FINAL SYNOPTIC CLINICAL STUDY REPORT

Study Title:	A Phase 2, Single-Center, Double-Blind, Placebo-Controlled, Randomized Study to Investigate the Effect of Ledipasvir/Sofosbuvir Fixed-Dose Combination on Cerebral Metabolism and Neurocognition in Treatment-Naive and Treatment-Experienced Subjects with Chronic Genotype 1 HCV Infection
Name of Test Drug:	Ledipasvir/Sofosbuvir (LDV/SOF) fixed-dose combination (FDC)
Dose and Formulation:	LDV/SOF FDC (90/400 mg)
Indication:	Hepatitis C virus infection
Sponsor:	Gilead Sciences, Inc 333 Lakeside Drive Foster City, CA 94404, USA
Study No.:	GS-US-337-1445
Phase of Development:	Phase 2
IND No.:	115268
EudraCT No.:	Not Applicable
ClinicalTrials.gov Identifier:	NCT02219685
Study Start Date:	25 August 2014 (First Subject Screened)
Study End Date:	07 April 2016 (Last Subject Observation)
Principal or Coordinating Investigator:	Name: Michael Curry, MD Affiliation: PPD
Gilead Responsible Medical Monitor:	Name: Benedetta Massetto, MD Telephone: PPD Fax: PPD
Report Date:	04 August 2016

STUDY SYNOPSIS

Study GS-US-337-1445
Gilead Sciences, Inc.
333 Lakeside Drive
Foster City, CA 94404
USA

Title of Study: A Phase 2, Single-Center, Double-Blind, Placebo-Controlled, Randomized Study to Investigate the Effect of Ledipasvir/Sofosbuvir Fixed-Dose Combination on Cerebral Metabolism and Neurocognition in Treatment-Naïve and Treatment-Experienced Subjects with Chronic Genotype 1 HCV Infection

Investigators: Michael Curry, MD

Study Centers: One site in the United States

Publications: Curry MP, Moczynski NP, Liu H, Stamm LM, Yun C, et al, The effect of Sustained Virologic Response on Cerebral Metabolism and Neurocognition in Patients With Chronic Genotype 1 HCV Infection. Poster SAT-215 presented at the European Association for the Study of the Liver, 13–17 April 2016, Barcelona, Spain.

Study Period:

25 August 2014 (First Subject Screened)
07 April 2016 (Last Subject Observation)

Phase of Development: Phase 2

Objectives:

The primary objectives of this study were as follows:

- To determine the effect of sustained virologic response (SVR) on cerebral metabolism as determined by magnetic resonance spectroscopy (MRS)
- To determine the effect of SVR on neurocognition as measured by neurocognitive tests

The secondary objectives of this study were as follows:

- To evaluate the antiviral efficacy of combination therapy with ledipasvir (LDV)/sofosbuvir (SOF) fixed dose combination (FDC) for 12 weeks in treatment-naive or treatment-experienced subjects, as measured by the proportion of subjects who attain sustained virologic response at 4, 12, and 24 weeks after discontinuation of therapy (SVR4, SVR12, and SVR24)
- To evaluate the safety and tolerability of LDV/SOF FDC administered for 12 weeks
- To evaluate the effect of SVR on health-related quality of life (HRQoL) as assessed through HRQoL surveys

- To evaluate the effect of SVR on mood as assessed through depression surveys

The exploratory objectives of this study were:

- To evaluate the effect of SVR on cerebral blood flow through arterial spin labeling of magnetic resonance images (MRI)
- To evaluate the effect of SVR on level of cytokines
- To identify or validate correlations that may exist across different brain function evaluations, including cerebral metabolism, neurocognition, HRQoL, and mood

Methodology: In this Phase 2, double-blind, placebo-controlled study, treatment-naive and treatment-experienced subjects without cirrhosis and with genotype 1 hepatitis C virus (HCV) infection were randomized and treated for 12 weeks with LDV/SOF or matching placebo. Randomization was stratified by treatment history (treatment naive vs treatment experienced). Approximately 40 subjects were to be randomized 2:1 to the following:

Group 1 (n = approximately 27): LDV/SOF FDC (90 mg/400 mg) once daily for 12 weeks

Group 2 (n = approximately 13): LDV/SOF matching placebo once daily for 12 weeks

All subjects completed a screening assessment within 42 days prior to baseline/Day 1. Study visits occurred at screening, baseline/Day 1, and on-treatment at Weeks 1, 2, 4, 8, and 12 for all treatment groups.

After unblinding at the posttreatment Week 4 visit, subjects initially randomized to Group 2 (placebo) were offered open-label treatment with LDV/SOF for 12 weeks (visits as defined above). Subjects were to initiate open-label LDV/SOF within 2 weeks of the posttreatment Week 4 visit.

All subjects who received LDV/SOF were to complete posttreatment follow-up visits at posttreatment Weeks 4, 12, and 24 following the last dose of study drug, regardless of treatment duration.

