GS-9857 in patients with chronic hepatitis C virus genotype 1–4 infection: a randomized, double-blind, dose-ranging phase 1 study

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SUMMARY. GS-9857, an inhibitor of the hepatitis C virus (HCV) nonstructural protein (NS) 3/4A, demonstrates potent antiviral activity against HCV genotypes 1–6 and improved coverage against commonly encountered NS3 resistance-associated variants (RAVs). In this study, the safety, tolerability, antiviral activity and pharmacokinetics (PK) of GS-9857 were evaluated in patients with chronic HCV genotype 1–4 infection. Patients with genotype 1–4 infection received placebo or once-daily GS-9857 at doses ranging from 50 to 300 mg for 3 days under fasting conditions. GS-9857 was well tolerated: all reported adverse events (AEs) were mild or moderate in severity. Diarrhoea and headache were the most commonly reported AEs. Grade 3 or 4 laboratory abnormalities were observed in 17% of patients receiving GS-9857; there were no Grade 3 or 4 abnormalities in alanine aminotransferase, aspartate aminotransferase or alkaline phosphatase levels. GS-9857 demonstrated potent antiviral activity in patients with chronic HCV infection, achieving mean and median maximum reductions in HCV RNA of ≥3 log10 IU/mL following administration of a 100-mg dose in patients with HCV genotype 1a, 1b, 2, 3 or 4 infection. The antiviral activity of GS-9857 was unaffected by the presence of pretreatment NS3 RAVs. In patients with genotype 1–4 infection, GS-9857 exhibited linear PK and was associated with a median half-life of 29–42 h, supporting once-daily dosing. Thus, the tolerability, efficacy and pharmacokinetic profile of GS-9857 support its further evaluation for treatment of patients with chronic HCV infection.

Keywords: GS-9857, hepatitis C virus, NS3/4A protease inhibitor.

Abbreviations: AE, adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AUC0–24, area under the plasma concentration vs time curve up to 24 h; AUCinf, area under the plasma concentration vs time curve extrapolated to infinity; AUClast, area under the plasma concentration vs time curve from zero to the last quantifiable concentration; BMI, body mass index; Cmax, maximum plasma concentration; CL/F, apparent oral clearance of drug following administration; Cmax, maximum plasma concentration; DAA, direct-acting antiviral; ECG, electrocardiogram; EC50, dose or systemic exposure required to produce 50% of the maximal drug induced anti-HCV activity; Emax, maximal anti-HCV activity; HCV, hepatitis C virus; LC/MS/MS, liquid chromatography tandem mass spectroscopy; LLOQ, lower limit of quantification; MedDRA, Medical Dictionary for Regulatory Activities; NS, nonstructural protein; PK, pharmacokinetics; PD, pharmacodynamics; RAV, resistance-associated variant; RNA, ribonucleic acid; t1/2, elimination half-life; Tlast, time of last observed quantifiable plasma drug concentration; Tmax, time of maximum plasma concentration; ULN, upper limit of normal; λz, terminal elimination rate constant.

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Maribel Rodriguez-Torres, who for many years has been a leading light in hepatitis C research, passed away on December 28, 2015. The authors sadly regret the loss of an esteemed colleague and dear friend.

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INTRODUCTION

In recent years, significant progress has been made in developing treatments for patients with chronic hepatitis C virus (HCV) infection [1]. With the introduction of direct-acting antiviral agents (DAAs), highly effective therapeutic regimens with sustained virologic response rates of >90% are now available for most patients [1–3]. Despite this progress, there remain unmet medical needs, specifically for shorter treatment regimens and for salvage therapies to treat patients who have not responded to existing regimens [2–4].

GS-9857 is a nonstructural protein (NS) 3/4A inhibitor with potent in vitro activity against HCV genotypes 1–6 and an improved resistance profile against commonly encountered genotype 1 NS3 resistance-associated variants (RAVs) relative to other HCV protease inhibitors [5]. In a previous study, GS-9857 was well tolerated and exhibited linear pharmacokinetics (PK) when administered as single or multiple doses ranging from 30 to 300 mg under fasting conditions to healthy volunteers [6]. GS-9857 is in development for possible use as a component of a combination DAA regimen for the treatment of chronic HCV.

