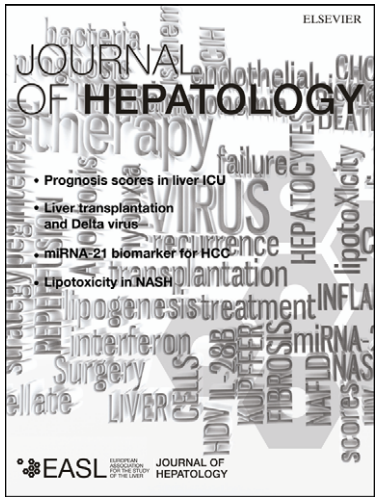
Accepted Manuscript




# A Phase 1, Randomized, Placebo-Controlled, Three-Day, Dose-Ranging Study of GS-5885, an NS5A Inhibitor, in Patients with Genotype 1 Hepatitis C

Eric J. Lawitz, Daniel Gruener, John M. Hill, Thomas Marbury, Lisa Moorehead, Anita Mathias, Guofeng Cheng, John O. Link, Kelly A. Wong, Hongmei Mo, John G. McHutchison, Diana M. Brainard

PII: S0168-8278(12)00095-5
DOI: 10.1016/j.jhep.2011.12.029
Reference: JHEPAT 4107

To appear in: *Journal of Hepatology*

Received Date: 1 October 2011
Revised Date: 20 December 2011
Accepted Date: 29 December 2011

Please cite this article as: Lawitz, E.J., Gruener, D., Hill, J.M., Marbury, T., Moorehead, L., Mathias, A., Cheng, G., Link, J.O., Wong, K.A., Mo, H., McHutchison, J.G., Brainard, D.M., A Phase 1, Randomized, Placebo-Controlled, Three-Day, Dose-Ranging Study of GS-5885, an NS5A Inhibitor, in Patients with Genotype 1 Hepatitis C, *Journal of Hepatology* (2012), doi: 10.1016/j.jhep.2011.12.029

# A Phase 1, Randomized, Placebo-Controlled, Three-Day, Dose-Ranging Study of GS-5885, an NS5A Inhibitor, in Patients with Genotype 1 Hepatitis C

Eric J. Lawitz, MD,[1] Daniel Gruener, MD,[2] John M. Hill, MD,[3] Thomas Marbury, MD,[4] Lisa Moorehead, MPH,[5] Anita Mathias, PhD,[5] Guofeng Cheng, PhD, [5] John O. Link, PhD,[5] Kelly A. Wong, PhD,[5] Hongmei Mo, MD,[5] John G. McHutchison, MD,[5] and Diana M. Brainard, MD[5]

From [1]Alamo Medical Research, San Antonio, Texas; [2]CRI Worldwide, Philadelphia, Pennsylvania; [3]Avail Clinical Research, DeLand, Florida; [4]Orlando Clinical Research Center, Orlando, Florida; and [5]Gilead Sciences, Inc., Foster City, California

**ClinicalTrials.gov identifier:** NCT01193478

**Word count**: 5,450

**Address correspondence to**: Eric Lawitz, MD, 621 Camden Street, Suite 202, San Antonio, TX 78258; Telephone: 210-253-3426: Fax: 210-227-6951; E-mail: lawitz@alamomedicalresearch.com

**Abbreviations:** AUC, area under the plasma concentration-time curve; BMI, body mass index; $EC_{50}$, 50% effective inhibitory concentration; $EC_{90}$, 90% effective inhibitory concentration; HCV, hepatitis C virus; PEG-IFN, peginterferon alfa; RBV, ribavirin

**Financial Support:** This trial was supported by Gilead Sciences.

**ABSTRACT**

**Background & Aims:** GS-5885 is an inhibitor of the hepatitis C virus (HCV) NS5A protein and exhibits potent suppression of genotype 1 HCV replicons. The safety, tolerability, pharmacokinetics, antiviral activity, and resistance profile of once-daily GS-5885 doses of 1-90 mg were evaluated in patients with chronic genotype 1 HCV.

**Methods:** Genotype 1 HCV-infected patients were randomized to 3 days of once-daily (QD) dosing with placebo (n=12) or GS-5885 1 mg (n=10), 3 mg (n=10), 10 mg (n=20), 30 mg (n=10), or 90 mg (n=10). Plasma samples for pharmacokinetics, HCV RNA, and NS5A sequencing were collected through Day 14.

**Results:** GS-5885 was well tolerated and resulted in median maximal reductions in HCV RNA ranging from 2.3 $\log_{10}$ IU/mL (1 mg QD) to 3.3 $\log_{10}$ IU/mL (10 mg QD in genotype 1b and 30 mg QD). $E_{max}$ modeling indicated GS-5885 30 mg was associated with >95% of maximal antiviral response to HCV genotype 1a. HCV RNA reductions were generally more sustained among patients with genotype 1b versus 1a. Three of 60 patients had a reduced response and harbored NS5A-resistant virus at baseline. NS5A sequencing identified residues 30 and 31 in genotype 1a, and 93 in genotype 1b as the predominant sites of mutation following GS-5885 dosing. Plasma pharmacokinetics were consistent with QD dosing.

**Conclusions:** During 3 days of monotherapy, low doses of GS-5885 demonstrated significant antiviral activity in genotype 1a and 1b HCV-infected patients. GS-5885 is currently being evaluated in combination direct antiviral regimens with and without peginterferon.

