FINAL CLINICAL STUDY REPORT

Study Title:	A Phase 2, Randomized, Double-Blind, Parallel-Group, Placebo-Controlled Study to Investigate the Safety, Tolerability, Pharmacokinetics, and Activity of GS-9450 in Adults with Non-Alcoholic Steatohepatitis (NASH)
Name of Test Drug:	GS-9450
Dose and Formulation:	1-, 5-, 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-228-0101
Phase of Development:	Phase 2
IND No.:	101,816
EudraCT No.:	2008-002361-31
Study Start Date:	21 August 2008 (First Subject Screened)
Study End Date	05 August 2009 (Last Subject Observation)
Principal or Coordinating Investigator:	Name: Pr Vlad Ratziu Affiliation: [redacted] [redacted] PPD
Gilead Responsible Medical Monitor:	Name: Elsa Mondou, MD Telephone: [redacted] PPD Fax: [redacted] PPD
Report Date:	17 June 2010

STUDY SYNOPSIS
Study GS-US-228-0101:
Gilead Sciences, Inc.
4 University Place
4611 University Drive
Durham, NC 27707
USA

Title of Study: Study GS-US-228-0101: A Phase 2, Randomized, Double-Blind, Parallel-Group, Placebo-Controlled Study to Investigate the Safety, Tolerability, Pharmacokinetics, and Activity of GS-9450 in Adults with Non-Alcoholic Steatohepatitis (NASH)
Investigators: Multicenter
Study Centers: There were a total of 41 study sites, including 35 sites in the United States and 6 sites in France.
Publications: Ratziu V, Chojkier M, Sheikh MY, Sanyal AJ, Lim JK, Conjeevaram H, et al. Safety, tolerability, and preliminary activity of GS-9450, a selective caspase inhibitor, in patients with non-alcoholic steatohepatitis (NASH) [Oral Presentation]. 45th Annual Meeting of the European Association for the Study of the Liver (EASL) 2010 April 14–18; Vienna, Austria.
Study Period: 21 August 2008 (First subject screened) 05 August 2009 (Last subject observation)
Phase of Development: Phase 2

Objectives:

This is a first proof-of-concept study conducted with GS-9450 administered to subjects with NASH.

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 NASH

The secondary objectives of this study were as follows:

- To investigate the pharmacokinetics of multiple oral doses of GS-9450 and its metabolites in subjects with NASH
- To investigate the activity of multiple oral doses of GS-9450 in subjects with NASH, as evidenced by: (1) change from baseline in cytokeratin-18 (CK–18) fragments, (2) change from baseline in alanine aminotransferase (ALT), and (3) change from baseline in other noninvasive biomarkers (including metabolic markers)

Methodology: This was a randomized, double-blind, parallel-group, placebo-controlled, multicenter study investigating the safety, tolerability, pharmacokinetics, and activity of multiple oral doses of GS-9450 in adults with NASH. Approximately 110 subjects, 18–75 years of age, with elevated ALT (> 60 U/L at screening), fatty liver on screening ultrasound, and biopsy-proven NASH were planned for randomization (1:1:1:1:1) to one of five parallel treatment groups (to obtain 22 subjects per treatment group) as follows: GS-9450 1 mg, 5 mg, 10 mg, 40 mg, or matching placebo administered by mouth once daily.

Qualifying subjects were stratified by the presence/absence of type 2 diabetes (i.e., on/off oral diabetic medication at study entry) and by geographic region.

Following randomization, subjects were required to return to the study site within five business days for a baseline visit, at which time they were dispensed study medication and entered a 4-week treatment period. Upon completion of the treatment period, subjects entered a 4-week off-treatment follow-up period. Each subject's participation in the study lasted up to approximately 12 weeks (inclusive of the screening, treatment, and off-treatment follow-up periods).

All available safety and tolerability data were reviewed by an independent Data Monitoring Committee (DMC) after 55 subjects (half of the total enrollment) had been randomized and treated and after 55 subjects had completed the study, including both the 4-week on-treatment and 4-week follow-up off-treatment periods. Continued enrollment was dependent upon satisfactory safety and tolerability profiles as determined by the DMC review.