Number of Subjects (Planned and Analyzed):

Planned:

- 40 subjects (approximately 27 subjects randomized to Group 1 [LDV/SOF] and 13 subjects randomized to Group 2 [placebo])

Analyzed:

- 40 subjects in the Full Analysis Set (26 subjects in Group 1 [LDV/SOF] and 14 subjects in Group 2 [placebo])
- 40 subjects in the Safety Analysis Set (26 subjects in Group 1 [LDV/SOF] and 14 subjects in Group 2 [placebo])

Diagnosis and Main Criteria for Inclusion: Eligible subjects were HCV treatment-naive or treatment-experienced males or nonpregnant/nonlactating females aged 18 to 60 years of age, with chronic genotype 1 HCV monoinfection and without cirrhosis, had screening HCV RNA levels $\geq 10^5$ IU/mL, and a body mass index (BMI) ≥ 18 kg/m^2.

Duration of Treatment: Subjects randomized to LDV/SOF received 12 weeks of LDV/SOF followed by a 24-week posttreatment period. Subjects randomized to placebo received 12 weeks of placebo, followed by 12 weeks of open-label LDV/SOF, and then followed by a 24-week posttreatment period. Between treatment with placebo and open-label LDV/SOF, subjects were followed off treatment for 4 to 6 weeks.

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

- **LDV/SOF** FDC was administered orally to subjects with genotype 1 HCV infection at a dose of 90/400 mg/day (1 tablet once daily)

The batch numbers of LDV/SOF administered in this study were DK1209B1R and DK1303B1.

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

- **LDV/SOF matching placebo** was administered orally to subjects with genotype 1 HCV infection (1 tablet once daily)

Placebo to match LDV/SOF tablets were identical in appearance to the active LDV/SOF tablets. The batch number of placebo-to-match LDV/SOF tablets was DK1207B1.

Criteria for Evaluation:

Efficacy: The effect of SVR on cerebral metabolism and neurocognition was evaluated by MRS and administration of neurocognitive tests. The MRS was performed on or within 7 days prior to baseline/Day 1 and at the posttreatment Week 4 visit. An optional MRS was performed at the posttreatment Week 24 visit for subjects who provided additional consent. The following neurocognitive tests were conducted on or within 7 days prior to baseline/Day 1 and at posttreatment Weeks 4 and 24: Digit Span (Forward Span, Backward Span, Sequencing Span) Wechsler Adult Intelligence Test IV (WAIS IV), Symbol Span, Wechsler Memory Scale IV (WMS IV), Symbol Search, Wechsler Adult Intelligence Test IV (WAIS IV), FAS fluency, Animal Naming, Hopkins Verbal Learning Test- Revised (multiple equivalent forms), Brief Visuospatial Memory Test- Revised (multiple equivalent forms), Grooved Pegs, Trail Making Test A and B, Delis Kaplan Executive Function System (D-KEFS) Color-Word Interference Test.

Blood samples to determine HCV RNA levels were collected from subjects at screening, baseline/Day 1 (predose), Weeks 1, 2, 4, 8, and 12 during treatment (or upon early termination), and posttreatment Weeks 4, 12, and 24. The COBAS® AmpliPrep/COBAS® TaqMan® HCV Quantitative Test, v2.0 was used to quantify HCV RNA in this study. The lower limit of quantitation (LLOQ) of the assay was 15 IU/mL. Plasma samples were collected at Day 1 and at each on-treatment and posttreatment visit for viral sequence analysis.

Pharmacokinetics: No pharmacokinetic assessments were performed for this study.

Safety: Safety assessments included monitoring of adverse events (AEs) and concomitant medications, clinical laboratory analyses, vital signs measurements, and physical examinations.

Other: The following HRQoL questionnaires were administered on or within 7 days prior to baseline/Day 1 and at posttreatment Weeks 4 and 24: Functional Assessment of Chronic Illness Therapy-Fatigue (FACIT-F), Work Productivity and Activity Impairment Questionnaire, Hepatitis C (WPAI), Short-Form (SF-36), and Chronic Liver Disease Questionnaire-HCV (CLDQ-HCV). The Beck Depression Inventory (BDI-II) and Beck Hopelessness Scale (BHS) surveys were also administered on or within 7 days prior to baseline/Day 1 and at posttreatment Weeks 4 and 24.

Statistical Methods: All tables, figures, and listings produced for this study are provided in Section 15.1 and Appendix 16.2. Documentation of statistical methods is provided in Appendix 16.1.9.

Subject Disposition: Subject disposition was summarized by group and overall, and included the number of subjects: screened, not randomized, randomized, randomized but never treated, in the Full Analysis Set, and in the Safety Analysis Set. Disposition also included the number and percentage of subjects who: completed blinded study treatment, completed open-label treatment, discontinued study (and the reasons for doing so), completed the study, and did not complete the study (and the reasons for doing so). Additionally, for subjects who completed treatment, a further categorization by available HCV RNA assessment at posttreatment Week 4 was provided.

Subject Demographics: Subject demographics were summarized by group and overall. Subject demographics were summarized for subjects in the Safety Analysis Set and for subjects in the Full Analysis Set. Subject demographics and baseline disease characteristics were summarized using descriptive statistics (sample size, mean, standard deviation [SD], median, first quartile [Q1], third quartile [Q3], minimum, and maximum) for continuous data and by number and percentage of subjects for categorical data.

Efficacy: Efficacy data were summarized by group and treatment as follows: Group 1 LDV/SOF, Group 2 Placebo, Group 2 LDV/SOF, and also for all subjects treated with LDV/SOF. The primary endpoints were change from baseline in MRS metabolic ratios and neurocognitive function at 4 weeks after discontinuation of therapy in the Full Analysis Set. The mean change from baseline in MRS metabolic ratios and neurocognitive function at 4 weeks after discontinuation of treatment was tested within each of the 2 treatment groups and between Groups 1 and 2.