**Keywords:** NS5A protein, viral nonstructural protein, hepatitis C, antiviral agents

**INTRODUCTION**

Treatment of genotype 1 chronic hepatitis C virus (HCV) infection with 48 weeks of peginterferon alfa-2a (PEG-IFN) and ribavirin (RBV) results in sustained virologic response (SVR) in 40%-52% of patients [1-4]. Addition of telaprevir or boceprevir, both HCV NS3 protease inhibitors, to the PEG-IFN and RBV regimen increases SVR rates to 67%-75% [5-10]. Therapy with PEG-IFN and RBV can cause side effects such as influenza-like fatigue, anemia, and depression, yet PEG-IFN and RBV remain the backbone of HCV therapy because monotherapy with telaprevir or boceprevir leads to the rapid emergence of viral resistance [11]. Development of antiviral agents targeting other HCV proteins to be used in combination may reduce the emergence of resistance and potentially allow for a successful HCV treatment regimen without PEG-IFN.

The NS5A protein plays a role in both viral RNA replication [12] and the assembly of HCV virions [13]. In HCV replicon cells, inhibition of NS5A results in the redistribution of NS5A from the endoplasmic reticulum to lipid droplets and appears to disrupt formation of new replication complexes [14]. Clinically, inhibition of NS5A has been associated with significant reductions in HCV RNA and enhanced SVR rates when combined with PEG-IFN and RBV [15,16].

GS-5885 is a novel NS5A inhibitor with $EC_{50}$ (50% effective inhibitory concentration) values of 34 pM against genotype 1a and 4 pM against genotype 1b replicons [17]. GS-5885 is stable in human liver microsomes and hepatocytes and has no inhibitory effect on the activities of major human CYP enzymes and low liability for induction via activation of xenobiotic receptors such as the aryl hydrocarbon receptor or pregnane X receptor (Gilead Sciences, data on file).

We examined the safety, tolerability, pharmacokinetics, and antiviral activity of once-

daily dosing of GS-5885 for 3 days in patients with chronic genotype 1 HCV. The GS-5885 doses evaluated ranged from 1 mg to 90 mg daily. Treatment-emergent changes in the NS5A genetic sequence were also assessed.

## PATIENTS AND METHODS

### Patients

Eligible patients were 18–65 years of age, with chronic infection with genotype 1a or 1b HCV virus and plasma HCV RNA ≥5 $\log_{10}$ IU/mL at screening. Patients were HCV treatment naïve and had a body mass index (BMI) of 19 to 35 kg/m$^2$ inclusive, creatinine clearance ≥70 mL/min, and a QTcF interval ≤450 msec. Patients with any of the following conditions or characteristics were excluded from participation: known cirrhosis, hepatic decompensation, excessive ongoing alcohol intake, Gilbert's syndrome, evidence of hepatocellular carcinoma, coinfection with HIV or hepatitis B virus, prothrombin time >1.5 × ULN, albumin <3 g/dL, alanine aminotransferase and aspartate aminotransferase levels >5 × ULN, total bilirubin >ULN, hemoglobin <11 g/dL, platelets <90,000/mm$^3$, or absolute neutrophil count <1,000 cells/mm$^3$ (<900 cells/mm$^3$ for African Americans). Concomitant prescription or non-prescription medications were prohibited during the study unless prior approval was received from the medical monitor. The only exception was the use of hormonal contraception; additional double barrier method contraception was mandated for all women of child bearing potential. All patients provided written informed consent before undertaking any study-related procedures.

### Study Design

This was a Phase 1, multi-center, randomized, double-blind, placebo-controlled, dose-escalation study that included 6 cohorts: 5 cohorts included only patients with genotype 1a HCV, and 1 cohort included patients with only genotype 1b HCV. Patients arrived at a study center the day prior to dosing initiation and were sequestered for approximately 5 days. In all cohorts, oral tablets of GS-5885 or matching placebo were administered in a fasted state once–daily for 3 days (Days 1-3). Doses of GS-5885 in individual cohorts were as follows: 1 mg, 3 mg, 10 mg (2

cohorts: genotype 1a and 1b), 30 mg, and 90 mg. Each cohort had 12 patients, 10 randomly assigned to active drug and 2 to placebo. Study treatment (GS-5885 or placebo) was assigned to patients according to a centralized randomization schedule generated via computer by the sponsor's Biometrics group. The study protocol was approved by each institution's review board prior to study initiation and was performed in accordance with Good Clinical Practice guidelines outlined by the International Conference on Harmonization.

**Safety Assessments**

From Baseline through the Day 14 Follow-up Visit, safety was evaluated on the basis of adverse events, physical examinations, clinical laboratory tests, vital signs, and ECG recordings. Concomitant medication intake was also recorded. Treatment-emergent adverse events were summarized by treatment, system organ class, and preferred term using the most current version of the Medical Dictionary for Regulatory Activities (MedDRA®).

**Pharmacokinetic and Pharmacodynamic Assessments**

Plasma samples for analysis of GS-5885 concentrations were drawn through 24 hours after the first dose on Day 1 and after dosing on Day 3. Additional blood samples were collected on Days 4, 5, 6, 7, 8, and 10. Concentrations of GS-5885 were determined in plasma using a validated LC/MS/MS assay with a lower limit of quantification of 1 ng/mL. Pharmacokinetic parameters were estimated via non-compartmental methods using Phoenix WinNonlin™ 6.0 (Pharsight Corporation, Sunnyvale, California, USA).

