SYNOPTIC CLINICAL STUDY REPORT

Study Title:	A Double-blind, Randomized, Placebo-Controlled, Phase 1/2 Study of the Safety, Pharmacokinetics, and Antiviral Activity of GS-9131 in Antiretroviral-Naive, HIV-1 Infected Subjects
Name of Test Drug:	GS-9131
Dose and Formulation:	30-mg tablets
Indication:	Human immunodeficiency virus- type 1 (HIV-1) infection
Sponsor:	Gilead Sciences, Inc. 333 Lakeside Drive Foster City, CA 94404 United States
Study No.:	GS-US-180-0104
Phase of Development:	Phase 1/2
IND No.:	Not applicable
EudraCT No.:	2008-002045-23
Study Start Date:	06 August 2008 (First Subject Screened)
Study End Date:	28 October 2008 (Last Subject Observation)
Principal or Coordinating Investigator:	Anton Pozniak, MD [redacted]
Gilead Responsible Medical Monitor:	Name: Jeff Enejosa, MD Phone: [redacted] Fax: [redacted]
Report Date:	15 July 2009

CONFIDENTIAL AND PROPRIETARY INFORMATION

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

STUDY SYNOPSIS

Gilead Sciences, Inc.
333 Lakeside Drive
Foster City, CA 94404
United States

Title of Study: A Double-blind, Randomized, Placebo-Controlled, Phase 1/2 Study of the Safety, Pharmacokinetics, and Antiviral Activity of GS-9131 in Antiretroviral-Naive, HIV-1 Infected Subjects

Investigators: Anton Pozniak, MD

Study Centers: PPD

Publications: None

Study Period:

06 August 2008 (First subject screened)
28 October 2008 (Last subject observation)

Phase of Development: Phase 1/2

Objectives:

The primary objective of this study was as follows:

- To investigate the safety, tolerability, and antiviral activity of GS-9131 administered orally as 10 consecutive once-daily doses in antiretroviral (ARV)-naive subjects chronically infected with HIV-1

Methodology: This was a single-center, randomized, double-blind, multiple-dose, placebo-controlled study evaluating the antiviral activity, safety, and pharmacokinetics of GS-9131 in ARV-naive subjects chronically infected with HIV-1. Eligible subjects were randomized in a 2:1 ratio to receive GS-9131 (30 mg) or matching placebo for 10 consecutive days. Subjects returned for follow-up visits on Days 14 and 21.

Plasma HIV-1 ribonucleic acid (RNA) levels were assessed at screening, baseline, and on Days 1 (predose), 2, 3, 4, 7, 10, 11, 14, and 21. CD4 and CD8 counts and percentages were assessed at screening, baseline, and on Days 2, 4, 7, 11, 14, and 21.

Methodology (Continued):

HIV-1 genotyping and phenotyping were performed by Monogram Biosciences, Inc. using plasma samples collected from each subject at baseline and Day 11. For the genotypic assay, the HIV-1 reverse transcriptase (RT) gene was sequenced to include the entire protease region (amino acids 1 to 99) and amino acids 1 to 305 of the RT region. The phenotypic assay assessed viral susceptibility to all FDA-approved nucleoside reverse transcriptase inhibitors (NRTIs), GS-9131, GS-9148, and the non-nucleoside reverse transcriptase inhibitors (NNRTIs) efavirenz (EFV), etravirine (ETV), and nevirapine (NVP). Plasma samples were also collected at Day 21 for future virology and/or pharmacokinetic analyses, if needed.

Adverse events (AEs), concomitant medications, and physical examinations (complete or symptom directed) were assessed at each visit. Laboratory assessments and serum pregnancy tests were performed at screening, baseline, and on Days 2, 4, 7, 11, 14, and 21. Weight and vital signs were assessed at screening, baseline, and on Days 2, 4, 7, 11, and 21. Electrocardiograms (ECGs) were assessed at screening, baseline, and on Days 2, 11, and 21.

The pharmacokinetics of GS-9131 and its metabolites, GS-9148 and GS-9148 diphosphate (GS-9148-DP), were assessed in plasma (GS-9131 and GS-9148) and in peripheral blood mononuclear cells (PBMCs; GS-9148-DP only) from blood samples collected over a 24-hour period during Days 1 to 2 and Days 10 to 11. Samples for plasma analysis were collected at 0 (predose), 10 and 30 minutes, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12, and 24 hours postdose. Samples for PBMC analysis were collected at 0 (predose), 1, 2, 6, 12, and 24 hours postdose. In addition, trough plasma and PBMC samples were collected on Days 4, 7, 14, and 21.