Magnetic resonance spectroscopy data were analyzed in the LCmodel program and measured in 3 specific areas of the brain (basal ganglia, frontal cortex, and dorsolateral prefrontal cortex). Three cerebral metabolic signals, with creatine level as a control metabolite, were as follows: 1) N-acetylaspartylglutamate (NAAG) + N-acetylaspartate (NAA), 2) choline (glycerophosphocholine [GCP] + phosphocholine [PCh]), and 3) myo-inositol (mI). The effect of SVR on cerebral metabolism was determined by MRS and was evaluated by Beth Israel Deaconess Medical Center (an external institute). Magnetic resonance spectroscopy data were summarized by treatment group and the cerebral metabolic signals for the 3 regions of the brain.

Selected neurocognitive function evaluations were analyzed for paired comparison within-treatment group (ie, memory raw score, working memory raw score, processing speed [and individual items], executive control [and individual items], motor [and individual items], and semantic access) and for comparison between treatment groups (ie, memory T score, attention scaled score [working memory], executive 1 processing speed, executive 2 conceptual shifting and initiation, and motor).

To evaluate within-treatment group changes from baseline to posttreatment time points, a paired t-test was used for both MRS metabolic ratios and neurocognitive function.

To evaluate the difference between the 2 treatment groups in the change from baseline to posttreatment Week 4 at the end of the blinded treatment phase, a t-test was used for MRS metabolic ratio data, and ANCOVA with treatment group and baseline values in the model was used for neurocognitive function data.

The secondary efficacy endpoints included the proportion of subjects with HCV RNA < LLOQ at 4, 12, and 24 weeks after treatment discontinuation (ie, SVR4, SVR12, and SVR24), the proportion of subjects with HCV RNA < LLOQ by study visit, HCV RNA (log_{10} IU/mL) and change from baseline in HCV RNA (log_{10} IU/mL) through the end of treatment, and the proportion of subjects with virologic failure. The point estimates of SVR4, SVR12, and SVR24 rates and their 2-sided 95% exact confidence intervals (CIs) based on the Clopper-Pearson method were provided for each treatment group as applicable {Clopper et al 1934}.

A summary table of virologic outcomes was provided that included the number and percentage of subjects with SVR12, overall virologic failure (with subgroups for on-treatment virologic failure and relapse), and other (those who did not achieve SVR12 and did not meet virologic failure criteria). In addition, a summary table of the number and percentage of subjects with HCV RNA < LLOQ at each posttreatment follow-up visit was provided; 95% Clopper-Pearson exact CIs were presented for the double-blind and open-label LDV/SOF treatment groups and the total proportion of subjects treated with LDV/SOF who achieved HCV RNA < LLOQ. SVR12 was also presented by randomization strata (ie, treatment experienced or treatment naive).

All continuous endpoints were summarized using descriptive statistics. All categorical endpoints were summarized by the number and percentage of subjects who met the endpoint definition.

Pharmacokinetics: Pharmacokinetic analyses were not conducted for this study.

Safety: Safety assessments included monitoring of AEs and concomitant medications, clinical laboratory analyses, vital signs measurements, and physical examinations for subjects in the Safety Analysis Set. Safety data were summarized by group (Group 1 and Group 2) and within Group 2, by treatment and overall for the treatment period. Data were also summarized for all subjects who had received LDV/SOF (both during the double-blind period and during the open-label period). Safety summaries included all data collected on or after the first dose date of study drug through the date of the last dose of any study drug plus 30 days. All AEs and laboratory abnormalities discussed in this clinical study report (CSR) were treatment emergent and are referred to as AEs for the purposes of this report. Adverse events and laboratory abnormalities were graded according to the Gilead Grading Scale for Severity of Adverse Events and Laboratory Abnormalities (Appendix 16.1.1, Appendix 3). Adverse events were coded using the Medical Dictionary for Regulatory Activities (MedDRA), Version 18.1.

Adverse events were summarized (by system organ class and/or preferred term) by analysis group for the number and percentage of subjects who had (1) any AE, (2) Grade 3 or above AEs, (3) Grade 2 or above AEs, (3) all treatment-related AEs, (5) nonserious AEs occurring in at least 5% of subjects in any treatment group, (6) any Grade 3 or above treatment-related AE, (7) Grade 2 or above treatment-related AEs, (8) serious adverse events (SAEs), (9) treatment-related SAEs, (10) AEs leading to permanent discontinuation from study drug, and (11) AEs leading to interruption of study drug. Data listings for all AEs, SAEs, deaths, Grade 3 or above AEs, and AEs leading to permanent discontinuation from LDV/SOF were provided.

Laboratory results were assigned toxicity grades of Grade 1 through Grade 4. Laboratory abnormalities were defined as values that increased at least 1 toxicity grade from baseline at any postbaseline time point to the date of the last dose of any study drug plus 30 days (ie, treatment emergent). The number and percentage of subjects by analysis group who had any graded laboratory abnormality or any Grade 3 or 4 laboratory abnormality were summarized. Laboratory data were summarized using descriptive statistics by analysis group with corresponding changes from baseline for alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, alkaline phosphatase, hemoglobin, reticulocytes, white blood cell (WBC) counts, neutrophils, lymphocytes, and platelets. Data listings of hematology and chemistry laboratory values and Grade 3 or 4 laboratory abnormalities were provided.

