

Overall, subjects were predominately male (70%), and the majority of subjects (80.8%) were white. At baseline, the mean age was 53 years, the mean BMI was 28.6 kg/m^2, and the mean creatinine clearance (CL_{cr}) was 100 mL/min (estimated by Cockcroft-Gault). Across the treatment groups, mean ALT ranged from 106 to 108 IU/L and mean AST ranged from 84 to 88 IU/L at study baseline.

Efficacy Results: The primary efficacy endpoint was histologic response defined as a ≥ 2-point decrease in Knodell necroinflammatory score with no concurrent worsening in the Knodell fibrosis score at Week 24. Per protocol, the on-treatment biopsy was to be performed anytime within 1 week prior to the protocol-designated Week 24 visit day relative to the baseline (Day 1) visit (ie, anytime 23 to 24 weeks after the first dose of study medication). Overall, 25% of subjects who had completed 23 weeks of dosing prior to the on-treatment biopsy achieved a histologic response. A higher percentage of subjects in the placebo group (10/26 subjects [38%]) achieved a histologic response after 23 weeks of dosing compared with subjects in the GS-9450 treatment groups (5/25 subjects [20%] and 4/25 subjects [16%] in the 10- and 40-mg GS-9450 dose groups, respectively). Of note, none of the subjects with potential DILI completed the on-treatment biopsy and therefore these subjects were not included in the analysis of histologic response.

Across all of the treatment groups, median ALT levels ranged from 85 to 88 IU/L at baseline. Alanine aminotransferase levels decreased over the first 4 weeks of treatment in both of the GS-9450 treatment groups (the median change from baseline was –27.0 IU/L at Week 4 in both the 10- and 40-mg groups). The reduction in ALT from baseline in the GS-9450 10-mg treatment group was generally maintained over Weeks 4 to 24 (median change from baseline of –31.5 IU/L at Week 24). In the GS-9450 40-mg group, ALT levels generally increased from Weeks 4 to 12 (median change from baseline of –7.0 IU/L at Week 12), and then decreased again by Week 16 (median change from baseline of –17.0 IU/L at Week 16). In the placebo group, ALT levels showed little change through Week 16 (median change from baseline of –1.0 IU/L at Week 16), and a slight decrease from Week 16 to Week 24 of the on-treatment period (median change from baseline of –9.0 IU/L at Week 24). Ad hoc analyses of the safety analysis set, with and without exclusion of subjects with potential DILI, confirmed that subjects receiving GS-9450 10 mg had a statistically ($p \leq 0.004$) greater reduction from baseline in the median ALT level compared to placebo-treated subjects at all on-treatment study visits up through Week 24; however, the GS-9450 40-mg treatment group did not maintain a statistically significant reduction in median ALT compared to placebo over the on-treatment period.

In the GS-9450 treatment groups (10 and 40 mg), median ALT levels increased towards baseline after cessation of treatment. The rebound in ALT levels is similar to what has been observed for other investigational caspase inhibitors (Pockros PJ, et al., Hepatology 2007;46 (2):324-9; Shiffman ML, et al., Aliment Pharmacol Ther 2010;31 (9):969-78). Across all of the study groups, median ALT levels ranged from 65.0 to 96.0 IU/L at the Week 30 visit and from 81.5 to 95.5 IU/L at the postdose Week 4 visit. For subjects who completed the study and underwent the Week 30 visit (off-treatment), the median change from baseline for ALT was –22.0 IU/L in the GS-9450 10-mg group, 2.0 IU/L in the GS-9450 40-mg group, and –8.0 IU/L in the placebo group. For subjects who underwent the off-treatment postdose Week 4 visit, the median change from baseline for ALT was 1.5 IU/L in the GS-9450 10-mg group, 0.5 IU/L in the GS-9450 40-mg group, and –5.0 IU/L in the placebo group.