Parameters estimated included area under the plasma concentration-time curve (AUC) of the dosing interval ($AUC_{tau,}$ after administration of the last dose), AUC extrapolated to infinity ($AUC_{\infty,}$ after administration of first dose), elimination rate constant ($\lambda_z$), and half-life ($T_{1/2}$). In

addition, maximum observed plasma concentration ($C_{max}$), last quantifiable concentration ($C_{last}$), and concentration at the end of dosing interval ($C_{tau}$) were also identified.

The pharmacokinetic/pharmacodynamic relationship between GS-5885 doses and HCV RNA levels were explored using a simple $E_{max}$ exposure-response curve (Phoenix WinNonlin™ 6.0). Appropriateness of the model was assessed visually using diagnostic and predictive check plots. Goodness of fit was assessed by using Akaike's information criterion and residual plots.

**Efficacy Assessments**

***HCV RNA.*** Plasma samples for determining HCV RNA levels were drawn at Screening; prior to the first dose on Day 1 (Baseline); at the following hours after the initial dose: 1, 2, 4, 8, 12, 24, 36, 48, 72, 84; and on the following days: 4, 5, 6, 7, 9, 11, and 14. HCV RNA levels were measured with the Roche COBAS® TaqMan® HCV Test v2.0 for use with the High Pure System, with a lower limit of quantification of 25 IU/mL.

***Viral Sequencing.*** Population sequencing of the HCV NS5A gene was performed for all patients at baseline (Day 1 prior to dosing), on Day 4, and on Day 14 by Virco BVBA (Beerse, Belgium). In addition, deep sequencing was performed at baseline for a few selected patients. For deep sequencing, 7 independent PCR reactions were performed to amplify the NS5A gene from one sample. The pool of PCR products was fragmented into smaller fragments (150-550 base pairs in length) and sequenced by 454 pyrosequencing technology developed by Virco BVBA.

**Endpoints and Statistical Analyses**

The primary efficacy endpoint was antiviral activity of GS-5885. Reduction in HCV RNA was summarized as continuous change from baseline in $\log_{10}$ HCV RNA. All statistical summaries and analyses were performed using SAS® software (SAS Institute, Cary, North Carolina, USA).

## RESULTS

### Study Population

Between August 2010 and January 2011, a total of 72 patients were randomized at 9 study centers in the United States. All patients except for one, who was lost to follow-up after Day 8, completed the study through the Day 14 follow-up visit. Overall, 72 percent (52/72) of patients were male, and the mean (SD) age was 48 (8.3) years old (Table 1). Mean pre-treatment HCV RNA levels were similar between dosing groups, with a range of 6.3 (30 mg GS-5885) to 6.8 $\log_{10}$ IU/mL (90 mg GS-5885 and placebo). Two patients (1 assigned to 10 mg GS-5885 and 1 assigned to placebo) were excluded from analyses because of study drug administration error.

### Safety Assessments

GS-5885 was well tolerated at all doses evaluated. No serious adverse events were reported, and no patients interrupted or discontinued dosing because of adverse events. From the 70 patients included in the safety analysis, 22 reported a total of 41 clinical adverse events (Table 2). Headache was the most common adverse event considered related to study drug (n=4/70, 6%). There was no dose-dependent trend apparent for any adverse events. All adverse events were mild or moderate in severity (2 moderate adverse events in the 1-mg and 30-mg groups and 4 moderate adverse events in the placebo group). In the 1-mg cohort, the 2 moderate adverse events were both rash; in the 30-mg cohort, the 2 moderate adverse events were headache and frequent urination. There were no clinically significant abnormalities as judged by physical examination, ECG, or clinical laboratory assessments.

### Pharmacokinetic Assessments

GS-5885 exhibited time-independent, near-linear pharmacokinetics (Figure 1 and Table 3). Maximal concentrations were achieved 4 to 6 hours (median $T_{max}$) after dosing across all dose

groups. At the 1-mg dose, all patients had quantifiable concentrations for up to 24 hours after dosing on Day 3. The median apparent plasma half-life across cohorts from 3 mg to 90 mg ranged between 22 to 50 hours, supporting once-daily dosing. Pharmacokinetic parameters of 10 mg GS-5885 were similar between HCV genotype 1a or 1b infection. Except at the 1-mg dose, plasma concentrations 24 hours following dosing well exceeded the protein-adjusted mean $EC_{90}$ for genotype 1 [18]. Specifically, mean plasma GS-5885 $C_{tau}$ on Day 3 for the 3-, 10-, 30-, and 90-mg doses were approximately 2.6-, 10.6-, 51.1- and 127.4-fold, respectively, higher than the protein-binding-adjusted mean $EC_{90}$ value against HCV genotype 1a (0.91 ng/mL).