Other: Other secondary endpoints included the change from baseline in neurocognitive function at 24 weeks after treatment discontinuation, and change from baseline in HRQoL questionnaires (ie, SF-36, CLDQ-HCV, FACIT-F, WPAI: Hepatitis C) and mood-related assessments (BDI-II and BHS) at 4 and 24 weeks after treatment discontinuation.

The same analyses as primary endpoints on neurocognitive function at postreatment Week 4 were repeated at posttreatment Week 24 as secondary endpoints.

For the SF-36, a Wilcoxon signed rank test was used to explore within treatment group changes from baseline to posttreatment time points. A Wilcoxon rank sum test was used to explore differences between treatment groups in change in status from baseline to each time point at blinded treatment phase. The same analyses were carried out for CLDQ-HCV (overall score), FACIT-F (trial outcome index and total score), and WPAI: Hepatitis C (% overall work impairment due to HCV for subjects who worked in the past week and % activity impairment due to hepatitis C for all subjects). The calculation algorithms for CLDQ-HCV, FACIT-F and WPAI:Hepatitis C are described in the statistical analysis plan (SAP) (Appendix 16.1.9, Appendix 3).

For the BDI-II and BHS scores, a paired t-test was used to evaluate within treatment group changes from baseline to posttreatment time points. ANCOVA with treatment group and baseline value in the model was used to evaluate the difference of the 2 treatment groups in change in status from baseline to posttreatment time points.

All the above analyses for neurocognitive function, HRQoL questionnaires, and depression survey were based on the Full Analysis Set.

SUMMARY OF RESULTS:

Subject Disposition and Demographics: A total of 40 subjects were randomized, received blinded study drug and were included in the Full Analysis Set and Safety Analysis Set: 26 subjects randomized and treated with LDV/SOF in Group 1 and 14 subjects randomized and treated with placebo in Group 2 (Section 15.1, Table 3). All subjects in Group 2 initiated treatment with open-label LDV/SOF after completing the double-blind treatment with placebo and the 4-week posttreatment period. All subjects (100%) completed study drug dosing. Overall, 2 subjects randomized to LDV/SOF did not complete the study and did not have posttreatment HCV RNA data after withdrawal; 1 subject (Subject PPD was lost to follow-up after posttreatment Week 4 and 1 subject (Subject PPD withdrew consent after posttreatment Week 12 (Appendix 16.2, Listings 5 and 12).

Overall, the mean age of subjects was 45 years (range 22 to 60 years); 47.5% (19 of 40 subjects) of subjects were male (Section 15.1, Table 5). The majority of subjects were white (90.0%; 36 subjects), and not of Hispanic or Latino ethnicity (92.5%; 37 subjects). The mean BMI was 25.9 kg/m^2 (range 20.0 to 36.4). Overall, no subject had cirrhosis and 42.5% (17 subjects) were treatment experienced. Of these treatment-experienced subjects, 52.9% (9 of 17 subjects) had prior virologic relapse or on-treatment virologic failure and 47.1% (8 of 17 subjects) were nonresponders. The majority of subjects had genotype 1a HCV infection (87.5%; 35 subjects) and had an IL28B non-CC allele (82.5%; 33 subjects). The overall mean (SD) baseline HCV RNA value was 6.3 (0.58) log_{10} IU/mL, and 70% (28 subjects) of subjects had baseline HCV RNA $\geq$ 800,000 IU/mL. Baseline demographic and disease characteristics were not statistically significantly different between Group 1 and Group 2. Similar results were observed when using the Full Analysis Set or the Full Analysis Set with the 1 subject in Group 1 who did not achieve SVR4 excluded (Section 15.1, Tables 5.1 and 5.2).

Analyses related to disposition, demographics, and study drug exposure are presented in Section 15.1, Tables 1 to 7, Figure 1, and Appendix 16.2, Listings 1 to 9.1.

Efficacy Results: The primary endpoints were change from baseline in MRS metabolic ratios and neurocognitive function at 4 weeks after discontinuation of treatment in the Full Analysis Set.

Overall, no statistically significant changes in MRS were observed except for a significant difference within the LDV/SOF Group 1 (from baseline to posttreatment Week 4) for the metabolite choline in the basal ganglia region of the brain (Table 1). Similar results were observed when the 1 subject randomized to LDV/SOF who did not achieve SVR4 was excluded from the analyses (Section 15.1, Table 44.1).

Table 1. GS-US-337-1445: Magnetic Resonance Spectroscopy Metabolic Ratios: Change from Baseline to Posttreatment Week 4 (Full Analysis Set)