Number of Subjects (Planned and Analyzed):
Planned: 110 subjects (22 subjects [to allow for 20 evaluable subjects] each in the GS-9450 1-mg, 5-mg, 10-mg, 40-mg, and matching placebo treatment groups)
Analyzed:

- Safety and activity analysis sets: 124 subjects (26 subjects each in the GS-9450 1- and 5-mg groups; 24 subjects in the GS-9450 10-mg group; 25 subjects in the GS-9450 40-mg group; and 23 subjects in the matching placebo group)
- Pharmacokinetic analysis set: 97 subjects (25 subjects in the GS-9450 1-mg group; 26 subjects in the GS-9450 5-mg group; and 23 subjects each in the GS-9450 10- and 40-mg groups)

Diagnosis and Main Criteria for Inclusion: Adult subjects (18–75 years of age) with ALT > 60 U/L at screening, fatty liver on screening ultrasound, and biopsy-confirmed NASH without cirrhosis were eligible for this study. A liver biopsy was required during screening if there was no prior biopsy ≤ 12 months old. Subjects were required to have a platelet count ≥ 75,000/mm^3, adequate hematologic function (absolute neutrophil count ≥ 1500/mm^3, hemoglobin ≥ 11.0 g/dL), and calculated creatinine clearance ≥ 70 mL/min. Subjects with noninsulin-dependent diabetes for < 10 years were eligible if stably managed for at least 6 months prior to screening. Subjects who had received glitazone treatment within 6 months of screening, or had diabetic peripheral neuropathy or gastroparesis were not eligible. Subjects were required to have had a stable weight (no weight loss > 4%) for 8 weeks prior to screening, and to have been willing to maintain consistent diet, food intake, and physical exercise during the study. Subjects with other forms of liver disease (including, but not limited to, alcoholic liver disease; viral or autoimmune hepatitis; α-1-antitrypsin deficiency; hemochromatosis; Wilson's disease; primary biliary cirrhosis; primary sclerosing cholangitis; prior exposure to organic solvents such as carbon tetrachloride; drug-induced liver disease; or other metabolic, hereditary, or infectious liver disease) were excluded from the study. Subjects with serological evidence of coinfection with hepatitis B virus (HBV), hepatitis C virus (HCV), or human immunodeficiency virus (HIV); or with evidence of hepatocellular carcinoma were excluded from the study.

Duration of Treatment: 28 days (total study duration was up to 12 weeks, including screening [maximum of 30 days], a 4-week treatment period, and a 4-week follow-up period)

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

GS-9450 1-mg capsule (Lot No. BF0807A1, BF0801A1)
GS-9450 5-mg capsule (Lot No. BF0802A1)
GS-9450 10-mg capsule (Lot No. BF0803A1)
GS-9450 40-mg capsule (Lot No. BF0804A1)

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. BF0805A1); administered by mouth once daily without regard to food

Criteria for Evaluation:

Activity: The primary activity endpoints were the absolute and percent changes from baseline in CK–18 fragments, ALT levels, and aspartate aminotransferase (AST) levels at Week 4.

The secondary activity endpoints were:

- Absolute and percent changes from baseline in CK–18 fragments, ALT levels, and AST levels at time points other than Week 4
- Change from baseline in noninvasive markers (C-peptide, free fatty acids, adiponectin, IL-6, TNFα, FibroTest, high-sensitivity C-reactive protein [CRP])
- Change from baseline for tests of immune function (absolute CD4 lymphocyte count, CD4/CD8 ratio)
- Change from baseline in body weight and body mass index (BMI)
- Change from baseline in serum lipid profile
- Percent of subjects with normal or normalized ALT
- Percent of subjects with normal or normalized AST

Pharmacokinetics: Steady-state pharmacokinetic parameters (C_{max}, T_{max}, C_{tau}, AUC_{tau}, λ_z, and $T_{1/2}$ of GS-9450 and its metabolites; and CL/F and V_z/F of GS-9450 only) were evaluated following multiple doses of GS-9450 (during Weeks 2 to 4). Trough concentrations were determined from samples collected during Weeks 1, 2, 3, and 4 of the treatment period of the study, and 1 week after the last dose of study drug (at the follow-up Week 1 visit).

Safety: Safety was evaluated by assessment of clinical laboratory tests, physical examinations, vital signs measurements, 12-lead electrocardiograms (ECGs), and AEs. Concomitant medication intake was also recorded.