**Efficacy Assessments**

***Antiviral response.*** Administration of GS-5885 resulted in rapid reductions in HCV RNA concentrations at all doses tested. By 8 hours post 1$^{st}$ dose, median reductions in HCV RNA were ≥2 $\log_{10}$ IU/mL in cohorts dosed at 3 mg and above. In all cohorts receiving ≥3 mg GS-5885 daily, median HCV RNA reductions were ≥2.5 $\log_{10}$ IU/mL at 12 hours and ≥3 $\log_{10}$ IU/mL by 36 hours. Overall, median maximal reductions from baseline were >3 $\log_{10}$ IU/mL for all dosing cohorts except the 1-mg group, which had a median (range) maximal reduction of 2.3 (0.95, 3.57) $\log_{10}$ IU/mL. Median (range) maximal HCV RNA reductions for the other groups were as follows: 3.1 (2.72, 3.66) for 3 mg, 3.2 (1.60, 3.67) for genotype 1a patients receiving 10 mg, 3.3 (2.28, 4.13) for genotype 1b patients receiving 10 mg, 3.3 (0.88, 4.17) for 30 mg, and 3.1 (1.48, 4.03) for 90 mg. For placebo, the median (range) maximal HCV RNA reduction was 0.3 (0.03, 0.66) $\log_{10}$ IU/mL. Median maximal HCV RNA reductions were similar for genotype 1a and 1b patients receiving 10 mg GS-5885; however, HCV RNA suppression was more sustained in genotype 1b patients (Figure 2). No patient in any cohort experienced viral rebound, defined as HCV RNA increase by >1 $\log_{10}$ from nadir during the dosing period. *IL28B* status (CC vs. non-

CC) did not appear to influence HCV RNA decline (data not shown).

***Exposure-response modeling.*** The simple $E_{max}$ model adequately described the relationship between GS-5885 exposure (assessed as AUC over the dosing interval) and maximum reductions in HCV RNA concentrations. $E_{max}$ values predicted by the model were close to the observed values. As seen in Figure 3, $E_{max}$ modeling indicated that exposures achieved following administration of 30 mg GS-5885 provide >95% of maximal antiviral response in genotype 1a infected subjects. Inter-subject variability in exposure/response varied across cohorts, with the highest variability observed at the 90-mg dose. However, fairly robust responses were observed through the range of exposures achieved.

***NS5A sequence analysis.*** Population sequencing performed for all patients at baseline revealed 5 patients with detectable pre-existing resistance-associated NS5A mutations. Four of these 5 were identified in GT1a patients, and 2 of the 4 had maximal HCV RNA reductions $\leq$1.6 $\log_{10}$ IU/mL (Figure 4). NS5A mutation Q30E/Q was identified in one of these patients (Figure 4A) and L31M in the other (Figure 4B). A third patient, dosed at 10 mg GS-5885, had a maximal HCV RNA reduction of -1.6 $\log_{10}$ IU/mL with no detectable resistance mutations by population sequencing, which has a limit of detection of ~25%. In this third patient (Figure 4C), 454 pyrosequencing of the baseline sample revealed that 12% of the viral species harbored an NS5A Y93C mutation. For all 3 of these patients, the mutations observed at baseline were predominant at Days 4 and 14. Plasma exposures of GS-5885 in these 3 patients were similar to others within their groups.

Treatment-emergent mutations in NS5A were assessed by population sequencing at Day 4 (approximately 24 hours after the last dose of GS-5885) and Day 14. In the patients with genotype 1a HCV, the spectrum of detected resistance mutations was complex (Table 4). Of the

10 HCV genotype 1a patients dosed with 1 mg GS-5885, 3 had resistance mutations detected at NS5A positions 30 or 31 by population sequencing at either Day 4 or Day 14. No change from baseline at known resistance amino acid positions (NS5A amino acids 28, 30, 31, 93) were detected for the remaining 7 patients at Day 4 and Day 14. Irrespective of racial background or ethnicity, all patients dosed at ≥3 mg GS-5885 had resistance mutations detected by population sequencing. The fold shift in $EC_{50}$ of GS-5885 versus these mutants suggests M28T had the lowest resistance and Y93H had the highest (Table 4). M28T and Q30H were only detected in the 1-, 3-, and 10-mg cohorts with HCV genotype 1a. At the 30- and 90-mg doses, these two mutations were not detected, which is consistent with suppression of lower-level resistant variants at higher doses. Conversely, the highest-level resistant variants among patients with genotype 1a virus, Y93C and Y93H, were selected more frequently in patients who received 90 mg GS-5885. The 2 resistance mutations with the broadest representation across dosing cohorts with genotype 1a HCV were Q30R and L31M, with Q30R having the highest frequency. All 10 patients with genotype 1b HCV, who received 10 mg GS-5885, harbored Y93H following treatment, consistent with Y93H being the primary GS-5885 resistance mutation selected in the genotype 1b replicon (data not shown).

**DISCUSSION**

This is the first report of the safety and antiviral activity of GS-5885 in HCV-infected patients. In this study of patients with genotype 1 HCV, 3 days of once-daily dosing with GS-5885 were well tolerated and led to substantial declines in HCV RNA during the dosing interval. The median maximal reduction in HCV RNA ranged from 2.3 $\log_{10}$ IU/mL (1 mg QD) to 3.3 $\log_{10}$ IU/mL (10 mg QD in genotype 1b and 30 mg QD). Median maximal reductions were >3 $\log_{10}$ IU/mL for all groups receiving 3 mg or higher GS-5885 daily, and the magnitude of viral suppression was similar between them. GS-5885 produced a rapid reduction in HCV RNA over the initial 12 hours of therapy, and the response was greater than has been reported for telaprevir or boceprevir monotherapies [19,20]. The kinetics of HCV RNA reductions over the first 24 hours after initiating GS-5885 were similar to those seen with a single dose of the NS5A inhibitor BMS-790052 [15]. Theoretically, a more rapid and profound reduction of HCV RNA may result in better suppression of wild type virus and reduce the likelihood of de novo resistant mutants during treatment. Whether GS-5885 provides an advantage in clinical response over other classes of HCV inhibitors warrants further study.