At Week 24, 6/29 subjects (21%) in the GS-9450 10-mg group, 11/28 subjects (39%) in the 40-mg group, and 4/27 subjects (15%) in the placebo group had normalized ALT (all

Efficacy Results (continued):

subjects had abnormal baseline ALT). In an ad hoc analysis, the percentage of subjects in the GS-9450 treatment groups (10 and 40 mg) with normalized ALT was significantly greater ($p \leq 0.013$; Fisher exact test) compared to the placebo group through 12 weeks of treatment. At Week 24, the percent of subjects with normalized ALT was greater for the GS-9450 groups (21% and 39% for the 10- and 40-mg groups, respectively) than for placebo (15%), but the differences were not statistically significant. Following the end of study treatment, the percentage of subjects with normalized ALT values decreased in the GS-9450 10- and 40-mg treatment groups.

In ad hoc Spearman correlation analyses, there was little to no association found between the change in ALT and the change in the total Knodell inflammatory score at the time of on-treatment biopsy in any of the treatment groups or overall. Similarly, little to no association was found in partial correlation analyses, adjusting for stratification by median overall baseline ALT and median overall baseline total Knodell inflammatory score.

A similar trend was observed for AST. Median AST levels ranged from 66.0 to 73.0 IU/L at baseline. Aspartate aminotransferase levels declined by Week 4 of therapy with GS-9450 (median change of −14.0 IU/L for the 10 mg group and −16.0 IU/L for the 40 mg group), but not with placebo (median change of +1.0 IU/L). The reduction in AST from baseline in the GS-9450 10-mg treatment group was generally maintained over Weeks 4 to 24 (median change from baseline of −21.5 IU/L at Week 24). In the GS-9450 40-mg group, a smaller reduction in median AST levels was observed over Weeks 12 to 24 (median change in AST from baseline of −7.0 IU/L at Week 12 and −8.5 IU/L at Weeks 24). In the placebo group, AST levels showed little change over the on-treatment period.

In the GS-9450 treatment groups, AST levels trended towards baseline levels after treatment was stopped. Across all of the study groups, median AST levels ranged from 56.0 to 73.0 IU/L at the Week 30 visit and from 65.5 to 74.5 IU/L at the postdose Week 4 visit. For subjects who completed the study and underwent the Week 30 visit (off-treatment), the median change from baseline for AST was −13.5 IU/L in the GS-9450 10-mg group, 2.0 IU/L in the GS-9450 40-mg group, and −8.5 IU/L in the placebo group. For subjects who underwent the off-treatment postdose Week 4 visit, the median change from baseline for AST was 0 IU/L in the GS-9450 10-mg group, −1.0 IU/L in the GS-9450 40-mg group, and −3.0 IU/L in the placebo group.

Overall, changes in $\log_{10}$ HCV RNA levels were minimal after 23 weeks of treatment in all treatment groups (median change in $\log_{10}$ HCV RNA at Week 24: 0.12, 0.28, and 0.14 IU/mL in the GS-9450 10-mg, 40-mg, and placebo groups, respectively [safety analysis set]).

Pharmacokinetic Results: A listing of the plasma concentrations for GS-9450 in 1080 analyzed samples is provided for the safety set by subject and sample collection time; no further analysis was conducted. The remaining 300 samples were not analyzed by the bioanalytical lab and are not reported.

Safety Results: A total of 307 subjects were randomized and treated in the study. Overall, the mean exposure to study drug was 124.7 ± 47.04 days (range: 1 to 197 days). The majority of subjects (85.3% [262/307 subjects]) received study drug for at least 71 to 98 days (Week 12). However, due to the early termination of the study by Gilead, only 49.8% of subjects received study drug through Week 20. A total of 105 subjects (34.2%) received 155 to 175 days (Week 24) of study treatment (including 35, 37, and 33 subjects in the GS-9450 10-mg, 40-mg, and placebo groups, respectively).