Subjects were to be withdrawn from the study if they experienced a ≥ Grade 3 AE or confirmed laboratory abnormality (with the exception of asymptomatic Grade 3 or 4 cholesterol and triglyceride increases or creatine kinase elevations unrelated to study drug), or a ≥ Grade 2 rash that was considered by the investigator to be related to the study drug.

Number of Subjects (Planned and Analyzed):
Planned: 18 subjects (12 active, 6 placebo)
Analyzed: 18 subjects (12 active, 6 placebo)

Diagnosis and Main Criteria for Inclusion: Eligible subjects were ARV-naive (≤ 14 days of total ARV exposure; no ARV therapy within 90 days prior to baseline) males and females (female subjects were nonpregnant and nonlactating), 18 to 65 years of age (inclusive), with chronic HIV-1 infection (screening plasma HIV-1 RNA 10,000 to 100,000 copies/mL and CD4 cell count ≥ 200 cells/mm^3) and weight ≥ 50 kg. At screening, subjects were hepatitis B and C negative, had adequate hepatic, renal, and hematologic functions, and were in good health, as determined by the investigator.

Duration of Treatment: 10 days

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

GS-9131: One 30-mg tablet of GS-9131 (Lot 180-104/01) administered orally, once daily (in the morning), with 240 mL of water. Doses were administered at the study center in a blinded fashion to fasted subjects (i.e., no food or liquids, except water, for at least 8 hours prior to dosing).

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

Placebo: One matching placebo tablet (Lot 180-104/01) administered orally, once daily (in the morning), with 240 mL of water. Doses were administered at the study center in a blinded fashion to fasted subjects (i.e., no food or liquids, except water, for at least 8 hours prior to dosing).

Criteria for Evaluation:

Efficacy:

The primary efficacy endpoint was the maximum reduction from baseline in HIV-1 RNA ($\log_{10}$ copies/mL) on measurements taken between Days 2 to 11.

Secondary efficacy endpoints were as follows:

- Time-weighted average change in HIV-1 RNA ($\log_{10}$ copies/mL) from baseline to Day 11 ($DAVG_2$)
- Change from baseline in HIV-1 RNA by study visit
- Change from baseline in CD4 and CD8 cell counts and percentages by study visit
- Proportion of subjects who achieved HIV-1 RNA values < 50 copies/mL at any postbaseline visit
- Proportion of subjects who achieved HIV-1 RNA values < 400 copies/mL at any postbaseline visit
- Number and percentage of subjects achieving an HIV-1 RNA decrease from baseline of ≥ 1.0 $\log_{10}$ copies/mL and ≥ 1.5 $\log_{10}$ copies/mL on Days 2 to 11

Pharmacokinetics:

The following plasma pharmacokinetic parameters were calculated for GS-9131 and GS-9148: C_{max}, T_{max}, C_{last}, C_{tau}, T_{last}, λ_Z, $AUC_{0\text{-}last}$, AUC_{inf}, AUC_{tau}, $\%AUC_{exp}$, $T_{1/2}$ (V_Z/F and CL/F for GS-9131 only). See Pharmacokinetic Abbreviations for definitions of the above parameters.

PBMC concentrations of GS-9148-DP were assessed, and GS-9148-DP pharmacokinetics were explored. Accumulation indices were assessed by comparison of selected plasma GS-9131 and GS-9148 pharmacokinetic parameters (AUC and C_{max}) between Day 1 and Day 10.

Safety:

Safety criteria included AEs, clinical laboratory tests, vital signs, and ECGs.

Statistical Methods

Efficacy: Efficacy data were summarized for subjects in the randomized and treated (RAT) analysis set, which included all randomized subjects who received at least one dose of study drug. Two-sided statistical tests were performed at $\alpha = 0.05$ for efficacy parameters.

The primary efficacy endpoint, the maximum reduction from baseline in HIV-1 RNA between Days 2 to 11, was compared between the placebo and GS-9131 treatment groups using a Wilcoxon rank sum exact test.

