Research Article

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A phase 1, randomized, placebo-controlled, 3-day, dose-ranging study of GS-5885, an NS5A inhibitor, in patients with genotype 1 hepatitis C

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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 *vs.* 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 was 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 with direct antiviral regimens with and without peginterferon.

Keywords: NS5A protein; Viral non-structural protein; Hepatitis C; Antiviral agents.

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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.

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 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 $\geqslant 5$ $\log_{10}$ IU/ml at screening. Patients were HCV treatment naïve and had a body mass index (BMI) of 19–35 kg/m^2 inclusive, creatinine clearance $\geqslant 70$ ml/min, and a QTcF interval $\leqslant 450$ ms. 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, co-infection with HIV or hepatitis B virus, prothrombin time >1.5 $\times$ ULN, albumin <3 g/dl, alanine aminotransferase and aspartate aminotransferase levels >5 $\times$ ULN, total bilirubin >ULN, hemoglobin <11 g/dl, platelets <90,000/mm^3, or absolute neutrophil count <1000 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 childbearing 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 patients with genotype 1a HCV only, and 1 cohort included patients with genotype 1b HCV only. 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, 3, 10 mg (2 cohorts: genotype 1a and 1b), 30, 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 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 h 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_{∞}, after administration of first dose), elimination rate constant (λ_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 was 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, seven 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.

End points and statistical analyses

The primary efficacy end point 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 nine study centers in the United States. All patients except one, who was lost to follow-up after day 8, completed the study through the day 14 follow-up visit. Overall, 72% (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 four moderate adverse events in the placebo group). In the 1-mg cohort, the two moderate adverse events were both rash; in the 30-mg cohort, the two 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 (Fig. 1 and Table 3). Maximal concentrations were achieved