Treatment-emergent AEs were defined as any AE that began on or after the date of first dose of study drug up to and including the date of last dose (on-treatment period) or after the date of last dose up to and including the last follow-up visit date (off-treatment follow-up period). Overall, there was a slightly higher incidence of AEs in the GS-9450 treatment groups compared with the placebo group. The overall incidence of treatment-emergent AEs was 80.2% and 81.6% in the GS-9450 10- and 40-mg groups, respectively, and was 70.9% for the placebo group. The frequency of AEs that were considered by the investigator to be related to study drug was similar in all 3 treatment groups (39.6% in the GS-9450 10-mg group, 33.0% in the GS-9450 40-mg group, and 35.9% in the placebo group).

The frequency of treatment-emergent AEs during the on-treatment period was 77.2% for the GS-9450 10-mg group, 80.6% for the GS-9450 40-mg group, and 67.0% for the placebo group. The most common AEs (occurring in ≥ 5% of subjects overall) during the on-treatment period were nausea, diarrhea, fatigue, upper respiratory tract infection, arthralgia, back pain, and headache. The frequency of AEs during study treatment did not appear to be dependent on GS-9450 dose. Most treatment-emergent AEs that occurred during the on-treatment period were considered by the investigator to be of mild or moderate intensity. Seventeen subjects had severe (Grade 3) AEs during the on-treatment period, including 6 subjects in the GS-9450 10-mg group, 4 subjects in the GS-9450 40-mg group, and 7 subjects in the placebo group. The majority of these severe AEs (across all treatment groups) were considered by the investigator to be related to study drug. Two severe AEs (subcutaneous abscess and facial bones fracture) were considered by the investigator to be SAEs. Both of these events were considered to be unrelated to study drug. While on-treatment, one subject had an AE of acute myocardial infarction that was assessed as Grade 4 (life threatening); this event was considered by the investigator to be serious and unrelated to the study drug.

The frequency of treatment-emergent AEs during the follow-up off-treatment period was 13.9% for the GS-9450 10-mg group, 20.4% for the GS-9450 40-mg group, and 15.5% for the placebo group. The most common AEs during the off-treatment period were nasopharyngitis, ALT increased, and AST increased. Most treatment-emergent AEs that occurred during the off-treatment period were also considered by the investigator to be of mild or moderate intensity. Four subjects had severe (Grade 3) AEs during the off-treatment period, including 2 subjects in the GS-9450 10-mg group (liver injury; increased ALT and AST), 1 subject in the GS-9450 40-mg group (blood bilirubin increased, ALT increased, and AST increased), and 1 subject in the placebo group (myocardial ischemia). These events were considered to be related to study drug, except for the event of myocardial ischemia. Two subjects had AEs that were assessed as Grade 4 (life threatening) while off-treatment.

Safety Results (continued):

PPD (GS-9450 40 mg) discontinued prematurely from the study on Day 29 due to an AE of complete AV block, which was considered resolved on the same day. PPD (GS-9450 10 mg) discontinued prematurely from the study on Day 127 due to the early termination of the study by the sponsor, and had a MI on study Day 147, which was considered resolved on Day 155. Both of these events were considered by the investigator to be serious and unrelated to the study drug.

The overall incidence of treatment-emergent AEs considered by the investigator to be related to study drug was 39.6% for the GS-9450 10-mg group, 33.0% for the GS-9450 40-mg group, and 35.9% for the placebo group. The most common study-drug related AEs (occurring in ≥ 2% subjects overall) were nausea, diarrhea, fatigue, ALT increased, arthralgia, myalgia, headache, dizziness, and pruritis. In general, the frequency of treatment-related AEs did not appear to be dependent on GS-9450 dose.