Statistical Methods:

Activity: The primary analysis set for activity analyses included all randomized subjects who received at least one dose of study drug. For the primary activity endpoint analyses, absolute and percent changes from baseline in CK-18 fragments, ALT levels, and AST levels at Week 4 were assessed by an analysis of covariance (ANCOVA) model adjusting for baseline values. For pair-wise treatment comparison involving placebo, GS-9450 40 mg once daily was compared first. The GS-9450 10 mg, 5 mg, and 1 mg once daily pair-wise comparisons to placebo were then considered sequentially to minimize the familywise Type I error. P-values and 95% confidence intervals (CIs) around the treatment group differences were calculated.

Observed values (baseline and postdose) and changes (absolute and percent) from baseline for ALT and AST at Day 3, Weeks 1 to 4 of the treatment period, and Weeks 1 to 4 of the

off-treatment (follow-up) period were summarized by treatment group. Observed values (baseline and postdose) and changes (absolute and percent) from baseline for CK–18 fragments, noninvasive markers, tests of immune function, body weight, BMI, and serum lipids at Weeks 2 and 4 of the treatment period and at 4 weeks following the last dose of study drug (Week 4 of the off-treatment period) were summarized by treatment group. Summary statistics for these endpoints included the following: n, mean, median, minimum, maximum, Q1, Q3, and standard deviation (SD). No formal statistical testing was performed for the secondary activity endpoints. The baseline and postdose observed values for all activity endpoints are provided in listings by subject.

The percentage of subjects with normal ALT or with normalized ALT (for those with abnormal baseline ALT), and the percentage of subjects with normal AST or with normalized AST at baseline and each postdose time point were summarized by treatment group.

As an exploratory analysis, the linear relationship between the difference between time-weighted average post baseline and baseline (DAVG) and percent measurable change from baseline for ALT at Weeks 2, 3, and 4 versus dose and AUC_{tau} at Week 4 was evaluated; as well as the linear relationship between DAVG for CK-18 fragments at Week 4 versus dose and AUC_{tau} at Week 4.

Figures of the observed values, absolute changes from baseline, and percent changes from baseline for ALT, AST, and CK–18 fragments by individual subject are provided for each active dose group and placebo. Summary figures of median (Q1, Q3); median (Q1, Q3) change from baseline; and median (Q1, Q3) percent change from baseline for ALT, AST, and CK–18 fragments are provided by treatment group.

There was no imputation of missing data for any of the activity endpoints.

Pharmacokinetics: The pharmacokinetic analysis set included all subjects who had evaluable pharmacokinetic profiles. Concentrations of GS-9450 and its metabolites were determined in plasma using validated bioanalytical assays. Steady-state plasma pharmacokinetic parameters of GS-9450 and its metabolites assessed during Weeks 2 to 4 included: C_{max}, T_{max}, C_{tau}, λ_z, $T_{1/2}$, and AUC_{tau}. In addition, V_z/F and CL/F were assessed for GS-9450 only. Trough concentrations were determined from samples collected during the treatment-period Weeks 1, 2, 3, and 4 visits, as well as at the off-treatment follow-up Week 1 visit. Plasma concentration-time data for each subject were analyzed using standard noncompartmental methods to compute pharmacokinetic parameters. Descriptive statistics (n, mean, SD, % coefficient of variation [CV], minimum, median, maximum, Q1, and Q3) and number of subjects with values below the limit of quantitation (BLQ) were calculated for the concentration from each sampling time. For pharmacokinetic parameters, the geometric mean, geometric mean 95% CI, and the mean and SD of the natural log-transformed values were provided in addition to the above-mentioned nine descriptive statistics.

Dose proportionality information was obtained by comparing plasma pharmacokinetic parameters of GS-9450 across all dose levels. The primary method for evaluation of dose proportionality was based on AUC_{tau} and C_{max} using a power model that was fitted using all fasted doses. The power model used a general equation $y = \beta_0 \times dose^{\beta_1}$ where y represents the dependent variables (e.g., AUC_{tau} and C_{max}). The exponent β_1 in the model was assessed

by regressing the natural log-transformed pharmacokinetic 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% CI.