We conducted a phase 1b study to evaluate the safety, tolerability, antiviral activity and PK of GS-9857 in patients with chronic HCV infection. The primary objectives of this study were to evaluate the safety and tolerability of multiple doses of GS-9857 and to determine the antiviral activity of GS-9857 in patients with genotype 1–4 HCV infection. Secondary objectives were to evaluate the PK and pharmacodynamics of GS-9857, to characterize plasma HCV ribonucleic acid (RNA) during treatment and after treatment discontinuation and to identify sequence changes in the NS3 coding region of HCV in the 48 weeks following the administration of multiple doses of GS-9857.

METHODS

Study design

This was a double-blind, multicentre, randomized, placebo-controlled study evaluating the safety, tolerability, antiviral activity and PK of GS-9857 in patients with chronic HCV infection (NCT02185794) (Fig. 1). The approved study protocol allowed for up to 10 unique dosing cohorts, of which the first three cohorts were placebo-controlled and dosed in a fasted state, while the remaining, adaptive cohorts did not include a placebo control and could be dosed in either a fasted or a fed state. The final study included seven cohorts; results from the five cohorts that received GS-9857 under fasting conditions are included here, while results from the two cohorts that received GS-9857 treatment under fed conditions will be reported separately.

Study participants

Male and female patients with chronic HCV infection, between 18 and 65 years (inclusive) of age, and with a body mass index (BMI) ranging from 19 to 34 kg/m² were eligible to participate in this study. At screening, eligible patients were required to have alanine aminotransferase (ALT) ≤5 × upper limit of normal (ULN), aspartate aminotransferase (AST) ≤5 × ULN, direct bilirubin ≤ULN, albumin ≥3.5 g/dL, international normalized ratio ≤1.5 × ULN, haemoglobin ≥11 g/dL for females and ≥12 g/dL for males, platelets ≥90 000/mm³ and creatinine clearance ≥70 mL/min. Patients were included in this study only if they had HCV RNA levels ≥5 log₁₀ IU/mL and HCV genotype (Covance Central Laboratory Services, Indianapolis, IN, USA) 1a, 1b, 2, 3 or 4 at screening and had not previously been treated with HCV NS3/4A protease inhibitors.

Patients were excluded from this study if they were co-infected with hepatitis B virus or human immunodeficiency virus; had evidence of cirrhosis; had chronic liver disease unrelated to HCV; or had any clinically relevant electrocardiogram (ECG) abnormalities at screening. Pregnant or lactating patients were also excluded, as were those with current or prior clinical hepatic decompensation or evidence of hepatocellular carcinoma; a history of syncope, palpitations or unexplained dizziness; a history of significant cardiac disease; or a family history of Long QT syndrome.
Procedures

Patients were screened within 30 days prior to the first study drug dose administration. Patients satisfying study criteria were randomized and admitted to the study facility on day–1 to commence dosing on baseline/day 1. Following treatment with the study drug once daily for 3 days (days 1, 2, and 3), patients were discharged from the study facility on day 4. Short-term follow-up occurred on days 5, 6, 7, 8, and 10, and long-term follow-up visits were scheduled for weeks 12, 24, and 48.

Patients with HCV genotype 1a, HCV genotype 2 and HCV genotype 3 received double-blinded GS-9857 (50, 100 or 300 mg for patients with HCV genotype 1a and 3, and 100 mg for patients with HCV genotype 2) or placebo once daily under fasting conditions for 3 days (Fig. 1). GS-9857 100 mg was administered once daily for 3 days under fasting conditions to patients with HCV genotype 1b and HCV genotype 4.

All patients underwent an overnight fast >10 h prior to dosing. Except for the 240 mL of water provided with the study dose, fluids were prohibited for 1 h before and 2 h after dosing. A meal was provided to all patients after the 4-h postdose plasma collection.

This study was approved by an institutional review board and conducted in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki. All patients enrolled in this study were required to understand and provide written informed consent prior to study participation.

Study assessments

Safety was assessed by monitoring adverse events (AEs) and performing clinical laboratory evaluations, vital signs measurements and 12-lead ECGs. AEs were categorized using the Medical Dictionary for Regulatory Activities, Version 18 (MeDRA, VA, USA).

To evaluate GS-9857 antiviral activity against HCV in patients with genotype 1–4 HCV infection, the primary efficacy endpoint, the change in HCV RNA from baseline to each postdose assessment up to day 10 by treatment and cohort: and the number and percentage of patients with HCV RNA <LLOQ, <LLOQ detected and <LLOQ target not determined at each postdose assessment, summarized by treatment.