There were a total of 11 treatment-emergent SAEs reported during the study. Seven SAEs occurred during the on-treatment period, including events of pneumonia, chest pain (2 events), acute MI, subcutaneous abscess, facial bones fracture, and erysipelas. None of these events were considered by the investigator to be related to study drug. The event of acute MI (PPD [GS-9450 10 mg]) led to discontinuation from the study. Four SAEs occurred during the off-treatment period, including events of myocardial ischemia, AV block complete, MI, and liver injury. These events were considered by the investigator to be unrelated to study drug or study procedure, except for the event of liver injury. PPD (GS-9450 10 mg) had a SAE of liver injury which was considered to be related to study drug and led to discontinuation from the study. The event of AV block complete (PPD [GS-9450 40 mg]) led to discontinuation from the study.

Overall, there were 12 subjects (3.9%) who experienced an AE leading to permanent discontinuation of study drug. Of these 12 subjects, 9 subjects permanently discontinued study drug due to AEs considered by the investigator to be related to study drug. In the GS-9450 10-mg group, study-drug related AEs leading to discontinuation of study drug included rash generalized, lipase increased, depression, and liver injury. In the GS-9450 40-mg group, study-drug related AEs leading to discontinuation of study drug included LFT abnormal, liver disorder, and hepatitis. In the placebo group, study-drug related AEs leading to discontinuation of study drug included hepatic enzyme increased and LFT abnormal. None of these events were considered to be a SAE, except for the event of liver injury.

The majority of graded laboratory abnormalities that occurred during the on- and off-treatment periods were Grade 1 or 2. The most common (≥ 5% of subjects overall) Grade 3/4 laboratory abnormalities observed were for ALT (11.1%), AST (8.5%), and GGT (5.2%). Treatment-emergent Grade 3 or 4 laboratory abnormalities that occurred in more than one subject during the on-treatment period included elevated ALT, elevated AST, elevated GGT, hyperglycemia, elevated serum amylase, and elevated serum lipase. Treatment-emergent Grade 3 or 4 laboratory abnormalities that occurred in more than one subject during the off-treatment period included elevated ALT, elevated AST, elevated GGT, hyperglycemia, elevated serum amylase, elevated serum lipase, and elevated total bilirubin.

Safety Results (continued):

Off-treatment, there were more Grade 3/4 transaminase elevations in the GS-9450 10- and 40-mg dose groups compared with the placebo group.

Overall, more subjects experienced a marked laboratory abnormality (defined as at least a 3-grade increase on or after the date of the first dose of study drug) in the GS-9450 40-mg group (12.6%) compared to the GS-9450 10-mg (4.0%) and placebo (4.9%) groups. The most frequent marked laboratory abnormalities were observed in AST and ALT values.

Additional assessments were also made to investigate possible cases of DILI. As a general screen for possible DILI, subjects who had a treatment-emergent Grade 3 or Grade 4 aminotransferase elevation that occurred simultaneously with any treatment-emergent graded bilirubin elevation (Grade 1 or higher) were evaluated. Overall, 7 subjects had a treatment-emergent Grade 3 or 4 ALT or AST elevation associated with a treatment-emergent Grade 1 or higher total bilirubin elevation during the study. Approximately one-half of these subjects (4/7) received placebo during the study. Two subjects in the GS-9450 40-mg group and one subject in the GS-9450 10-mg group met these criteria. These 3 GS-9450-treated subjects also met the criteria for Hy's Law (discussed below). However, there was a quantitative difference in the apparent severity of injury between the GS-9450 and placebo subjects. Among the GS-9450-treated subjects ([redacted PPD]), peak ALT changes from baseline were 7-, 8-, and 26-fold baseline and peak total serum bilirubin values were 6.8, 4.0, and 11.0 mg/dL, respectively. Among the placebo-treated subjects ([redacted PPD]), peak ALT changes from baseline were 1.9-, 1.4-, 1.1-, and 2.8-fold baseline and peak total serum bilirubin values were 3.1, 2.4 (in a subject with baseline total bilirubin of 2.2 mg/dL), 1.5, and 1.5 mg/dL, respectively.