Safety: The safety analysis set included all randomized subjects who received at least one dose of study drug. Treatment-emergent AEs were coded using the Medical Dictionary for Regulatory Activities (MedDRA® version 11). A treatment-emergent AE was 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 of study drug (on-treatment period) or began after the date of last dose of study drug up to and including the last follow-up date (off-treatment follow-up period). Safety data from the on- and off-treatment study periods were analyzed separately, and combined (overall). Safety data were summarized by incidence (frequencies) of treatment-emergent AEs and laboratory abnormalities or descriptive statistical summaries (n, mean, SD, median, Q1, Q3, minimum, and maximum). Adverse events were also summarized by relationship to study drug and severity (for the on-treatment and off-treatment study periods and overall). Treatment-emergent SAEs and any AEs that caused permanent discontinuation from the study were summarized by treatment group, and are provided in listings.

Continuous laboratory data were summarized (n, mean, SD, median, Q1, Q3, minimum, and maximum) for the observed values and the absolute and percent changes from baseline (the last available measurement prior to dosing) by treatment and study visit. Categorical laboratory data were summarized by the number and percentage of subjects with the given response for each treatment and study visit. The incidence of treatment-emergent laboratory abnormalities, defined as values that increased at least one toxicity grade from baseline at any time up to and including the date of last dose of study drug (on-treatment period) or during the off-treatment follow-up period (after the date of last dose of study drug up to and including the last follow-up date), were summarized by treatment group. If baseline was missing, then it was treated as normal and any graded post-dose abnormality (i.e., at least Grade 1) was considered treatment emergent. Laboratory data from on- and off-treatment study periods were analyzed separately and combined (overall).

Individual data for 12-lead ECG, vital signs measurements, and physical examination findings are listed by subject and summarized by incidence (frequencies) of events/abnormalities or descriptive statistical summaries (n, mean, SD, median, Q1, Q3, minimum, and maximum), as appropriate.

SUMMARY – RESULTS:

Subject Disposition and Demographics: A total of 124 subjects (26 subjects each in the GS-9450 1- and 5-mg groups; 24 subjects in the GS-9450 10-mg group; 25 subjects in the GS-9450 40-mg group; and 23 subjects in the matching placebo group) were randomized and treated in the study; 119 subjects completed the study as planned. Five subjects discontinued the study early, including 2 subjects (1 subject each in the GS-9450 5- and 10-mg groups) who were lost to follow-up; 2 subjects (1 subject each in the GS-9450 10- and 40-mg groups) who withdrew consent; and 1 subject (GS-9450 40-mg group) who discontinued due to a treatment-related AE of moderate facial flushing.

Overall, across all treatment groups, subjects were predominately male (54.2%–80.8% of subjects), and the majority of subjects were white (76.9%–100% of subjects). Across the treatment groups, mean age ranged from 42 to 49 years, mean BMI ranged from 32.9 to 34.7 kg/m^2, and mean creatinine clearance at screening ranged from 96 to 102 mL/min (estimated by Cockcroft-Gault). The majority of subjects did not have type 2 diabetes (absent type 2 diabetes in 78.3% to 88.0% of subjects across all treatment groups).

Activity Results: The primary activity endpoints were the absolute and percent change from baseline in ALT, AST, and CK-18 fragment levels at Week 4.

Across the GS-9450 treatment groups (1, 5, 10, and 40 mg), ALT levels decreased rapidly over the first week of treatment, with median changes from baseline ranging from −19.0 to −46.0 U/L. A reduction in ALT levels from baseline in the GS-9450 treatment groups was generally maintained over Weeks 2 to 4 of the on-treatment period. At Week 4, a greater reduction in ALT levels from baseline was observed in the GS-9450 treatment groups (median changes from baseline ranging from −16.0 to −38.0 U/L across the GS-9450 treatment groups [mean −47 U/L for 40-mg group]) compared with the placebo group (median change from baseline of −5.0 U/L). The greatest median percent change in ALT at Week 4 occurred among subjects who received GS-9450 40 mg (–42%). Consistent with this finding, the highest proportion of subjects achieving ALT normalization was noted in the GS-9450 40-mg group (44.0% and 34.8% at Weeks 1 and 4, respectively).