Secondary efficacy endpoints were analyzed as follows:

- $DAVG_2$ was defined as the time-weighted average between the first postbaseline HIV-1 RNA value through the last available value up to Day 11, minus the baseline HIV-1 RNA value (baseline HIV-1 RNA was calculated as the geometric mean of baseline and Day 1 [predose] measurements). $DAVG_2$ was compared between the treatment groups using the Wilcoxon rank sum exact test.
- Change from baseline in HIV-1 RNA and change from baseline in CD4 and CD8 cell counts and percentages were compared between the treatment groups using the Wilcoxon rank sum exact test.
- Fisher's exact test was used to compare differences between the treatment groups for the proportion of subjects with HIV-1 RNA < 50 copies/mL and < 400 copies/mL at any postbaseline visit, and for the number and percentage of subjects achieving an HIV-1 RNA decrease of ≥ 1.0 $\log_{10}$ copies/mL and ≥ 1.5 $\log_{10}$ copies/mL from baseline on Days 2 to 11.

Pharmacokinetics: Single-dose and steady-state pharmacokinetics of GS-9131 and GS-9148 were evaluated for subjects in the pharmacokinetic analysis set (i.e., subjects who received at least one dose of GS-9131, had pharmacokinetic samples collected, and for whom pharmacokinetic parameters were evaluable).

Plasma pharmacokinetic parameters for GS-9131 and GS-9148 (C_{max}, T_{max}, C_{last}, C_{tau}, T_{last}, λ_Z, $AUC_{0\text{-}last}$, AUC_{inf}, AUC_{tau}, $\%AUC_{exp}$, $T_{1/2}$ [V_Z/F and CL/F for GS-9131 only]) were estimated using standard noncompartmental methods. The parameters were listed and summarized by analyte (GS-9131 and GS-9148).

Accumulation indices for GS-9131 and GS-9148 after single and multiple dosing of GS-9131 were assessed using analysis of variance methods. Plasma concentration data for GS-9131 and GS-9148 and PBMC concentration data for GS-9148-DP were listed for the GS-9131 treatment group and summarized by nominal time point. Plots of mean and median concentration-time data for each analyte were generated.

Statistical Methods (Continued):

Safety: Safety data were summarized for subjects in the RAT analysis set. Treatment-emergent AEs were coded using the Medical Dictionary for Regulatory Activities (MedDRA), version 11.0, and were summarized by treatment group. Clinical laboratory test results were summarized by treatment group and by maximum postbaseline toxicity grade. ECG assessment outcomes (normal versus abnormal) were summarized by visit and treatment group. Vital signs and body weight were summarized by visit and treatment group. All safety data, including AEs, laboratory results, ECG findings, vital signs, concomitant medications, and abnormal physical examination results were listed by subject.

SUMMARY - RESULTS:

Of 18 randomized and treated subjects (12 in GS-9131 group, 6 in placebo group), all completed 10 days of study drug treatment, all were male, 17 were white and 1 was [redacted] PPD, and the mean (SD) age in years was 35 (6.5) in the GS-9131 group and 38 (5.2) in the placebo group. Mean (SD) baseline HIV-1 RNA levels (log_{10} copies/mL) were 4.67 (0.308) in the GS-9131 group and 4.56 (0.130) in the placebo group.

Efficacy Results:

Results for the maximum reduction from baseline in HIV-1 RNA (primary efficacy endpoint), and results for selected secondary efficacy endpoints are presented in the following table.

Parameter[a]	GS-9131 (N=12)	Placebo (N=6)	p-value
Maximum reduction from BL[b] in HIV-1 RNA (log_{10} copies/mL) (Median [Q1, Q3])	-1.03 (-1.37, -0.76)	-0.10 (-0.43, -0.05)	0.001[c]
Time-weighted average change from BL[b] in HIV-1 RNA (log_{10} copies/mL) to Day 11 ($DAVG_2$) (Median [Q1, Q3])	-0.67 (-0.83, -0.51)	0.00 (-0.24, 0.23)	< 0.001[c]
HIV-1 RNA < 400 copies/mL at any postbaseline visit (% of subjects)	0	0	—
HIV-1 RNA < 50 copies/mL at any postbaseline visit (% of subjects)	0	0	—
Proportion of subjects achieving an HIV-1 RNA decrease from baseline of ≥ 1.0 log_{10} copies/mL (% of subjects)	58.3	0	0.038[d]

a HIV-1 RNA was reported using the HIV RNA PCR Taqman – CL assay.