Plasma samples for resistance surveillance were collected at day 1, and at 24, 48, 72 and 96 h following the first dose; and on the mornings of days 7 and 10. The NS3 gene was amplified and sequenced by deep sequencing using MiSeq technology (DL Diagnostic Laboratory, Rijswijk, the Netherlands) with a 1% assay cut-off. NS3 RAVs were defined as changes from the genotype consensus sequence and included the following substitutions: V36A/G/M/L/M, Q41R, F43L/S, T54A/C/G/S, V55A/I, Q80K/R/L, S122R, R155C/G/K/M/T/Q/S/W, A156F/G/N/P/T/V/S, D168A/E/F/G/H/I/N/K/L/P/V/T/Y (Q is referenced in genotype 3) and I/V170A/T/L/E/V.

For pharmacokinetic evaluation, blood samples were collected after the administration of the first and the third dose at the following time points: predose, and at 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 16, 24 h postdose. Additionally, samples were collected at approximately 48, 72, 96, 120 and 168 h following the third dose. Plasma concentration of GS-9857 was determined using validated high performance liquid chromatography tandem mass spectroscopy (LC/MS/MS) bioanalytical methods. The assays were performed by inVentiv Health Clinical Lab, Inc. (Princeton, NJ, USA), for GS-9857. Pharmacokinetic parameters were estimated using WinNonlin software (Certara, NJ, USA) with standard noncompartmental methods.

The pharmacokinetic parameters calculated included area under the plasma concentration vs time curve from zero to the last quantifiable concentration (AUClast), area under the plasma concentration vs time curve extrapolated to infinity (AUCinf), area under the plasma concentration vs time curve up to 24 h (AUC0–24), maximum plasma concentration (Cmax), observed plasma concentration at 24 h postdose (C24), last observed quantifiable plasma drug concentration (Clast), time of maximum plasma concentration (Tmax), time of last observed quantifiable plasma drug concentration (Tlast), elimination half-life (t1/2), terminal elimination rate constant (λz) and apparent oral clearance of drug following administration (CL/F). Dose proportionality was evaluated based on AUC0–24, C24 and Cmax on days 1 and 3 using both the power model and the analysis of variance method. Accumulation indices for GS-9857 were summarized by comparing day 3 to day 1 for AUC0–24, C24 and Cmax.

Statistics

Formal power or sample size calculations were not performed to determine cohort size for this exploratory study. A sample size of two to eight patients per active treatment
group per cohort was deemed sufficient for the characterization of HCV RNA, PK and safety assessments. Statistical summaries and analyses were performed using SAS® software (SAS Institute, Cary, NC, USA). The efficacy analysis included all enrolled patients who received ≥1 dose of the study drug, and had ≥1 HCV RNA assessment. The safety analysis set included all patients receiving ≥1 dose of the study drug. The pharmacokinetic analysis set included all patients who received ≥1 dose of study drug and had ≥1 postdose concentration value for the analyte.

RESULTS

Patient disposition and demographics

Of the 67 patients who received treatment, 65 patients completed day 10 of follow-up. Of the two patients who discontinued prior to day 10 of the study, one with genotype 3 infection withdrew consent following treatment with 1 dose of GS-9857 100 mg, and a second patient with genotype 1a infection treated with GS-9857 300 mg was lost to follow-up after completion of study treatment. There were 31 patients who did not complete the 48 weeks of long-term follow-up: 4 were discontinued at the investigator’s discretion, 19 withdrew consent and 8 were lost to follow-up. The safety and efficacy analyses sets included 67 patients, whereas the PK analysis set consisted of 59 patients.

The mean age of study participants was 49 years, and their mean BMI was 27.5 kg/m² (Table 1). Most patients included in this study were male (70%) and white (69%). At baseline, genotype 1a (42%) subtype was the most prevalent among patients, followed by genotype 3a (31%), 1b (9%) and 2b (9%). Genotypes 2a/2c, 4 and 4a/4c/4d were each present in 3% of patients. The viral RNA load was comparable across treatment groups, and the mean viral RNA load for the safety population at baseline was 6.3 log₁₀ IU/mL.