A dose response pattern for ALT was observed during study treatment. The greatest activity was observed at a GS-9450 dose of 40 mg QD based on the difference from placebo in the absolute and percent changes from baseline in ALT at Week 4 using an ANCOVA model. Linear regression of ALT levels, both the DAVG and percent measurable change (change from baseline/[baseline − 20 U/L]), versus dose and versus GS-9450 AUC_{tau} were statistically significant (significance level < 0.05) for all on-treatment weeks tested (Weeks 2, 3, and 4), with $p \leq 0.0091$ for ALT versus dose at all time points and $p \leq 0.0065$ for ALT versus GS-9450 AUC_{tau} at Weeks 2 and 4. Both the DAVG ALT and the percent measurable change in ALT at Week 4 versus dose were highly significant ($p < 0.0001$). An outlier value of 600 for percent measurable change of ALT at Week 4 was excluded from the assessments of the linear relationship between the percent measurable change in ALT at Week 4 and the GS-9450 dose or AUC_{tau}.

Alanine aminotransferase increased relatively quickly following the last dose of study

treatment. Offset of the ALT reduction produced by GS-9450 occurred within 1 week of stopping study medication and was evident at the first off-treatment follow-up visit (median changes from baseline in ALT levels ranged from −0.5 to −7.5 U/L for the GS-9450 groups at the follow-up off-treatment Week 1 visit). For the GS-9450 1-, 5-, and 10-mg groups, subsequent ALT levels (off-treatment Weeks 2, 3, and 4) were similar to baseline ALT levels. For the GS-9450 40-mg group, median ALT levels increased to modestly above baseline levels at off-treatment Weeks 2, 3, and 4. In the placebo group, ALT levels showed little change over the on- and off-treatment periods.

A decrease in AST levels was also observed during treatment, with the greatest effect observed at the 40-mg dose of GS-9450. Across the GS-9450 treatment groups (1, 5, 10, and 40 mg), AST levels decreased over the first week of treatment, with median changes from baseline ranging from −11.0 to −18.0 U/L across the GS-9450 treatment groups. However, by on-treatment Week 4, a smaller reduction in AST levels from baseline was observed in the GS-9450 treatment groups (median changes from baseline for AST ranging from −3.0 to −10.0 U/L at Week 4). The placebo group had a median change from baseline of −4.5 U/L at Week 4. The greatest median percent change in AST at Week 4 occurred among subjects who received GS-9450 40 mg (−23%). Notably the proportion of subjects with normal AST in the 40-mg GS-9450 group increased from 20.0% at baseline to 47.8% at Week 4, with a peak of 77.3% at Day 3.

A dose response pattern in AST levels was also observed during study treatment, with the greatest activity observed at a GS-9450 dose of 40 mg QD. However, the differences between each of the GS-9450 groups (40, 10, 5, and 1 mg) and placebo for the absolute and percent changes from baseline in AST at Week 4 using an ANCOVA model were not significant ($p > 0.05$ for all comparisons). Abnormal AST was not an entry criterion.

Aspartate aminotransferase increased relatively quickly following the last dose of study treatment; increasing to modestly above baseline levels in all GS-9450 treatment groups within 1 week of stopping study medication (median changes from baseline in AST levels ranged from 3.0 to 9.0 U/L for the GS-9450 groups at the follow-up off-treatment Week 1 visit). For the GS-9450 1-, 5-, and 10-mg groups, subsequent AST levels (off-treatment Weeks 2, 3, and 4) were similar to or above baseline AST levels. In the placebo group, AST levels showed a slight decrease over both the on- and off-treatment periods.

Changes observed for CK-18 fragments over the on-treatment period did not demonstrate a clear dose response pattern. Cytokeratin-18 fragment values declined in the two highest GS-9450 dose groups (10 and 40 mg) during treatment (median change from baseline of −161 U/L (33% reduction) and −143 U/L (24% reduction), respectively), compared to the GS-9450 5-mg (−18 U/L [2%]), 1-mg (−47 U/L [11%]), and placebo (−10 U/L [3%]) groups. However, the differences between each of the GS-9450 groups (40, 10, 5, and 1 mg) and placebo for the absolute and percent changes from baseline in CK-18 fragments at Week 4 using an ANCOVA model were not significant ($p > 0.05$ for all comparisons). Following the last dose of study treatment, the level of CK-18 fragments increased relatively quickly for the two highest dosing groups; increasing to above baseline levels within 1 week of stopping study medication.