	Group 1 LDV/SOF 12 Weeks (N = 26)	Group 2 Placebo 12 Weeks (N = 14)	Group 1 (LDV/SOF) vs Group 2 (Placebo) p value
NAA+NAAG			
Basal Ganglia			
Mean (SD) Baseline Level	1.24 (0.129)	1.28 (0.151)	0.38
Mean (SD) Change from Baseline to FU–4	−0.03 (0.085)	−0.01 (0.147)	0.58
Within-group p value	0.092	0.82	—
Frontal Cortex			
Mean (SD) Baseline Level	1.45 (0.150)	1.50 (0.274)	0.45
Mean (SD) Change from Baseline to FU–4	−0.03 (0.201)	−0.09 (0.279)	0.48
Within-group p value	0.44	0.27	—
Dorsolateral Prefrontal Cortex			
Mean (SD) Baseline Level	1.68 (0.165)	1.71 (0.180)	0.70
Mean (SD) Change from Baseline to FU–4	0.00 (0.154)	−0.01 (0.142)	0.96
Within-group p value	0.88	0.85	—
Myoinositol			
Basal Ganglia			
Mean (SD) Baseline Level	0.53 (0.090)	0.54 (0.172)	0.68
Mean (SD) Change from Baseline to FU–4	0.02 (0.110)	0.00 (0.157)	0.70
Within-group p value	0.49	0.98	—
Frontal Cortex			
Mean (SD) Baseline Level	0.78 (0.168)	0.77 (0.224)	0.82
Mean (SD) Change from Baseline to FU–4	−0.01 (0.207)	0.08 (0.156)	0.21
Within-group p value	0.88	0.095	—
Dorsolateral Prefrontal Cortex			
Mean (SD) Baseline Level	0.75 (0.102)	0.75 (0.102)	0.95
Mean (SD) Change from Baseline to FU–4	−0.02 (0.112)	0.00 (0.104)	0.59
Within-group p value	0.31	0.92	—

	Group 1 LDV/SOF 12 Weeks (N = 26)	Group 2 Placebo 12 Weeks (N = 14)	Group 1 (LDV/SOF) vs Group 2 (Placebo) p value
Choline			
Basal Ganglia			
Mean (SD) Baseline Level	0.26 (0.025)	0.26 (0.028)	0.99
Mean (SD) Change from Baseline to FU–4	−0.01 (0.020)	0.00 (0.029)	0.54
Within-group p value	0.021	0.54	—
Frontal Cortex			
Mean (SD) Baseline Level	0.29 (0.029)	0.29 (0.051)	0.56
Mean (SD) Change from Baseline to FU–4	0.01 (0.033)	0.00 (0.055)	0.57
Within-group p value	0.27	0.97	—
Dorsolateral Prefrontal Cortex			
Mean (SD) Baseline Level	0.28 (0.031)	0.27 (0.047)	0.64
Mean (SD) Change from Baseline to FU–4	0.00 (0.029)	0.01 (0.023)	0.63
Within-group p value	0.43	0.17	—

FU–4 = posttreatment follow-up Week 4
The p-values for the within-treatment group comparisons were based on paired t-tests.
Between-treatment comparisons were based on 2 sample t-test assuming equal variances.
Three spectral linewidths divided by creatine values at 3 regions (basal ganglia, frontal cortex, dorsolateral prefrontal cortex) were analyzed: NAA+NAAG = N-Acetylaspartate+N-Acetylaspartylglutamate (NAA+NAAG), myoinositol (mI), and choline (glycerophosphocholine [GPC]+phosphocholine [PCh]); spectroscopy results were expressed as metabolic ratio with creatine (Cr) used as control metabolite.
Source: Section 15.1, Table 44

Changes in neurocognitive function are summarized in Section 15.1, Tables 9 to 11.1. In the between-treatment group comparisons from baseline to posttreatment Week 4, there were no statistically significant differences between LDV/SOF and placebo treatment on memory T score, attention scaled score, executive 1 processing speed, executive 2 conceptual shift and initiation, and motor (Table 2). There was no impact on the results when the 1 subject in Group 1 who did not achieve SVR4 was excluded from the analysis (Section 15.1, Table 10.1).

Table 2. GS-US-337-1445: Summary of Neurocognitive Function: Between Group Comparison (Full Analysis Set)

		Change from Baseline to Posttreatment Week 4	Group 1 (LDV/SOF) vs Group 2 (Placebo)
Neurocognitive Domain	**Mean (SD) Baseline**	**Mean (SD) Change**	**p value**
Memory T Score			0.0795
Group 1 LDV/SOF	198.31 (41.02)	−3.88 (19.15)	
Group 2 Placebo	200.50 (25.75)	7.93 (23.63)	
Attention Scaled Score			0.7007
Group 1 LDV/SOF	33.00 (7.44)	0.73 (4.29)	
Group 2 Placebo	30.43 (5.12)	1.43 (3.34)	
Executive 1 Processing Speed			0.2677
Group 1 LDV/SOF	73.23 (10.76)	1.96 (5.71)	
Group 2 Placebo	73.50 (10.11)	4.00 (7.27)	
Executive 2 Conceptual Shift and initiation			0.9870
Group 1 LDV/SOF	214.12 (60.35)	−13.04 (21.03)	
Group 2 Placebo	224.86 (41.07)	−12.43 (15.38)	
Motor			0.3388
Group 1 LDV/SOF	142.81 (20.53)	−10.00 (17.47)	
Group 2 Placebo	144.29 (14.70)	−6.00 (17.16)	

Between-group p-values were from type III sums of squares of ANCOVA model for change from baseline with treatment group and baseline as covariates.
Source: Section 15.1, Table 10

In the within-treatment group comparisons, there was a statistically significant difference from baseline through posttreatment Week 4 for LDV/SOF Group 1 for the domains processing speed, executive control, and motor; however, placebo Group 2 also had statistically significant changes from baseline through posttreatment Week 4 for the domains working memory raw score, processing speed, and executive control (Table 3). For the open-label LDV/SOF Group 2, the only domain that was statistically significant at posttreatment Week 4 was memory raw score (Section 15.1, Table 9). At posttreatment Week 24, a statistically significant difference was observed for both LDV/SOF Group 1 and open-label LDV/SOF Group 2 for the following domains: memory raw score and processing speed; however, motor was also statistically significant at posttreatment Week 24 for LDV/SOF Group 1. Similar results were observed when the 1 subject in Group 1 who did not achieve SVR4 was excluded from the analysis (Section 15.1, Table 9.1). The only difference was that at posttreatment Week 24, the domain executive control was statistically significant for LDV/SOF Group 1 ($p = 0.0469$).