Safety

Table 2 presents treatment-emergent AEs occurring in patients by treatment group. Overall, 11 patients (16.4%) experienced AEs, 9 of whom were dosed with GS-9857 (9/59; 15.3%) and 2 of whom received placebo (2/8; 25.0%). The incidence of treatment-related AEs was 6.8% (4/59) for patients receiving GS-9857 and 12.5% (1/8) for patients receiving placebo. No serious AEs, AEs leading to study drug discontinuation, or deaths occurred during the study. All AEs were mild or moderate in severity. The most common AEs were diarrhoea, occurring in 5.1% (3/59) of

| Table 1 Demographic and baseline characteristics |
|-----------------------------------------------|
| Placebo (N = 8) | GS-9857 50 mg (N = 14) | GS-9857 100 mg (N = 30) | GS-9857 300 mg (N = 15) | Total (N = 67) |
| Age in years, mean (range) | 52 (41–58) | 49 (34–62) | 52 (30–64) | 43 (30–56) | 49 (30–64) |
| Male, n (%) | 7 (87.5) | 9 (64.3) | 21 (70.0) | 10 (66.7) | 47 (70.1) |
| BMI in kg/m², mean (range) | 26.3 (21.5–32.9) | 29.0 (25.4–32.9) | 27.5 (20.1–33.6) | 26.6 (19.4–34.0) | 27.5 (19.4–34.0) |
| Race, n (%) | | | | | |
| White | 6 (75.0) | 10 (71.4) | 18 (60.0) | 12 (80.0) | 46 (68.7) |
| Black or African American | 2 (25.0) | 4 (28.6) | 12 (40.0) | 3 (20.0) | 21 (31.3) |
| Ethnicity, n (%) | | | | | |
| Non-Hispanic/Latino | 6 (75.0) | 7 (50.0) | 18 (60.0) | 7 (46.7) | 38 (56.7) |
| Hispanic/Latino | 2 (25.0) | 7 (50.0) | 12 (40.0) | 8 (53.3) | 29 (43.3) |
| HCV RNA (log₁₀ IU/mL); mean (range) | 6.6 (5.8–7.0) | 6.0 (5.8–7.0) | 6.4 (5.1–7.2) | 6.0 (4.7–7.1) | 6.3 (4.7–7.2) |
| HCV genotype, n (%) | | | | | |
| 1a | 4 (50.0) | 8 (57.1) | 8 (26.7) | 8 (53.3) | 28 (41.8) |
| 1b | 0 | 0 | 6 (20.0) | 0 | 6 (9.0) |
| 2a/2c | 0 | 0 | 2 (6.7) | 0 | 2 (3.0) |
| 2b | 2 (25.0) | 0 | 4 (13.3) | 0 | 6 (9.0) |
| 3a | 2 (25.0) | 6 (42.9) | 6 (20.0) | 7 (46.7) | 21 (31.3) |
| 4 | 0 | 0 | 2 (6.7) | 0 | 2 (3.0) |
| 4a/4c/4d | 0 | 0 | 2 (6.7) | 0 | 2 (3.0) |

BMI, body mass index; HCV, hepatitis C virus; SD, standard deviation; RNA, ribonucleic acid.
patients receiving GS-9857 and in 12.5% (1/8) of patients receiving placebo, and headache, occurring in 1.7% (1/59) of patients receiving GS-9857 and in 25.0% (2/8) of patients treated with placebo. The incidence of AEs was not correlated with the dose of the study drug.

Grade 3 laboratory abnormalities were detected in 15.3% (9/59) of patients, and Grade 4 abnormalities were detected in 1.7% (1/59) of patients receiving GS-9857 (Table S1). No placebo-treated patients exhibited Grade 3 or Grade 4 abnormalities in laboratory evaluations. The incidence of Grade 3 and Grade 4 laboratory abnormalities was not correlated with GS-9857 dose, nor was dose associated with AEs. Analysis of vital signs measurements and physical examination findings by treatment group or of ECG measurements by individual patient did not reveal clinically significant changes during the study relative to baseline.

**Efficacy**

**Antiviral response**

Administration of GS-9857 daily for 3 days resulted in a rapid decline in HCV RNA from pretreatment levels at all doses and across all genotypes (mean and median maximum HCV RNA reduction >3 log_{10} IU/mL), except among patients with genotype 3a infection who received GS-9857 50 mg (Table 3; Fig. 2). At all postdose assessments, HCV RNA levels were not appreciably different from baseline levels for any of the patients who received placebo.