This study also examined a number of supplementary biomarkers, including markers of

inflammation (high-sensitivity CRP, IL-6, and TNFα); metabolic/endocrine measures (insulin, homeostasis model of insulin resistance [HOMA-IR; nondiabetics], adiponectin, and C-peptide); the FibroTest (a noninvasive marker of hepatic fibrosis utilizing a proprietary algorithm based on total bilirubin, GGT, α_2-macroglobulin, apolipoprotein A1, and haptoglobin); and markers of immune function (CD4, CD8, and the CD4/CD8 ratio). Overall, there were no notable changes in these supplementary biomarkers in any of the treatment groups over the on- and off-treatment study periods. There were also no discernable changes in serum lipids (triglycerides, low- or high-density lipoprotein [LDL, HDL], cholesterol, and free fatty acids), body weight, or BMI during the study.

Pharmacokinetic Results: Key GS-9450 pharmacokinetic parameters obtained following a single GS-9450 dosing interval during on-treatment Weeks 2 to 4 are summarized in the table below.

Pharmacokinetic Parameter, Mean (SD)	GS-9450 1 mg (N=25)	GS-9450 5 mg (N=26)	GS-9450 10 mg (N=23)	GS-9450 40 mg (N=23)
C_{max} (ng/mL)	1.52 (0.744)	7.42 (2.598)	15.06 (5.044)	74.53 (32.130)
T_{max} (h)[a]	2.92 (0.42, 6.02)	2.00 (0.50, 6.08)	2.12 (0.92, 6.00)	2.00 (0.50, 4.00)
AUC_{tau} (ng·h/mL)	16.92 (7.969)	71.04 (19.199)	146.44 (48.983)	530.00 (168.483)
$T_{1/2}$ (h)[a, b]	14.29 (7.78, 40.26)	14.27 (4.80, 53.51)	14.15 (7.58, 41.74)	12.63 (7.34, 42.43)

[redacted] were excluded from the pharmacokinetic analysis set due to lack of steady state pharmacokinetic sampling.

a Median (minimum, maximum)

b Summary values for $T_{1/2}$ calculated for n = 24 and n = 25 for the 1 and 5 mg treatment groups, respectively

Following oral administration of a GS-9450 dose under fasting conditions, GS-9450 reached peak concentrations in approximately 2 to 3 hours (median T_{max}). Multiple-dose $T_{1/2}$ values ranged between 13 to 14 hours (median values). Exposures were generally dose-proportional over the dose range tested. GS-9473 and GS-9471 were the major metabolites in human plasma.

Exploratory pharmacokinetic/pharmacodynamic modeling exercises revealed that inhibitory E_{max} models provided best-fit of GS-9450 exposure for $DAVG_{wk4}$ in ALT and $DAVG_{wk4}$ in CK–18 fragments data obtained from the study. The $DAVG_{wk4}$ in ALT data was reasonably described by the model. However, a weaker relationship was observed for the $DAVG_{wk4}$ in CK–18 fragment data. The mean exposures produced by 10- and 40-mg doses appeared to project a therapeutic advantage above lower doses for $DAVG_{wk4}$ in ALT. For both evaluations of GS-9450 activity assessed in this study ($DAVG_{wk4}$ in ALT and CK–18 fragments), the mean exposure produced in the group receiving the highest dose (40 mg) appeared to mediate an ALT and CK-18 fragment response at least 82% of the maximal effect achievable with GS-9450.

Safety Results: 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 last follow-up visit date. The overall

incidence of treatment-emergent AEs, including both the on- and off- treatment periods, ranged from 83.3% to 88.5% in the GS-9450 treatment groups and was 65.2% for the placebo group.