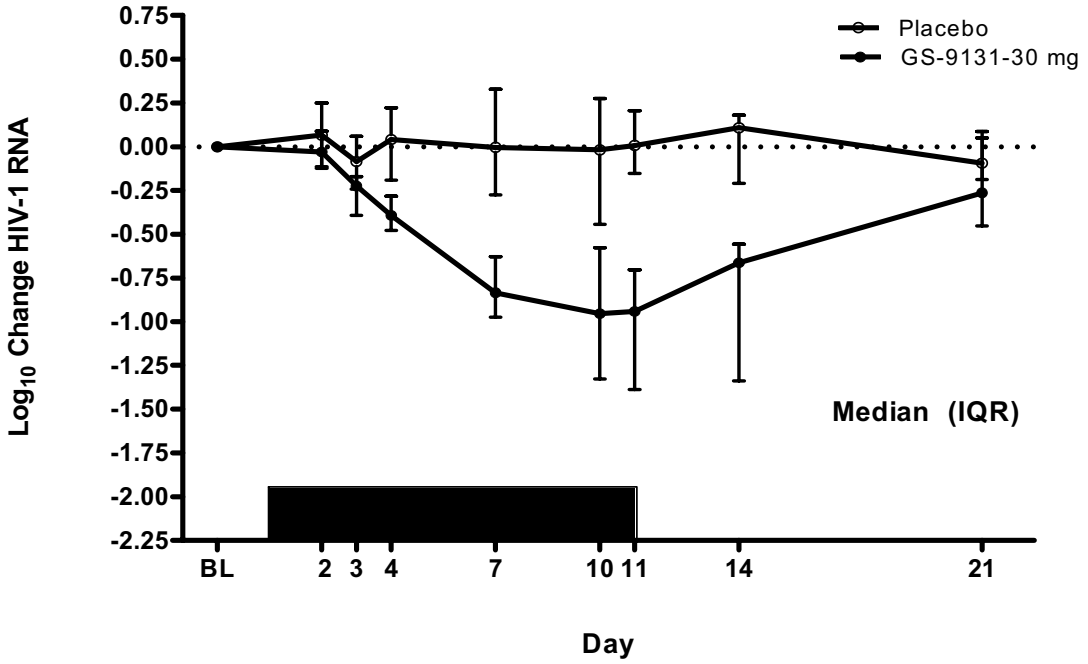
b Baseline (BL) HIV-1 RNA was calculated as the geometric mean of baseline and Day 1 (predose) measurements.

c The p-value is from a two-sided Wilcoxon rank sum exact test.

d The p-value is from a Fisher's exact test.

As displayed in the following figure, HIV-1 RNA levels in the GS-9131 group steadily decreased over the dosing period, and the maximum reduction from baseline in HIV-1 RNA (log_{10} copies/mL) up to Day 11 (primary endpoint) was significantly greater in the GS-9131 group than in the placebo group (p = 0.001).

Efficacy Results (Continued):



Through Day 7, the GS-9131 group showed significantly greater time-weighted average reductions from baseline in HIV-1 RNA levels than the placebo group ($DAVG_1$, $p < 0.001$), with the time-weighted average reduction from baseline becoming most pronounced by Day 11 ($DAVG_2$). No subjects in either treatment group achieved HIV-RNA levels < 400 or < 50 copies/mL at any postbaseline visit.

Seven of twelve (58.3%) subjects in the GS-9131 group compared to zero of six subjects in the placebo group achieved at least a 1.0 $\log_{10}$ decrease from baseline in HIV-1 RNA from Days 2 to 11 ($p = 0.038$). Two (16.7%) subjects in the GS-9131 group compared to zero subjects in the placebo group achieved at least a 1.5 $\log_{10}$ decrease from baseline in HIV-1 RNA from Days 2 to 11.

Changes from baseline in CD4 counts and percentages were not significantly different between the treatment groups. Changes from baseline in CD8 counts and percentages were not significantly different between the treatment groups, with exception of CD8 percentages assessed on Day 14 ($p = 0.040$). This difference was assessed as not clinically significant.

Virology Results:

The resistance analyses showed that subjects lacked primary drug resistance mutations at baseline, a finding that is consistent with the ARV-naive status of the subjects at screening. Overall, no RT mutations associated with NRTI or GS-9131 resistance developed in subjects treated with GS-9131. In addition, HIV-1 showed full phenotypic susceptibility to GS-9131 from baseline through Day 11 for subjects in the GS-9131 group.

Pharmacokinetics Results:

Results for selected plasma GS-9131 and GS-9148 pharmacokinetic parameters (mean [% coefficient of variation]) are presented in the following table.