Median HCV RNA levels were approximately 6 log_{10} IU/mL at baseline. Following treatment with 100 mg of GS-9857, median maximum decline in all groups was >3 log_{10} IU/mL (Table 3). The median maximum HCV RNA reduction was 4.5 log_{10} IU/mL for patients with HCV

### Table 2 Adverse events

| AE, n (%)  | Placebo (N = 8) | GS-9857 50 mg (N = 14) | GS-9857 100 mg (N = 30) | GS-9857 300 mg (N = 15) | Total (N = 67) |
|------------|-----------------|------------------------|------------------------|------------------------|---------------|
| Any event  | 2 (25.0)        | 2 (14.3)               | 5 (16.7)               | 2 (13.3)               | 11 (16.4)     |
| AEs leading to treatment discontinuation | 0 | 0 | 0 | 0 | 0 |
| Deaths     | 0               | 0                      | 0                      | 0                      | 0             |
| SAE        | 0               | 0                      | 0                      | 0                      | 0             |
| AEs (≥2 of patients overall) |                 |                        |                        |                        |               |
| Diarrhoea  | 1 (12.5)        | 0                      | 2 (6.7)                | 1 (6.7)                | 4 (6.0)       |
| Headache   | 2 (25.0)        | 0                      | 1 (3.3)                | 0                      | 3 (4.5)       |
| Treatment-related AEs | 1 (12.5) | 0 | 3 (10.0) | 1 (6.7) | 5 (7.5) |

AEs, adverse events; SAE, serious AE. Multiple AEs were counted once only per patient in each treatment group.

### Table 3 HCV RNA decline from baseline

| HCV genotype | Treatment (Placebo or GS-9857) (N) | Mean maximum decline in HCV RNA (log_{10} IU/mL) (SD) | Median maximum decline in HCV RNA (log_{10} IU/mL) | Q1, Q3 | Min, max |
|--------------|------------------------------------|-----------------------------------------------------|---------------------------------------------------|-------|----------|
| Genotype 1a  | Placebo (4)                        | 0.5 (0.3)                                           | 0.3                                               | 0.3, 0.6 | 0.2, 1.0 |
|              | 50 mg (8)                          | 4.2 (0.3)                                           | 4.1                                               | 3.9, 4.4 | 3.8, 4.6 |
|              | 100 mg (8)                         | 4.5 (0.6)                                           | 4.5                                               | 4.3, 4.8 | 3.4, 5.5 |
|              | 300 mg (8)                         | 4.0 (0.8)                                           | 4.0                                               | 3.7, 4.3 | 2.7, 5.5 |
| Genotype 1b  | 100 mg (6)                         | 4.0 (0.4)                                           | 3.9                                               | 3.8, 4.1 | 3.5, 4.8 |
| Genotype 2   | Placebo (2)                        | 0.3 (0.2)                                           | 0.3                                               | 0.2, 0.4 | 0.2, 0.4 |
|              | 100 mg (6)                         | 3.5 (0.5)                                           | 3.6                                               | 3.1, 3.7 | 2.9, 4.2 |
| Genotype 3   | Placebo (2)                        | 0.6 (0.6)                                           | 0.6                                               | 0.2, 1.0 | 0.2, 1.0 |
|              | 50 mg (6)                          | 1.8 (0.4)                                           | 1.6                                               | 1.4, 2.2 | 1.4, 2.3 |
|              | 100 mg (6)                         | 3.3 (1.2)                                           | 3.2                                               | 2.8, 3.8 | 1.6, 5.2 |
|              | 300 mg (7)                         | 3.7 (0.4)                                           | 3.6                                               | 3.5, 4.1 | 3.0, 4.3 |
| Genotype 4   | 100 mg (4)                         | 4.0 (0.7)                                           | 4.1                                               | 3.4, 4.6 | 3.0, 4.7 |

HCV, hepatitis C virus; SD, standard deviation; Q1, quartile 1; Q3, quartile 3; RNA, ribonucleic acid.

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Fig. 2 Median change from baseline hepatitis C virus (HCV) RNA over time in patients with HCV genotype 1–4 infection following administration of GS-9857 at 0 (day 1), 24 (day 2) and 48 (day 3) hours. (a) Genotype 1a. (b) Genotype 1b. (c) Genotype 2. (d) Genotype 3. (e) Genotype 4. GT, genotype; HCV, hepatitis C virus.

genotype 1a HCV receiving 100 mg of GS-9857, 3.9 log_{10} IU/mL for patients with HCV genotype 1b infection receiving 100 mg of GS-9857, 3.6 log_{10} IU/mL for patients with HCV genotype 2 receiving 100 mg of GS-9857, 3.2 log_{10} IU/mL for patients with HCV genotype 3a receiving 100 mg of GS-9857 and 4.1 log_{10} IU/mL for patients with HCV genotype 3a receiving 100 mg of GS-9857 and 4.1 log_{10} IU/mL for
patients with genotype 4 HCV 100 mg of GS-9857. Patients with HCV genotype 3 receiving GS-9857 50 mg or 100 mg for 3 days had more rapid virologic rebound after treatment than patients with other HCV genotypes (Fig. 2).