The frequency of treatment-emergent AEs during the 4-week on-treatment period was 76.9% for the GS-9450 1-mg group, 84.6% for the GS-9450 5-mg group, 75.0% for the GS-9450 10-mg group, 80.0% for the GS-9450 40-mg group, and 56.5% for the placebo group. The frequency of AEs did not appear to be dependent on GS-9450 dose. The most common AEs (occurring in ≥ 6 subjects [5%] overall) during the on-treatment period were diarrhea, nausea, abdominal pain upper, abdominal pain, dyspepsia, fatigue, arthralgia, back pain, and headache. Most treatment-emergent AEs that occurred during the on-treatment period were considered by the investigator to be of mild or moderate intensity. Severe AEs that occurred during the on-treatment period included the following: abscess, type 2 diabetes mellitus, back pain, arthralgia, osteoarthritis, pain in the extremity, and carpal tunnel syndrome. All of these events were considered to be unrelated to study drug, except for the event of pain in the extremity.

The overall incidence of treatment-emergent AEs considered by the investigator to be related to study drug was 26.9% for the GS-9450 1-mg group, 38.5% for the GS-9450 5-mg group, 37.5% for the GS-9450 10-mg group, 32.0% for the GS-9450 40-mg group, and 17.4% for the placebo group. The most common study-drug related AEs (occurring in ≥ 2 subjects overall) were diarrhea, dry mouth, abdominal pain, constipation, dyspepsia, nausea, vomiting, fatigue, asthenia, decreased appetite, headache, and somnolence. The frequency of study drug-related AEs did not appear to be dependent on GS-9450 dose.

The frequency of treatment-emergent AEs during the follow-up off-treatment period was 38.5% for the GS-9450 1-mg group, 42.3% for the GS-9450 5-mg group, 41.7% for the GS-9450 10-mg group, 36.0% for the GS-9450 40-mg group, and 52.2% for the placebo group. The most common AEs (occurring in ≥ 6 subjects [5%] overall) during the off-treatment period were fatigue and headache. Most treatment-emergent AEs that occurred during the off-treatment period were also considered by the investigator to be of mild or moderate intensity. Severe AEs that occurred during the off-treatment period included the following: increased blood creatine phosphokinase, abdominal pain, pancreatitis, pancreatitis relapsing, benign prostatic hyperplasia, cystitis, and migraine (events of pancreatitis and pancreatitis relapsing were considered SAEs and are described below). None of these severe events were considered to be related to study drug.

There were no deaths reported during the study. There were no SAEs during the on-treatment period of the study; however, there were 3 SAEs reported during the off-treatment period by 2 subjects. PPD in the GS-9450 1-mg group experienced an SAE of pancreatitis on Day 47 (onset was 18 days after the last 1-mg dose of GS-9450), which resolved on Day 49. Subsequently, the subject had an SAE of relapsing pancreatitis (concurrent with a moderate AE of intestinal obstruction) on Day 51, which resolved on Day 53. These events were considered by the investigator to be unrelated to study drug. This subject had a history of multiple (~20) prior episodes of pancreatitis and a history of dilatation/stenting of the sphincter of Oddi. PPD in the GS-9450 10-mg group experienced an SAE of hypothyroidism that occurred after study completion and was reported to Gilead Sciences after the database lock. This event was reported by the investigator as related to study drug.

However, antithyroglobulin antibody levels were subsequently tested in screening and baseline samples and were found to be high.

One subject ([redacted] [GS-9450 40-mg]) discontinued from the study due to a treatment-related AE of moderate facial flushing which started on Day 1. This subject received her last dose of study drug on Day 9, and the event resolved on Day 25.

Overall, the majority of graded laboratory abnormalities that occurred during the study were Grade 1 or 2. The overall percentage of subjects who had Grade 3 or 4 laboratory abnormalities ranged from 23.1% to 36.0% across the GS-9450 treatment groups. The placebo group had the highest incidence (43.5% of subjects) of treatment-emergent Grade 3 and 4 laboratory abnormalities during the study.

The percentage of subjects with at least one treatment-emergent Grade 3 or 4 laboratory abnormality during the on-treatment period ranged from 11.5% to 16.7% across the GS-9450 treatment groups and was 34.8% in the placebo group. 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 serum glucose, elevated serum lipase, urine blood, and urine glucose. None of these Grade 3–4 laboratory abnormalities resulted in discontinuation of study drug treatment. Overall, there was no clearly evident on-treatment pattern of clinically relevant changes in laboratory assessments and no specific laboratory abnormalities produced by GS-9450.