The inferential statistical results should be interpreted with caution since no multiple comparison adjustment was applied to control for type I error.

Table 3. GS-US-337-1445: Summary of Neurocognitive Function - Within Group Comparisons (Full Analysis Set)

Neurocognitive Domain	Mean Change from Baseline to Posttreatment Week 4			Mean Change from Baseline to Posttreatment Week 24		
	Mean (SD) Baseline N = 26	Mean Change (SD)	p value	Mean (SD) Baseline N = 24	Mean Change (SD)	p value
Memory Raw Score						
Group 1 LDV/SOF	71.62 (14.87)	−0.96 (6.66)	0.4685	74.04 (12.03)	−5.79 (10.18)	0.0105
Group 2 Placebo	70.79 (8.97)	4.14 (9.00)	0.1088	74.93 (8.66)	—	—
Working Memory Raw Score						
Group 1 LDV/SOF	45.88 (8.88)	1.81 (5.54)	0.1084	46.38 (9.08)	1.71 (5.50)	0.1415
Group 2 Placebo	42.21 (7.24)	2.79 (4.66)	0.0435	45.00 (6.49)	—	—
Processing Speed						
Group 1 LDV/SOF	94.96 (15.12)	4.27 (7.06)	0.0050	94.25 (15.03)	6.96 (9.06)	0.0010
Group 2 Placebo	94.71 (13.62)	4.71 (7.44)	0.0338	99.43 (11.62)	—	—
Executive Control						
Group 1 LDV/SOF	168.00 (52.72)	−9.31 (19.73)	0.0239	169.79 (54.13)	−14.88 (36.45)	0.0575
Group 2 Placebo	176.64 (37.46)	−13.07 (16.98)	0.0129	163.57 (35.06)	—	—
Motor						
Group 1 LDV/SOF	142.81 (20.53)	−10.00 (17.47)	0.0073	143.54 (21.20)	−9.21 (17.74)	0.0182
Group 2 Placebo	144.29 (14.70)	−6.00 (17.16)	0.2134	138.29 (20.99)	—	—
Semantic Access						
Group 1 LDV/SOF	22.92 (5.79)	−0.15 (4.21)	0.8537	23.00 (5.85)	0.38 (4.03)	0.6528
Group 2 Placebo	20.79 (4.19)	0.21 (4.63)	0.8651	21.00 (2.69)	—	—

Within group p-values were from paired t-test.
Source Section 15.1, Table 9

Secondary efficacy endpoints included the proportion of subjects who achieved SVR4, SVR12, and SVR24 in the LDV/SOF Group 1 and the open-label LDV/SOF Group 2 (Section 15.1,

Table 12). The proportion of subjects who achieved SVR4, SVR12, SVR24, as well as virologic outcomes is shown in Table 4. Overall, 95.0% (38 of 40) of subjects achieved SVR12.
Two subjects randomized to LDV/SOF failed to achieve SVR12: 1 treatment-experienced subject (Subject PPD had posttreatment virologic failure (relapse) at posttreatment Week 4, and 1 treatment-naive subject (Subject PPD was lost to follow-up after posttreatment Week 4 and could not be assessed for SVR12 (categorized as "other") (Appendix 16.2, Listing 12). Subject PPD with virologic relapse had a calculated adherence of 63.1% indicating noncompliance to study drug (Appendix 16.2, Listing 9.2).

One subject (Subject PPD randomized to LDV/SOF achieved SVR12 but then withdrew consent after the posttreatment Week 12 visit and was missing an HCV RNA value for SVR24 (Appendix 16.2, Listing 12). The subject was considered as achieving success at the SVR24 assessment according to the imputation rule prespecified in the SAP.

Table 4. GS-US-337-1445: Proportion of Subjects with SVR4, SVR12, SVR24, and Virologic Outcomes (Full Analysis Set)

	Group 1 LDV/SOF 12 Weeks N = 26	Group 2 OL LDV/SOF 12 Weeks N = 14	Total LDV/SOF 12 Weeks N = 40
SVR4	25/26 (96.2%)	14/14 (100.0%)	39/40 (97.5%)
95% CI	80.4% to 99.9%	76.8% to 100.0%	86.8% to 99.9%
SVR12	24/26 (92.3%)	14/14 (100.0%)	38/40 (95.0%)
95% CI	74.9% to 99.1%	76.8% to 100.0%	83.1% to 99.4%
SVR24	24/26 (92.3%)	14/14 (100.0%)	38/40 (95.0%)
95% CI	74.9% to 99.1%	76.8% to 100.0%	83.1% to 99.4%
Overall Virologic Failure	1/26 (3.8%)	0/14	1/40 (2.5%)
Relapse	1/26 (3.8%)	0/14	1/40 (2.5%)
On Treatment Virologic Failure	0/26	0/14	0/40
Other	1/26 (3.8%)	0/14	1/40 (2.5%)