Of the 59 patients receiving GS-9857 treatment, 4 (6.8%) had HCV RNA <LLOQ for at least 1 measurement through the day 10 assessment: one patient with genotype 1a infection receiving GS-9857 100 mg, one patient with genotype 1a infection receiving GS-9857 300 mg, one patient with genotype 1b infection receiving GS-9857 100 mg and one patient with genotype 3a infection receiving GS-9857 300 mg. All patients had HCV RNA return to near baseline levels during follow-up.

Resistance analysis
At baseline, sequencing results were available for 66 of the 67 patients enrolled in the study (Table 4). NS3 RAVs were detected in 16 of 66 patients (24.2%) at baseline; of these patients, 13 of 58 (22.4%) were treated with GS-9857, while 3 of 8 (37.5%) received placebo. Among those with NS3 RAVs who received GS-9857, 9 of 24 (37.5%) patients were infected with HCV genotype 1a, 1 of 6 (16.7%) with genotype 1b and 3 of 19 (15.8%) with genotype 3. Treatment with GS-9857 resulted in similar mean maximal viral load reduction in patients with or without the presence of NS3 RAVs at baseline (Table 4).

Postbaseline sequencing results were available for 61 of 67 enrolled patients; among these patients, 53 received GS-9857 for 3 days, whereas 8 received placebo. Sequencing was not possible for six patients: 4 due to assay failure and 2 due to insufficient HCV RNA sample concentration (<1000 IU/mL). Postbaseline NS3 RAVs were detected in 14 (26.4%) of the 53 patients receiving GS-9857, but not in any of the patients receiving placebo. Within a genotype, the postbaseline emergence of NS3 RAVs did not appear to be related to the treatment dose. A156T/V RAVs were the most frequent substitutions to emerge in patients and were detected in patients with genotype 1a or 1b infection but not detected in patients with genotype 3a infection (data not shown). Postbaseline RAVs were not detected in any of the patients with genotype 2 or 4 HCV infection following treatment with GS-9857.

Pharmacokinetic results
Plasma concentrations were measurable up to 24 h post-dose for all patients after single or multiple dosing, except for one patient receiving GS-9857 100 mg. GS-9857 plasma concentration vs time profiles were similar after 1 or 3 days of dosing, regardless of HCV genotype. Administration of GS-9857 50–300 mg resulted in a dose-proportional increase in $C_{\text{max}}$ and $AUC_{0-24}$. Median $T_{\text{max}}$ ranged from 1.3 to 4.0 h following the first dose, and 1.8–5.0 h after three doses (Table S2). The median $t_{1/2}$ on day 3 ranged from approximately 29–42 h across cohorts. Consistent with the half-life of GS-9857, significant accumulation was observed across dose levels evaluated.

Exposure–response relationships
Anti-HCV activity in patients with genotype 1a, 1b, 2 or 4 infection was not correlated with GS-9857 doses of 50, 100 or 300 mg, whereas median antiviral response exhibited a relationship with GS-9857 dose in patients with genotype 3 infection. This exposure–response relationship could be adequately described using a simple maximal anti-HCV activity ($E_{\text{max}}$) model that used $AUC_{0-24}$ on day 3 of treatment. Similar models using GS-9857 $C_{\text{max}}$ or $C_{24}$ provided comparable exposure–response relationships.

Table 4 Number of patients with NS3 RAVs at baseline and postbaseline time points through day 10

| GT 1a | GT 1b | GT 2 | GT 3 | GT 4 | Placebo |
|-------|-------|------|------|------|---------|
| 50 mg (N = 8) | 100 mg (N = 8) | 300 mg (N = 8) | 100 mg (N = 6) | 100 mg (N = 6) | 300 mg (N = 7) | 100 mg (N = 4) | Placebo (N = 8) |
| Patients with baseline HCV sequences, n | 8 | 8 | 8 | 6 | 5 | 6 | 6 | 7 | 4 | 8 |
| Patients with baseline RAVs, n | 3 | 4 | 2 | 1 | 0 | 2 | 1 | 0 | 0 | 3 |
| Patients with postbaseline HCV sequences, n | 7 | 8 | 7 | 6 | 5 | 5 | 6 | 6 | 3 | 8 |
| Patients with postbaseline NS3 RAVs, n | 3 | 1 | 1 | 4 | 0 | 2 | 1 | 2 | 0 | 0 |