The percentage of subjects with at least one treatment-emergent Grade 3 or 4 laboratory abnormality during the off-treatment period ranged from 19.2% to 28.0% across the GS-9450 treatment groups and was 26.1% in the placebo group. The only discernable difference between the treatment groups was a modest increase in Grade 3 ALT elevations in the GS-9450 40-mg dose group compared with the other treatment groups. Grade 3 ALT elevations were observed in 4 subjects in the GS-9450 40-mg group versus one subject each in the placebo and GS-9450 1-, 5-, and 10-mg groups. 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 serum glucose, urine blood, and urine glucose (only the latter was Grade 4). None of the subjects had total bilirubin values ≥ 2 mg/dL concurrently with treatment-emergent Grade 3–4 ALT or AST values during the on- or off-treatment periods.

Three subjects reported AEs related to hepatic laboratory parameters, including events of abnormal liver function tests, increased hepatic enzymes, and increased transaminases. All of these events were considered by the investigator to be of mild or moderate severity and unrelated to study drug. These 3 subjects had bilirubin values within normal limits at the time of these events.

Mean changes from baseline values for vital signs and ECG findings were generally minimal across all treatment groups. No subject had a physical examination finding that was considered to be clinically significant. No pregnancies were reported during the study.

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

- Following 4 weeks of once-daily treatment, a greater reduction in ALT levels from baseline was observed in the GS-9450 treatment groups compared with the placebo group. The greatest median percent change in ALT at Week 4 occurred among subjects who received GS-9450 40 mg (–42%; mean absolute change –47 U/L). A dose response pattern for ALT was observed during study treatment. Linear regression of DAVG and percent measurable change for ALT levels versus GS-9450 dose (40, 10, 5, 1, and 0 mg) and versus GS-9450 AUC_{tau} showed statistically significant linear relationships for all on-treatment weeks tested (Weeks 2, 3, and 4) and was highly significant at Week 4 ($p < 0.0001$), indicating high correlation of increasing activity with increasing GS-9450 dose/exposure.

- A decrease in AST levels was also observed during treatment, with the greatest effect observed at the 40-mg dose of GS-9450 (median percent change in AST at Week 4 of –23%). However, the differences between each of the GS-9450 groups (40, 10, 5, and 1 mg) and placebo for the absolute and percent changes from baseline in AST at Week 4 using an ANCOVA model were not significant.

- Changes observed for CK–18 fragments over the on-treatment period did not demonstrate a clear dose response pattern. Cytokeratin-18 fragment values declined in the two highest GS-9450 dose groups (10 and 40 mg) during treatment. However, the differences between each of the GS-9450 groups and placebo for the absolute and percent changes from baseline in CK–18 fragments at Week 4 using an ANCOVA model were not significant.

- Overall, there were no notable changes in the markers of inflammation, hyperinsulinemia, hepatic fibrosis (FibroTest), and immune function examined in any of the treatment groups over the on- and off-treatment study periods. There were also no discernable changes in serum lipids, body weight, or BMI in any of the treatment groups during the study.

- After administration of oral GS-9450 under fasting conditions, GS-9450 C_{max} typically occurred between 2 to 3 hours (median T_{max}). Multiple-dose $T_{1/2}$ values ranged between 13 to 14 hours (median values). Exposures were generally dose-proportional over the dose range tested. GS-9473 and GS-9471 were the major metabolites in human plasma.

- Inhibitory E_{max} models provided best-fit of GS-9450 exposure for $DAVG_{wk4}$ in ALT and $DAVG_{wk4}$ in CK-18 fragments data obtained from the study. The $DAVG_{wk4}$ in ALT data was reasonably described by the model. However, a weaker relationship was observed for the $DAVG_{wk4}$ in CK-18 fragment data. Mean exposures produced by a 40-mg dose elicited superior $DAVG_{wk4}$ in CK-18 fragment response compared to other doses; responses in $DAVG_{wk4}$ in ALT were greater in the 40-mg group; however, 10-mg doses produced a response in $DAVG_{wk4}$ ALT.

- Administration of GS-9450 at doses of 1, 5, 10, and 40 mg was considered generally well tolerated in subjects with NASH through 28 days of treatment, with no SAEs, only 1 discontinuation due to AE (moderate flushing), and fewer treatment-emergent Grade 3/4 laboratory abnormalities in the 40-mg dose group than placebo during the treatment period.