OL = open label
HCV RNA was analyzed using COBAS AmpliPrep/COBAS TaqMan HCV Quantitative Test v2.0 with limit of quantitation 15 IU/mL.
Relapse = confirmed HCV RNA ≥ LLOQ during the posttreatment period having achieved HCV RNA < LLOQ at last on-treatment visit.
SVRx was sustained virologic response (HCV RNA < LLOQ) x weeks after stopping study treatment.
A missing SVR value was imputed as a success if it was bracketed by values that were termed successes (ie, '<LLOQ TND' or '<LLOQ detected'); a missing SVR24 was imputed as a success if SVR12 was a success; otherwise, the missing SVR value was imputed as a failure.
The exact 95% CI for the proportion within treatment group was based on the Clopper-Pearson method.
On-Treatment Virologic Failure = Breakthrough (confirmed HCV RNA ≥ LLOQ after having previously had HCV RNA < LLOQ while on treatment), Rebound (confirmed > 1 log10IU/mL increase in HCV RNA from nadir while on treatment), or Non-response (HCV RNA persistently ≥ LLOQ through 8 weeks of treatment). Other = subject who did not achieve SVR12 and did not meet virologic failure criteria
Source: Section 15.1, Tables 12 and 16

HCV RNA levels (log_{10} IU/mL) declined rapidly in the LDV/SOF treatment groups. At Week 2 the overall mean (SD) change from baseline was −5.10 (0.559) log_{10} IU/mL and by Week 4, 97.5% (39 of 40) of subjects on LDV/SOF achieved HCV RNA < LLOQ (Section 15.1, Table 14 and Table 15).

All efficacy analyses are provided in Section 15.1, Tables 9 to 16, Tables 44 and 44.1, Figures 2 to 7, and in Appendix 16.2, Listings 11, 12, 13.5, 13.6, 13.7.

Virologic Resistance Analysis:

Virologic resistance analysis was performed on a sample from the 1 subject who experienced virologic relapse. Table 5 shows the nonstructural (NS)5A and NS5B resistance-associated variants (RAVs) detected at baseline and posttreatment in this subject. This subject with genotype 1a HCV infection had the L31M and Y93H NS5A RAVs at baseline, with the Y93H enriching to a full mutant at the time of virologic failure. No NS5B nucleoside inhibitor RAVs were observed at either time point in this subject.

Table 5. GS-US-337-1445: Subjects with Virologic Failure

Subject	HCV GT	Treatment Group	Pretreatment NS5A RAVs	Posttreatment NS5A RAVs	Pretreatment NS5B NI RAVs	Posttreatment NS5B NI RAVs
00519-74110	1a	LDV/SOF 12 weeks	L31M (> 99%) Y93H (21.5%)	L31M (> 99%) Y93H (> 99%)	None	None

GT = genotype; NI = nucleoside inhibitor; RAV = resistance-associated variant

Pharmacokinetic Results: Pharmacokinetic analyses were not conducted for this study.

Safety Results: The mean (SD) duration of exposure to study drug was 12.1 (0.15) weeks and 12.1 (0.16) weeks in the LDV/SOF Group 1 and placebo Group 2, respectively (Section 15.1, Table 6). Exposure to open-label LDV/SOF in Group 2 was 12.2 (0.21) weeks. Across all treatment groups (ie, LDV/SOF Group 1, placebo Group 2, and open-label LDV/SOF Group 2), all subjects completed study drug (Section 15.1, Tables 2 and 3; Appendix 16.2, Listing 5). The proportion of subjects with calculated ≥ 90% adherence was 96.2% (25 of 26) of subjects in the LDV/SOF Group 1, 100.0% in the placebo Group 2, and 100.0% in the open-label LDV/SOF Group 2 (Section 15.1, Table 8). The 1 subject with virologic relapse in the LDV/SOF Group 1 had a calculated adherence rate of < 80% (Appendix 16.2, Listing 9.2).

Adverse Events and Serious Adverse Events

The majority of subjects experienced at least 1 AE: 84.6% (22 of 26) of subjects in the LDV/SOF Group 1, 85.7% (12 of 14) of subjects in the placebo Group 2, and 64.3% (9 of 14) of subjects in the open-label LDV/SOF Group 2 (Section 15.1, Table 22).

The most frequently reported AEs that occurred in > 2 subjects were nasopharyngitis (23.1%; 6 of 26), nausea (19.2%; 5 of 26), fatigue (19.2%; 5 of 26), and headache (19.2%, 5 of 26) in LDV/SOF Group 1; fatigue (42.9%; 6 of 14) and nasopharyngitis (21.4%; 3 of 14) in the placebo Group 2; diarrhea (21.4%, 3 of 14), muscle spasms (21.4%, 3 of 14), and headache (21.4%, 3 of 14) in the open-label LDV/SOF Group 2 (Section 15.1, Table 23).

Most AEs reported in the study were Grade 1 or Grade 2 in severity (Section 15.1, Table 26, Appendix 16.2, Listings 14 and 15). No subject experienced a Grade 4 AE, and 1 subject

experienced a Grade 3 AE. Subject PPD in the LDV/SOF Group 1 experienced the Grade 3 AE of bruxism beginning on Day 2. The AE was considered related to study drug and resolved on Day 8. Study drug was continued without interruption.