GT, genotype; HCV, hepatitis C virus; NS3, nonstructural protein 3; RAVs, resistance-associated variants.© 2016 The Authors. Journal of Viral Hepatitis Published by John Wiley & Sons Ltd
DISCUSSION

In this randomized, phase 1b study, the safety, antiviral efficacy and PK of GS-9857 at doses ranging from 50 to 300 mg were assessed in patients with chronic genotype 1–4 HCV infection. GS-9857 was generally well tolerated by patients with chronic HCV infection when administered once daily for 3 days. All AEs were of mild or moderate severity, with diarrhoea and headache occurring most commonly during this study. Although increased incidence of rash, pruritus, nausea and anaemia have been commonly reported with the use of certain protease inhibitor-based regimens [7–10], no such events were observed during this study. Grade 3 or Grade 4 laboratory abnormalities occurred in 16.9% of patients receiving GS-9857 and were not associated with GS-9857 dose or AEs. Notably, assessments of ALT, AST and alkaline phosphatase levels did not reveal any Grade 3 or Grade 4 abnormalities, indicating that GS-9857 administration was not associated with significant changes in liver function. Physical examination and ECG evaluation did not reveal clinically significant findings.

Treatment with GS-9857 at doses of 100 mg or 300 mg resulted in $>3 \log_{10}$ IU/mL median maximal reduction in viral load from baseline across all genotypes, including genotype 3, indicating that GS-9857 exhibited potent antiviral activity in HCV-infected patients. This observation is consistent with in vitro data showing that GS-9857 has pan-genotypic activity against genotype 1 to 6 replicons, with the half-maximal effective concentration ranging from 1.5 to 6.6 nM [5]. With the exception of patients with genotype 3a infection who received GS-9857 doses of <100 mg, a rapid and consistent reduction in HCV RNA was achieved and maintained through day 10. For patients with genotype 3a infection who received GS-9857 50 mg, HCV RNA reductions occurred more slowly.

This study characterized substitutions at positions 36, 41, 43, 54, 55, 80, 122, 155, 156, 168 and 170, which have been reported to be associated with resistance to NS3/4A protease inhibitors [11–17]. Prior to the start of GS-9857 treatment, 22.4% of patients who were sequenced harboured NS3 RAVs. Treatment with GS-9857 resulted in similar mean maximal viral load reductions in patients with and without NS3 RAVs, indicating that the antiviral efficacy of GS-9857 was maintained in the presence of mutations commonly associated with resistance to treatment. The majority of patients (73.6%) who were sequenced after 3 days of GS-9857 treatment did not have emergent NS3 RAVs, and there were no emergent NS3 RAVs in patients with genotype 2 or 4 infection. A156V or A156T were the most prevalent substitutions to emerge in patients with genotype 1a or 1b infection. In vitro data indicate that GS-9857 has potent activity against the most common genotype 1 RAVs except A156T [5]; this substitution is associated with high levels of resistance (≥100-fold) but low viral fitness (1.5% compared to wild-type genotype 1a replicon) [12]. The overall low frequency of emergent NS3 RAVs suggests that GS-9857 has a relatively high barrier to resistance as compared with other first-generation protease inhibitors, which may be a substantial benefit in the context of retreatment of patients who have failed a prior treatment with a DAA-based regimen.

Administration of GS-9857 at doses ranging from 50 to 300 mg under fasting conditions resulted in dose-proportional increases in exposure. The median half-life across cohorts ranged from approximately 29–42 h, supporting once-daily dosing. Consistent with its long median half-life, significant accumulation of GS-9857 exposure was observed. GS-9857 plasma PK was similar among patients with genotype 1a, 1b, 2, 3 or 4 HCV infection.

Patients with cirrhosis or chronic liver disease were excluded from this preliminary investigational study. As such, the lack of significant safety concerns observed may be the result of selection for a study population less prone to AEs. Further evaluations of GS-9857 in patients with more advanced disease or additional complications will be necessary to confirm the findings of this study.