In vitro replicon data demonstrate that the antiviral activity of GS-5885 is less against genotype 1a than 1b (mean $EC_{50}$ versus 1a = 34 pM; mean $EC_{50}$ versus 1b = 4 pM). Although the mean maximal HCV RNA reductions with 10 mg GS-5885 in genotype 1a and 1b patients were not vastly different (3.2 vs. 3.3 $\log_{10}$ IU/mL), viral suppression lasted longer after treatment cessation in the 1b patients. Furthermore, the pattern of resistance mutations was significantly different between 1a and 1b patients, suggesting that antiviral activity differs in these two patient populations [21].

Exposure-response analyses indicate that GS-5885 doses ≥30 mg provide >95% of

maximal antiviral responses in patients with genotype 1a HCV. The plasma $C_{trough}$ concentrations of GS-5885 at 30 mg and 90 mg were 230-fold and 572-fold higher than the protein-adjusted genotype 1a $EC_{50}$, respectively. The results suggest that GS-5885 dosing beyond 90 mg is unlikely to cause further meaningful reductions in HCV RNA.

Three patients in the current study had substantially reduced HCV RNA reductions as compared with median values in their respective cohorts. These patients were subsequently found to have variants in their baseline NS5A sequence that are known to confer resistance to GS-5885. Whether these findings are clinically relevant depends in part on the frequency of these mutations in HCV-infected patients. Studies assessing the prevalence of known HCV drug-resistance mutations in untreated HCV-infected patients have focused on NS3 protease inhibitors or NS5B non-nucleoside inhibitors, and their results indicate dominant resistant mutations are common [22,23]. Few data are available regarding the prevalence of NS5A resistance mutations in genotype 1a and 1b infected patients. In the European HCV database of over 500 genotype 1a sequences, the reported prevalence of mutations Y93C and L31M is 0.2% and Q30E is 0.0% [24,25]. Mutants observed in this study retained full susceptibility to other classes of antivirals, including direct acting anti-HCV agents and interferon (data not shown).

The resistance profile for GS-5885 mapped primarily to residues 28, 30, 31, and 93 of HCV NS5A. In genotype 1a patients, resistance mutations were spread across these residues, whereas in genotype 1b patients, Y93H was the sole resistance variant noted by population sequencing. For genotype 1a patients, NS5A, M28T, and Q30H resistance mutations were not detected in patients receiving GS-5885 ≥30 mg. This is consistent with a lower-fold resistance observed with these 2 mutations against the genotype 1a replicon when compared with other variants (Table 4). Plasma $C_{trough}$ concentrations of GS-5885 at 30 mg or 90 mg were higher than

protein-adjusted $EC_{50}$ values for these 2 mutants (Tables 3 and 4). While the replication capacity of the genotype 1a M28T mutant is 31%, the replication level of the Q30H mutant is substantially higher at 75% of the wild-type 1a replicon [15]. Suppression of these two mutants with GS-5885 at 30 mg or higher potentially increases the resistance barrier of GS-5885-containing combination regimens.

The picomolar potency, pharmacokinetic properties supporting once daily dosing, and current safety profile of NS5A inhibitors make them an attractive target for drug development [26]. In addition, GS-5885 demonstrated additive to synergistic antiviral activity in vitro when combined with IFN, RBV, and multiple other classes of direct-acting antivirals including GS-9451 (protease inhibitor), GS-9669 (non-nucleoside polymerase inhibitor), GS-9190 (non-nucleoside polymerase inhibitor), and GS-6620 (nucleoside analog) (Gilead Sciences, data on file). Therefore, GS-5885 in combination with PEG/RBV or direct-acting antivirals is currently being evaluated in Phase 2 studies. The antiviral activity, including subtype differences, observed with GS-5885 is similar to BMS-790052, the only other NS5A inhibitor also in Phase 2 development [15]. The pattern of resistance mutations selected by GS-5885 is also similar to that of BMS-790052 [15], where the Y93H mutation dominated with genotype 1b virus, and mutations at residues 28, 30, 31, and 93 were predominant with genotype 1a virus. Furthermore, the levels of resistance against the Q30R, Y93C, and Y93H single mutations in genotype 1a were similar between GS-5885 and BMS-790052. However, the in vitro activity as measured by $EC_{50}$ against other resistant mutations varies for the two compounds. In genotype 1b virus, BMS-790052 is significantly more active against the Y93H mutation (0.05 nM for BMS-790052 vs. 5 nM for GS-5885) [24]. In contrast, in genotype 1a, GS-5885 is 4-5 times more active against the

M28T and Q30H mutations than BMS-790052, while the latter is approximately 2 times more potent against the L31M mutation.

In conclusion, data from the current study demonstrate that GS-5885 is a potent, once-daily inhibitor of HCV replication in vivo and is well tolerated in treatment-naïve genotype 1 HCV infected patients. These results confirm NS5A as an important target for direct acting anti-HCV agents and support further clinical development of GS-5885. GS-5885 is currently being evaluated in multiple combination therapies with and without PEG-IFN alfa-2a in Phase 2 studies in treatment-naive and treatment-experienced HCV-infected patients.