Parameter	GS-9131		GS-9148	
	Single Dose (n=12)	Steady-State (n=12)	Single Dose (n=12)	Steady-State (n=12)
Plasma AUC (ng•h/mL)[a]	109.8 (28.1)	249.8 (25.7)	525.5 (32.7)	410.7 (23.3)
Plasma C_{max} (ng/mL)	179.4 (41.2)	385.6 (32.2)	30.6 (18.4)	47.3 (18.4)
Plasma C_{last} (ng/mL)	1.13 (68.6)	NA	6.78 (30.3)	NA
Plasma C_{tau} (ng/mL)	NA	—[b]	NA	9.7 (27.7)
$T_{1/2}$ (hr)[c]	0.36 (0.28, 0.57)	0.48 (0.46, 0.73)	20.51 (14.53, 25.83)	23.84 (21.92, 31.49)

NA=not applicable

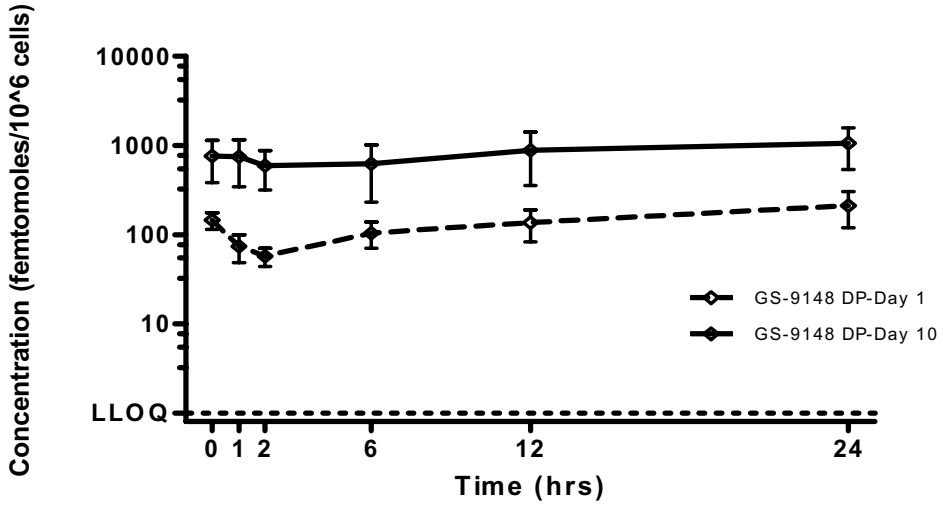
a AUC_{inf} is presented for single dose administration of GS-9131 and GS-9148; AUC_{tau} is presented for multiple dose administration of GS-9131 and GS-9148.

b Concentrations for GS-9131 were below limit of quantitation for all subjects at t = 24 hours; therefore, C_{tau} is not presented.

c Data presented as Median (Q1, Q3).

The pharmacokinetic parameter estimates of GS-9131 and GS-9148 after single dose administration of GS-9131 were similar to values seen previously in Study GS-US-180-0101. Consistent with values seen in preclinical studies, the prodrug (GS-9131) was rapidly hydrolyzed and exhibited a short plasma half-life, while the free phosphonate (GS-9148) exhibited a mean half-life of > 20 hours. GS-9148 exhibited near-linear pharmacokinetics, with accumulation ratios of nearly one (AUC geometric mean ratio was 79.35 [90% confidence interval: 70.42, 89.41]).

The figure below presents the Mean (SD) PBMC concentrations of GS-9148-DP observed after single and multiple dosing of GS-9131.



Pharmacokinetic Results (Continued)

In accordance with preclinical data, single and multiple doses of GS-9131 resulted in high concentrations of the antiviral active species, GS-9148-DP, in PBMCs. The maximum concentration (Mean[SD]: 1186 [450.1] fmol/10^6 cells) of GS-9148-DP was achieved 6 to 24 hours postdose.

Safety and Tolerability Results:

Seventeen subjects (11 [91.7%] in the GS-9131 group, 6 [100.0%] in the placebo group) reported at least one AE, and 15 subjects (9 [75.0%] in the GS-9131 group, 6 [100.0%] in the placebo group) experienced AEs considered by the investigator to be related to study drug. No treatment-related AEs ≥ Grade 3, SAEs, pregnancies, deaths, or AEs leading to study drug interruption or premature study drug discontinuation were reported.

All AEs were mild or moderate in severity. The most frequent AEs were as follows: fatigue (4 [33.3%] in the GS-9131 group, 4 [66.7%] in the placebo group); headache (6 [50.0%] in the GS-9131 group, 1 [16.7%] in the placebo group); nausea (4 [33.3%] in the GS-9131 group, 1 [16.7%] in the placebo group); abnormal dreams (3 [25.0%] in the GS-9131 group, 2 [33.3%] in the placebo group); and hypersomnia (2 [16.7%] in the GS-9131 group, 2 [33.3%] in the placebo group). These AEs were also the AEs that were the most frequently considered to be related to study drug.