In summary, administration of multiple doses of GS-9857 was well tolerated and resulted in a robust decline of HCV RNA levels in patients with genotype 1–4 HCV infection. Notably, the potent antiviral activity of GS-9857 was preserved in the presence of commonly observed NS3 mutations associated with resistance to protease inhibitors. GS-9857 demonstrated linear PK when administered at doses ranging from 50 to 300 mg under fasting conditions. The median half-life of GS-9857 ranged from 29 to 42 h, conducive to once-daily dosing. Lastly, GS-9857 has demonstrated additive antiviral activity when evaluated in vitro in combination with sofosbuvir or velpatasvir [5]. Together, these data support the further development of once-daily GS-9857 in combination with other DAs for the treatment of patients with chronic HCV infection.

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REFERENCES

1 American Association for the Study of Liver Diseases/Infectious Diseases
2 Society of America HCV Guidance Panel. Hepatitis C guidance: AASLD-IDSA recommendations for testing, managing, and treating adults

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infected with hepatitis C virus. Hepatology 2015; 62: 932–954.
2 Lam BP, Jeffers T, Younoszai Z, Fazel Y, Younossi ZM. The changing landscape of hepatitis C virus therapy: focus on interferon-free treatment. Therap Adv Gastroenterol 2015; 8: 298–312.
3 Holmes JA, Thompson AJ. Interferon-free combination therapies for the treatment of hepatitis C: current insights. Hepat Med 2015; 7: 51–70.
4 Solbach P, Wedemeyer H. The new era of interferon-free treatment of chronic hepatitis C. Viszeralmedizin 2015; 31: 290–296.
5 Taylor J, Appleby T, Barauskas O et al. P0899: preclinical profile of the pan-genotypic HCV NS3/4A protease inhibitor GS-9857. J Hepatol 2015; 62: S681.
6 Kirby B, Yang J, Yang C et al. P0861: evaluation of the pan-genotypic HCV NS3/4A protease inhibitor GS-9857 in healthy volunteers. J Hepatol 2015; 62: S663.
7 INCIVEK® (Telaprevir) Capsules, for Oral Use. Full prescribing information. Cambridge, MA: Vertex Pharmaceuticals Incorporated, 2013.
8 OLYSIO® (Simeprevir) Capsules, for Oral Use. Full prescribing information. Titusville, NJ: Janssen Products, LP, 2015.
9 VICTRELIS® (Boceprevir) Capsules, for Oral Use. Full prescribing information. Whitehouse Station, NJ: Merck & Co. Inc., 2015.
10 VIEKIRA PAK® (Ombitasvir, Paritaprevir, and Ritonavir Tablets; Dasabuvir Tablets), Co-Packaged for Oral Use. Full prescribing information. North Chicago, IL: AbbVie Inc., 2014.
11 Sarrazin C, Kiefer TL, Bartels D et al. Dynamic hepatitis C virus genotypic and phenotypic changes in patients treated with the protease inhibitor telaprevir. Gastroenterology 2007; 132: 1767–1777.
12 Tong X, Chuse R, Skelton A, Chen T, Wright-Minogue J, Malcolm BA. Identification and analysis of fitness of resistance mutations against the HCV protease inhibitor SCH 503034. Antiviral Res 2006; 70: 28–38.
13 Reesink HW, Fanning GC, Farha KA et al. Rapid HCV-RNA decline with once daily TMC435: a phase I study in healthy volunteers and hepatitis C patients. Gastroenterology 2010; 138: 913–921.
14 Tong X, Bogen S, Chase R et al. Characterization of resistance mutations against HCV ketoamide protease inhibitors. Antiviral Res 2008; 77: 177–185.
15 Lenz O, Verbinnen T, Lin TI et al. In vitro resistance profile of the hepatitis C virus NS3/4A protease inhibitor TMC435. Antimicrob Agents Chemother 2010; 54: 1878–1887.
16 Susser S, Welsch C, Wang Y et al. Characterization of resistance to the protease inhibitor boceprevir in hepatitis C virus-infected patients. Hepatology 2009; 50: 1709–1718.
17 Palanisamy N, Danielsson A, Kokkula C et al. Implications of baseline polymorphisms for potential resistance to NS3 protease inhibitors in Hepatitis C virus genotypes 1a, 2b and 3a. Antiviral Res 2013; 99: 12–17.

SUPPORTING INFORMATION
Additional Supporting Information may be found in the online version of this article:

Table S1. Grade 3 or 4 laboratory abnormalities
Table S2. Pharmacokinetic parameters of GS-9857 following single-dose and multiple-dose administration