**ACKNOWLEDGMENTS**

The authors thank the patients for their participation in this study. We are also grateful to the following investigators for their study participation: Steven Komjathy, MD, Charles River Clinical Services, Tacoma, Washington; Michael DeMicco, MD, Advanced Clinical Research Institute, Anaheim, California; Abel Murillo, MD, Elite Research Institute, Miami, Florida; Frederick Jenkin, MD, Synergy, National City, California; and Kenneth Kim, MD, West Coast Clinical Trials, Cypress, California. The authors also acknowledge the assistance of Jennifer King, PhD, in the preparation of the manuscript.

**REFERENCES**

[1] Manns M.P., McHutchison J.G., Gordon S.C., Rustgi V.K., Shiffman M., Reindollar R., et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet 2001; 358: 958-965.

[2] Fried M.W., Shiffman M.L., Reddy K.R., Smith C., Marinos G., Gonçales FL. Jr, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 2002; 347: 975-982.

[3] Hadziyannis S.J., Sette H. Jr, Morgan T.R., Balan V., Diago M., Marcellin P., et al. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. Ann Intern Med. 2004; 140: 346-355.

[4] McHutchison J.G., Lawitz E.J., Shiffman M.L., Muir A.J., Galler G.W., McCone J., et al. Peginterferon alfa-2b or alfa-2a with RBV for treatment of hepatitis C infection. N Engl J Med 2009; 361: 580-593.

[5] Hezode C., Forestier N., Dusheiko G., Ferenci P., Pol S., Goeser T., Bronowicki J.P., et al. Telaprevir and peginterferon with or without ribavirin for chronic HCV infection. N Engl J Med 2009; 360: 1839-1850.

[6] McHutchison J.G., Everson G.T., Gordon S.C., Jacobson I.M., Sulkowski M., Kauffman R., et al. Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. N Engl J

Med 2009; 360: 1827-1838.

[7] Kwo P.Y., Lawitz E.J., McCone J., Schiff E.R., Vierling J.M., Pound D., et al. Efficacy of boceprevir, an NS3 protease inhibitor, in combination with peginterferon alfa-2b and ribavirin in treatment-naive patients with genotype 1 hepatitis C infection (SPRINT-1): an open-label, randomised, multicentre phase 2 trial. Lancet 2010; 376: 705-716.

[8] Poordad F., McCone J. Jr, Bacon B.R., Bruno S., Manns M.P., Sulkowski M.S., et al. Boceprevir for untreated chronic HCV genotype 1 infection. N Engl J Med 2011; 364: 1195-1206.

[9] Jacobson I.M., McHutchison J.G., Dusheiko G.M., Di Bisceglie A.M., Reddy R., Bzowej N.H., et al. Telaprevir for previously untreated chronic hepatitis C infection. N Engl J Med. 2011; 364: 2405-2416.

[10] Sherman K.E., Flamm S.L., Afdhal N.H., Nelson D.R., Sulkowski M.S., Everson G.T., et al. Telaprevir in cominbation with peginterferon alfa-2a and ribavirin for 24 or 48 weeks in treatment-naïve genotype 1 HCV patients who achieved an extended rapid viral response: Final results of the phase 3 ILLUMINATE Study. Hepatology. 2010; 52(Suppl 1): Abstract LB-2.

[11] Sarrazin C., Zeuzem S. Resistance to direct antiviral agents in patients with hepatitis C virus infection. Gastroenterology 2010; 138: 447-462.

[12] Evans M.J., Rice C.M., Goff S.P. Phosphorylation of hepatitis C virus nonstructural protein 5A modulates its protein interactions and viral RNA replication. Proc Natl Acad Sci U S A 2004; 101: 13038-13043.

[13] Tellinghuisen T.L., Foss K.L., Treadaway J. Regulation of hepatitis C virion production via phosphorylation of the NS5A protein. PLoS pathogens 2008; 4: e1000032.

[14] Targett-Adams P., Graham E.J., Middleton J., Palmer A., Shaw S.M., Lavender H., et al. Small molecules targeting hepatitis C virus-encoded NS5A cause subcellular redistribution of their target: insights into compound modes of action. J Virol 2011; 85: 6353-6368.

[15] Gao M., Nettles R.E., Belema M., Snyder L.B., Nguyen V.N., Fridell R.A., et al. Chemical genetics strategy identifies an HCV NS5A inhibitor with a potent clinical effect. Nature 2010; 465: 96-100.

[16] Pol S., Ghalib R.H., Rustgi V.K., Martorell C., Everson G.T., Tatum H.A., et al. First report of SVR12 for a NS5A replication complex inhibitor BMS-790052 in combination with PEG-IFNa-2A and RBV: Phase 2A trial in treatment-naïve HCV-genotype-1 subjects. J Hepatol 2011; 54(Suppl 1): S544-S545.

[17] Lawitz E., Gruener D., Hill J., Marbury T., Komjathy S., DeMicco M., et al. Three-day, dose ranging study of the HCV NS5A inhibitor GS-5885. J Hepatol 2011; 54(Suppl 1): S481-S482.

[18] Mo H., Yang C., Wang K., Wang Y., Huang M., Murray B., et al. Estimation of inhibitory quotient using a comparative equilibrium dialysis assay for prediction of viral response to hepatitis C virus inhibitors. J Viral Hepat 2011; 18: 338-348.

[19] Reesink H.W., Zeuzem S., Weegink C.J., Forestier N., van Vliet A., van de Wetering de Rooij J., et al. Rapid decline of viral RNA in HCV patients treated with VX-950: a phase 1b, placebo-controlled, randomized study. Gastroenterology 2006; 131: 997-1002.