Three subjects in the placebo group experienced a treatment-emergent Grade 3 laboratory abnormality: one subject (PPD) had elevated blood in urine, one subject (PPD) had elevated neutrophils (retest done at local laboratory was normal), and one subject (PPD) had elevated creatine kinase (CK) due to physical exertion. The elevated neutrophil and CK events qualified as marked laboratory abnormalities (Grade 0 at baseline to Grade 3 postbaseline).

One subject (PPD) in the GS-9131 group experienced greater than 1-grade elevations in alanine aminotransferase (ALT) and aspartate aminotransferase (AST). At screening and baseline, Subject PPD had Grade 1 elevations in ALT and AST. While on treatment with GS-9131, ALT and AST both increased to Grade 4 by Days 14 and 21, respectively. At screening, Subject PPD was found to be immune to hepatitis A and B, and hepatitis C (HCV) viral antibodies were negative. Given the subject's hepatitis serology status and increases in ALT and AST, HCV RNA polymerase chain reaction (PCR) was performed and showed low-level HCV viremia: 17,131 and 28,505 copies/mL on Days 16 and 23, respectively. However, the HCV PCR was undetectable (< 15 copies/mL) on Day 39. The investigator assessed the subject's ALT and AST elevations as related to HCV infection and not GS-9131.

Five abnormal ECG assessments (3 in GS-9131 group, 1 in placebo group) were reported from baseline through Day 21; none were determined to be clinically significant.

No clinically significant changes in body weight or vital signs were observed.

CONCLUSIONS:

Efficacy: GS-9131 administered once daily to 12 ARV-naive subjects over a period of 10 days produced substantial antiviral activity, as demonstrated by steady decreases in average postbaseline HIV-1 RNA levels through Day 11. No subjects in either treatment group achieved virologic suppression at HIV RNA < 400 or < 50 copies/mL during the 21-day study period. No clinically significant changes in CD4 and CD8 counts and percentages were observed.

Virology: Genotypic and phenotypic resistance analyses indicate that subjects treated with GS-9131 did not develop resistance to GS-9131 during the study.

Pharmacokinetics: The plasma pharmacokinetic parameter estimates of GS-9131 and GS-9148 after single dose administration of GS-9131 were similar to values previously seen in Study GS-US-180-0101. GS-9148 exhibited near-linear plasma pharmacokinetics following multiple dosing. Single and multiple doses of GS-9131 resulted in high concentrations of GS-9148-DP in PBMCs, which were associated with a 1.03 $\log_{10}$ median reduction in HIV-RNA.

Safety: GS-9131 was generally well-tolerated by the HIV-1 infected, ARV-naive subjects treated in this study. No deaths, pregnancies, or SAEs occurred in this study, and no subjects interrupted or discontinued study drug due to AEs. All AEs were mild or moderate in severity. Four subjects (1 [8.3%] in GS-9131 group, 3 [50%] in placebo group) had treatment-emergent Grade 3 or 4 laboratory abnormalities; none were considered related to study drug.

Pharmacokinetic Abbreviations: $\mathbf{C_{max}}$: the maximum observed concentration of drug in plasma; $\mathbf{T_{max}}$: the time (observed time point) of C_{max}; $\mathbf{C_{last}}$: the last observed quantifiable concentration of drug in plasma; $\mathbf{C_{tau}}$: the observed drug concentration at the end of the dosing interval; $\mathbf{T_{last}}$: the time (observed time point) of C_{last}; λ_z: terminal elimination rate constant; $\mathbf{AUC_{0\text{-}last}}$: the area under the concentration versus time curve from time zero to the last quantifiable concentration; $\mathbf{AUC_{inf}}$: The area under the concentration versus time curve extrapolated to infinite time, calculated as $AUC_{0-last} + (C_{last}/\lambda_z)$; $\mathbf{AUC_{tau}}$: the area under the concentration versus time curve over the dosing interval; $\mathbf{\%AUC_{exp}}$: percentage of AUC extrapolated between AUC_{0-t} and AUC_{inf}; $\mathbf{T_{1/2}}$: the estimated terminal elimination half-life of drug in plasma; $\mathbf{V_Z/F}$: the apparent volume of distribution of the drug; **CL/F:** the apparent oral clearance after administration of the drug: $CL/F = dose/AUC_{inf.}$