Possible Hy's Law cases (ie, any elevated ALT or AST of > 3 x ULN, ALP < 2 x ULN, and increased bilirubin ≥ 2 x ULN), which are predictive of possible DILI, were also examined. A total of 5 subjects (1 subject in the GS-9450 10-mg group and 2 subjects each in the GS-9450 40-mg and placebo groups) had laboratory tests results from at least one study visit (on- or off-treatment) that met the Hy's Law criteria. The same observation (as made in the discussion of possible DILI cases above) regarding the qualitative difference in clinical laboratory abnormalities between GS-9450 and placebo subjects can be made for this group of possible Hy's Law cases.

Overall, mean changes from baseline values for pulse rate, systolic and diastolic blood pressure, respiration rate, and temperature were generally minimal across all treatment groups. Vital sign-related findings that were reported as treatment-emergent AEs included tachycardia, bradycardia, and hypertension. All of these events were considered to be of mild or moderate severity, and none of these events were SAEs. One pregnancy was reported during the study. [redacted PPD] (placebo) discontinued prematurely from the study on Day 7 due to a positive pregnancy test; however, subsequent testing failed to confirm pregnancy and it was the conclusion of the investigator that the original test was a false positive. There were no deaths or study-drug overdoses reported during the study.

CONCLUSIONS: From the overall results of this study, the following conclusions were made:

- A higher percentage of subjects in the placebo group (38%) achieved a histologic response (defined as a ≥ 2-point decrease in Knodell necroinflammatory score, with no concurrent worsening in the Knodell fibrosis score) after 23 weeks of dosing compared with subjects in the GS-9450 treatment groups (20% and 16% in the 10- and 40-mg GS-9450 dose groups, respectively).

- GS-9450 was associated with transient improvement in ALT values. Alanine aminotransferase and AST levels transiently decreased over the first 4 weeks of treatment in both of the GS-9450 treatment groups (10 and 40 mg). Reductions in ALT and AST from baseline in the GS-9450 10-mg treatment group were generally maintained over Weeks 4 to 24 (median changes from baseline of −31.5 IU/L and −21.5 IU/L at Week 24, respectively). In the GS-9450 40-mg group, ALT and AST levels were more variable over Weeks 4 to 24, with median changes from baseline of −16.5 IU/L and −8.5 IU/L at Week 24, respectively. In the placebo group, ALT levels showed a slight decrease from Week 16 to Week 24 of the on-treatment period (median change from baseline of −9.0 IU/L at Week 24) and AST levels showed little change over the on-treatment period.

- Improvements in ALT did not correlate with improved Knodell necroinflammation score, Knodell fibrosis score, or with improvement in the composite primary endpoint.

- The most common study-drug related AEs (occurring in ≥ 2% subjects overall) were nausea, diarrhea, fatigue, ALT increased, arthralgia, myalgia, headache, dizziness, and pruritis. In general, the frequency of treatment-related AEs did not appear to be dependent on GS-9450 dose.

- Seven subjects had a treatment-emergent Grade 3 or 4 ALT or AST elevation associated with a treatment-emergent Grade 1 or higher total bilirubin elevation during the study. Approximately half of these subjects (4/7) received placebo during the study. Two subjects in the GS-9450 40-mg group and one subject in the GS-9450 10-mg group met these criteria, and also met Hy's Law criteria. However, the severity of liver injury appeared to be greater in subjects who received GS-9450 compared with subjects who were treated with placebo.

- There were 5 possible cases of Hy's Law (ie, any elevated ALT or AST of > 3 x ULN, ALP < 2x ULN, and increased bilirubin ≥ 2 x ULN) during the study, including 1 subject in the GS-9450 10-mg group and 2 subjects each in the GS-9450 40-mg and placebo groups who had laboratory tests results from at least one study visit that met these criteria. Due to these possible cases of DILI, the study was terminated early to ensure subject safety.