No subject experienced an SAE or an AE leading to the interruption or discontinuation of study drug (Section 15.1, Tables 30 to 32). There were no subject deaths or pregnancies reported during the study (Appendix 16.2, Listings 19 and 20).

All AE data are presented in Tables 22 to 39 and Listings 14 to 20.

Clinical Laboratory Results

No subject experienced a Grade 4 laboratory abnormality and 3 subjects (1 subject in each treatment group) experienced a Grade 3 laboratory abnormality (Section 15.1, Tables 41 and 42). Subject PPD randomized to LDV/SOF Group 1, who had Grade 1 lipase (105 U/L) at screening, experienced a single Grade 3 lipase value at Week 12 (457 U/L) that returned to within normal range by posttreatment Week 4. Lipase was within normal range at all other on-treatment visits except for Week 2 where the value was Grade 1 (Appendix 16.2, Listing 22). The only AE reported at the time of the Grade 3 lipase abnormality was Grade 1 fatigue which had been ongoing since Day 15 (Appendix 16.2, Listing 14). Study drug dosing was not changed and the subject completed the study. Subject PPD randomized to placebo, experienced a single Grade 3 lipase value at Week 2 (383 U/L) after switching to open-label LDV/SOF Group 2. The subject also had a Grade 1 elevation at Week 1 while on placebo; all other lipase values during both the placebo and the treatment phase were within normal range. Adverse events ongoing at the time of the Grade 3 lipase abnormality were Grade 1 diarrhea and Grade 1 dyspepsia. Study drug dosing was not changed and the subject completed the study. Subject PPD randomized to placebo experienced a single Grade 3 creatine kinase abnormality at the posttreatment Week 4 visit (2527 U/L) after discontinuing placebo treatment; all other creatine kinase values were within normal range except for the open-label Week 1 value (206 U/L [high, < Grade 1]). The only AE reported at the time of the Grade 3 elevation was Grade 1 nasopharyngitis. Study drug dosing was not changed and the subject completed the study (Appendix 16.2, Listing 14).

All laboratory results are provided in Section 15.1, Tables 40.1 to Table 42, Figures 8.1 to 8.10, and Appendix 16.2, Listings 21 to 27.2.

Vital Signs Measurements

Overall, mean systolic blood pressure, diastolic blood pressure, and heart rate measurements remained relatively constant over time (Section 15.1, Tables 43.1 to 43.3).

Other Results: All HRQoL questionnaires (SF-36, CLDQ-HCV, FACIT-F, WPAI: Hepatitis C) and mood-related assessments (BDI-II and BHS) are summarized in Section 15.1, Tables 17 to 21.1. No statistically significant differences between LDV/SOF Group 1 and placebo Group 2 were observed for the 8 domains of the SF-36, or for the physical component and mental component summaries at posttreatment Week 4 (Section 15.1, Table 17). The LDV/SOF Group 1 showed significant improvement from baseline to posttreatment Week 4 for the following domains and component scores: bodily pain and vitality. No statistically significant improvement was observed for any of the domain scores in the placebo Group 2 from baseline to posttreatment Week 4. The LDV/SOF Group 1 showed significant improvement from baseline to posttreatment Week 24 for the following domains and component scores: role physical, bodily

pain, general health, vitality, role emotional, mental health, and the mental component score.

For the CLDQ-HCV, FACIT-F, and WPAI: Hepatitis C HRQoL questionnaire overall scores, there were statistically significant differences at posttreatment Week 4 in the between-group comparisons favoring LDV/SOF over placebo (Section 15.1, Tables 18 to 20, respectively). The LDV/SOF Group 1 showed significant improvement from baseline to posttreatment Weeks 4 and 24 in the CLDQ-HCV and FACIT-F scores but not in the WPAI score. No statistically significant improvement was observed in placebo Group 2 for any of the HRQoL questionnaires.

All HRQoL data should be interpreted with caution as multiple endpoints were tested, and the study was not powered to test for these endpoints.

For the BDI-II mood assessment survey, a statistically significant difference was observed favoring LDV/SOF over placebo in the mean change from baseline at posttreatment Week 4; however, there was no significant difference in the mean change from baseline at posttreatment Week 4 for the BHS survey between LDV/SOF and placebo (Section 15.1, Table 21).

CONCLUSIONS: The conclusions of this Phase 2 study were as follows:

- Overall, no significant difference in cerebral metabolism of multiple metabolites measured by MRS in the basal ganglia, frontal and prefrontal cortex, or in neurocognitive tests, was observed among subjects treated with LDV/SOF compared to placebo.
- Treatment with LDV/SOF for 12 weeks resulted in rapid and sustained viral suppression. No subjects experienced on-treatment virologic breakthrough.
- In treatment-naive and treatment-experienced subjects with genotype 1 HCV infection, LDV/SOF administered once daily for 12 weeks resulted in a high SVR12 rate (95.0%) with a low relapse rate (2.5% [1 subject with drug adherence < 80%]).
- NS5A RAVs, but not NS5B RAVs were observed in the single subject experiencing virologic relapse.
- Treatment with LDV/SOF was safe and well tolerated with no subject discontinuing treatment due to an AE and no subject with an SAE, Grade 4 AE, or Grade 4 laboratory abnormality.