[20] Sarrazin C., Rouzier R., Wagner F., Forestier N., Larrey D., Gupta S.K., et al. SCH 503034, a novel HCV protease inhibitor, plus pegylated intereron alpha-2b for genotype 1 nonresponders. Gastroenterology 2007; 132: 1270-1278.

[21] Nettles R.E., Sevinsky H., Chung E., Burt D., Xiao H., Marbury T.C., et al. BMS-790052, a first-in-class potent hepatitis C virus NS5A inhibitor, demonstrates multiple-dose proof-of-concept in subjects with chronic GT1 HCV infection. Hepatology 2010; 52(Suppl S1): Poster 1881.

[22] Kuntzen T., Timm J., Berical A., Lennon N., Berlin A.M., Young S.K., et al. Naturally occurring dominant resistance mutations to hepatitis C virus protease and polymerase inhibitors in treatmet-naïve patients. Hepatology 2008; 48: 1769-1778.

[23] Gaudieri S., Rauch A., Pfafferott K., Barnes E., Cheng W., McCaughan G., et al. Hepatitis C virus drug resistance and immune-driven adaptations: relevance to new antiviral therapy. Hepatology 2009; 49: 1069-1082.

[24] Fridell R.A., Qui D., Wang C., Valera L., Gao M. Resistance analysis of the hepatitis C virus NS5A inhibitor BMS-790052 in an in vitro replicon system. Antimicrob Agents Chemother 2010; 54: 3641-3650.

[25] Combet C., Garnier N., Charavay C., Grando D., Crisan D., Lopez J., et al. euHCVdb: the European hepatitis C virus database. Nucleic Acids Res 2007; 35: D363-366.

[26] Schlutter J. Therapeutics: new drugs hit the target. Nature 2011; 474: S5-S7.

**Table 1. Patient Demographics and Baseline Characteristics**

| Demographic Characteristic | GS-5885 | | | | | Placebo |
|---|---|---|---|---|---|---|
| | 1 mg (n=10) | 3 mg (n=10) | 10 mg (n=20) | 30 mg (n=10) | 90 mg (n=10) | (n=12) |
| Age at baseline (years) | | | | | | |
| Mean (SD) | 49 (9.0) | 47 (7.9) | 50 (9.5) | 47 (10.5) | 49 (6.3) | 47 (6.2) |
| Male, n (%) | 9 (90) | 7 (70) | 16 (80) | 7 (70) | 6 (60) | 7 (58) |
| Race, n (%) | | | | | | |
| White | 5 (50) | 5 (50) | 14 (70) | 8 (80) | 7 (70) | 8 (67) |
| Black | 4 (40) | 5 (50) | 6 (30) | 2 (2) | 3 (30) | 3 (25) |
| Other | 1 (10) | 0 | 0 | 0 | 0 | 1 (8) |
| Body mass index ($kg/m^2$) | | | | | | |
| Mean (SD) | 27.3 (4.05) | 26.9 (3.68) | 26.2 (4.02) | 25.7 (3.84) | 27.5 (3.35) | 26.5 (3.46) |
| Serum creatinine clearance (mL/min) | | | | | | |
| Mean (SD) | 96.6 (15.17) | 96.9 (24.44) | 90.3 (16.59) | 83.1 (13.25) | 84.8 (8.85) | 90.0 (14.67) |
| $Log_{10}$ HCV RNA (IU/mL) | | | | | | |
| Mean (SD) | 6.7 (0.64) | 6.4 (0.47) | 6.6 (0.56) | 6.3 (0.49) | 6.8 (0.52) | 6.8 (0.51) |
| HCV genotype, n (%) | | | | | | |
| 1a | 10 (100) | 10 (100) | 10 (50) | 10 (100) | 10 (100) | 10 (83) |
| 1b | | | 10 (50) | | | 2 (17) |
| *IL28B* genotype[a], n | | | | | | |
| CC | 1 | 2 | 4 | 1 | 0 | 2 |
| CT | 5 | 2 | 10 | 6 | 0 | 2 |
| TT | 0 | 6 | 3 | 2 | 0 | 4 |

BMI, body mass index (weight [kg]/height [$cm^2$]); HCV, hepatitis C virus
Creatinine clearance estimated by Cockcroft-Gault equation.
[a]Not obtained for every subject.

**Table 2. Treatment-Emergent Adverse Events in ≥2 Patients Across Treatment Groups**

| | GS-5885 | | | | | Placebo |
|---|---|---|---|---|---|---|
| | **1 mg (n=10)** | **3 mg (n=10)** | **10 mg (n=19)** | **30 mg (n=10)** | **90 mg (n=10)** | **(n=11)** |
| Patients with ≥1 adverse event, n (%) | 4 (40) | 5 (50) | 5 (26) | 3 (30) | 1 (10) | 4 (36) |
| Total number of events, n | 11 | 7 | 7 | 4 | 2 | 10 |
| Adverse events occurring in ≥2 patients, n (%) | | | | | | |
| Nausea | 1 (10) | | 1 (5) | | | 1 (9) |
| Upper respiratory tract infection | | 1 (10) | 1 (5) | | | |
| Difficult blood draw | | 3 (30) | | | | |
| Back pain | | | 1 (5) | | | 1 (9) |
| Headache | 1 (10) | 2 (20) | 2 (11) | 1 (10) | | |
| Somnolence | 1 (10) | | | 1 (10) | | 1 (9) |
| Dizziness | 1 (10) | | | | | 1 (9) |
| Urinary frequency | | | | 2 (20) | | |
| Rash | 2 (20) | | | | | |

**Table 3. Mean Pharmacokinetic Parameters of GS-5885 at Day 1 and Day 3 in Genotype 1 HCV-Infected Patients**

| | GS-5885 | | | | |
|---|---|---|---|---|---|
| | **1 mg (n=10)** | **3 mg (n=10)** | **10 mg (n=19)[a]** | **30 mg (n=10)** | **90 mg (n=10)** |
| **Day 1** | | | | | |
| $C_{max}$ (ng/mL) | 1.2 (57.2) | 4.5 (33.3) | 18.7 (36.6) | 67.0 (45.9) | 166.5 (44.5) |
| $C_{last}$ (ng/mL) | 1.1 (11.9) | 1.5 (43.7) | 5.8 (41.6) | 22.5 (53.1) | 59.2 (52.9) |
| $AUC_{inf}$ (ng•h/mL) | 30.9 (51.8)[b] | 103.1 (85.1) | 388.1 (39.0) | 1491.0 (57.0) | 4137.4 (67.1) |
| **Day 3** | | | | | |
| $C_{max}$ (ng/mL) | 2.2 (39.7) | 6.1 (56.6) | 25.3 (40.0) | 103.3 (57.5) | 247.7 (45.4) |
| $C_{tau}$ (ng/mL) | 0.3 (161.0) | 2.4 (73.6) | 9.7 (41.5) | 46.5 (62.7) | 115.9 (42.6) |
| $AUC_{tau}$ (ng•h/mL) | 34.0 (29.8)[c] | 89.7 (54.6) | 368.8 (39.0) | 1592.4 (59.5) | 3815.5 (42.1) |
| $T_{1/2}$ (h) | 13.0 (7.7, 17.8) | 22.8 (13.1, 36.8) | 39.9 (28.5, 47.2) | 41.7 (25.8, 53.4) | 49.7 (37.8, 54.3) |

Data are presented as mean (CV %); $T_{1/2}$ presented as median (Q1, Q3).
[a]All cohorts comprised HCV genotype 1a patients, except for the 10 mg GS-5885 cohort, which comprised both genotype 1a and 1b patients.
[b]n=6.
[c]n=8.

**Table 4. Frequency of GS-5885 Resistance Mutations Detected by Population Sequencing Across Dosing Cohorts**

| | | Number of Patients that Developed NS5A Mutations (Change from Baseline) at Day 4 and/or Day 14[a] | | | | | |
|---|---|---|---|---|---|---|---|
| **Mutation** | **SDM Fold Shift vs 1a-H77** | **1 mg GT1a** | **3 mg GT1a** | **10 mg GT1a** | **30 mg GT1a** | **90 mg GT1a** | **10 mg GT1b** |
| M28T | 25 | - | 6 | 1 | - | - | - |
| Q30H | 73 | 1 | 3 | 1 | - | - | - |
| L31M | 140 | 1 | 6 | 4 | 5 | 4 | - |
| Q30R | 170 | 3 | 9 | 7 | 7 | 7 | - |
| Y93C | 327 | - | 1 | 3 | 2 | 5 | - |
| Y93H | 3309 | - | - | - | 1 | 2 | - |
| Y93H | 1319[b] | - | - | - | - | - | 10 |

[a] Mutations determined by population sequencing and occur in combinations in some patients

[b] Y93H in genotype 1b-con-1 replicon compared with wild type 1b-con-1

**FIGURE LEGENDS**

**Figure 1. Mean Plasma Concentration-Time Profile of GS-5885 After 3 Days of Once-Daily Dose Administration of 1-90 mg in HCV-Infected Patients.** Vertical error bars represent standard deviation around the mean. Time zero on the Y axis is the time at which the Day 3 dose was administered.

**Figure 2**. **Median $Log_{10}$ HCV RNA Change from Baseline.** Patients received GS-5885 or placebo once daily for 3 days. Arrows indicate time of dosing.

**Figure 3. GS-5885 Exposure and Changes in HCV RNA ($log_{10}$ IU/mL).** Exposure–response relationship was examined using an $E_{max}$ model (Phoenix WinNonlin v 6.0). Horizontal error bars represent standard deviation around the mean exposure; vertical error bars represent standard deviation around the mean response.

**Figure 4. Plasma HCV RNA Versus Time for Patients with Baseline Drug Resistance Mutations (DRMs).**

HCV RNA IU/mL $log_{10}$ change from baseline is plotted over time (hours) for three GT1a patients with baseline DRMs (filled symbols) and compared to the median (open symbols) of the remaining patients in each respective cohort. The presence of each mutation at baseline, Day 4, and Day 14 is indicated on each graph. Time of GS-5885 dosing is indicated below the X-axis. (A) Patient dosed at 30 mg GS-5885 with a mixture of Q30E/Q by population sequencing at baseline. Q30E is predominant at Days 4 and 14. (B) Patient dosed at 90 mg GS-5885 with a predominant L31M mutation at baseline, Day 4, and 14. (C) Patient dosed at 10 mg GS-5885

with 12% Y93C present at baseline. Y93C is predominant by population sequencing at Days 4 and 14.