Research Article

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A randomized, double-blind, multiple-dose study of the pan-genotypic NS5A inhibitor samatasvir in patients infected with hepatitis C virus genotype 1, 2, 3 or 4

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Background & Aims: Samatasvir is a pan-genotypic inhibitor of the hepatitis C (HCV) non-structural protein 5A (NS5A). This study evaluated the antiviral activity, pharmacokinetics and safety of samatasvir monotherapy in treatment-naïve subjects infected with HCV genotype 1–4.

Methods: Thirty-four genotype 1 and thirty genotype 2, 3 or 4 subjects were randomized to receive for 3 days placebo or samatasvir 25–100 mg per day. Plasma samples for HCV RNA, pharmacokinetics and sequencing were collected up to day 10.

Results: Samatasvir achieved potent antiviral activity across genotypes: mean maximum reductions from baseline were 3.2–3.6 (genotype 1a), 3.0–4.3 (genotype 1b), 3.2–3.4 (genotype 3), and 3.6–3.9 (genotype 4) $\log_{10}$/ml respectively; no viral rebound was observed during the 3-day treatment period. For genotype 2 HCV, samatasvir was active in subjects with *NS5A* L31 polymorphism at baseline (individual range 2.5–4.1 $\log_{10}$/ml), but showed minimal activity in those with baseline M31 polymorphism. Samatasvir exhibited a long plasma half-life of approximately 20 h which supports once daily dosing. Samatasvir was well tolerated in all subjects with no safety-related discontinuations or serious adverse events. The most common adverse events included constipation, nausea and headache and occurred at similar frequency in active and placebo subjects. All events were mild or moderate in intensity. There were no patterns or dose dependence of adverse events, vital signs, laboratory parameters or electrocardiograms.

Conclusions: Samatasvir 25–100 mg monotherapy for 3 days was well tolerated and induced a rapid and profound reduction in plasma HCV RNA in subjects infected with HCV genotype 1–4. Samatasvir is being evaluated in combination with other direct-acting antiviral agents in subjects with HCV infection.

Keywords: Samatasvir; IDX719; NS5A; Chronic hepatitis C; Pan-genotypic antiviral activity; Direct-acting antiviral agents; Pharmacokinetics.

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Abbreviations: HCV, hepatitis C virus; NS5A, nonstructural protein 5A; DAA, direct-acting antiviral agent; EC_{50}, 50% effective concentration; CYP, cytochrome P450; HBV, hepatitis B virus; HIV, human immunodeficiency virus; QD, once daily; BID, twice daily; AE, adverse event; BMI, body mass index; HCC, hepatocellular carcinoma; ECG, electrocardiogram; SAE, serious adverse event; PK, pharmacokinetic(s); AM, morning; PM, evening; C_{max}, maximum concentration; T_{max}, time to C_{max}; C_τ, predose trough concentration; AUC, area under curve; $t_{1/2}$, half-life; EC_{90}, 90% effective concentration.

Introduction

Direct-acting antiviral agents (DAAs) have radically reshaped the treatment paradigm of chronic hepatitis C virus (HCV) infection. While pegylated interferon still remains as an essential component of the current optimal treatment regimens containing either telaprevir or boceprevir, major efforts are being devoted towards the development of interferon-free all oral regimens by combining multi-class DAAs with or without ribavirin. A number of newer DAAs with improved safety profile and antiviral activity are expected to soon receive regulatory approval, bringing better treatment options to HCV-infected patients [1].

Amongst various classes of DAAs, non-structural protein 5A (NS5A) replication complex inhibitors have thus far been the most potent in suppressing viral replication [2,3]. These compounds have been shown to induce multi-log reductions in plasma HCV RNA within h of a single low dose [4,5]. While NS5A inhibitors are most active against HCV genotype 1b, many showed much less replicon activity against other genotypes, particularly genotype 2 and genotype 3 [2,3]. Considering the high prevalence of multiple HCV genotypes across many geographic regions, it is highly desirable for a DAA to possess pan-genotypic antiviral activity [6]. In that context, several newer NS5A inhibi-

tors with *in vitro* pan-genotypic antiviral activity are being developed (samatasvir, ACH-3102, GS5816, PPI668) [2,3]. To our knowledge, among these candidates, samatasvir, as a single agent, was the first to demonstrate pan-genotypic activity in HCV-infected patients [5].

Samatasvir (IDX719), a novel NS5A inhibitor of HCV replication, exhibits potent and pan-genotypic anti-HCV activity with *in vitro* 50% effective concentration (EC_{50}) values ranging from 2 to 24 pM against HCV of genotypes 1a, 1b, 2a, 3a, 4a, and 5a. There is only a 12-fold shift in EC_{50} values from the most sensitive genotype 4a to the least sensitive genotype 2a. With a 50% cytotoxicity concentration >50 μM, samatasvir has a high selectivity index of at least 2,000,000 [7,8]. Fig. 1 illustrates the chemical structure of samatasvir.

Samatasvir showed limited or no inhibition of human CYP enzymes or human transporters, and underwent very limited metabolism *in vitro*. In replicon studies, samatasvir demonstrated additive antiviral activity with other HCV therapeutic agents and no negative pharmacodynamic interaction with commonly used antiviral agents against hepatitis B (HBV) and human immunodeficiency virus (HIV). Together, these favorable characteristics make samatasvir an ideal component of all-oral DAA regimens [8].

Samatasvir was evaluated in a two-part clinical study. Part one included single-dose escalation and repeat dose administration in healthy subjects and an exploratory single-dose administration in subjects infected with HCV genotype 1, 2 or 3. Results from part one, reported elsewhere, showed that single and repeat doses of samatasvir up to 100 mg in healthy volunteers and single doses up to 100 mg in HCV-infected subjects were well-tolerated and achieved pharmacologically relevant drug exposure. Samatasvir exhibited dose-proportional plasma exposure and long plasma half-life, supporting once daily (QD) dosing [5]. Single doses of samatasvir demonstrated substantial pan-genotypic antiviral activity of up to 3.7 $\log_{10}$ IU/ml in patients with genotype 1, 2 or 3 HCV [5].

Part two of the study, reported here, evaluated the safety, pharmacokinetics (PK) and antiviral activity of samatasvir as a single agent following multiple doses up to 100 mg daily for 3 days in subjects infected with HCV genotype 1, 2, 3 or 4.

Materials and methods

Study design

This was a multicenter, randomized, double-blind, placebo-controlled, parallel-panel, multiple-dose study of samatasvir as a single agent dosed for 3 days in treatment-naïve patients with chronic HCV genotype 1, 2, 3 or 4. Thirty-four patients with genotype 1 HCV were randomized to receive either samatasvir (n = 28) or placebo (n = 6): 25 mg and 50 mg QD cohorts each had 8 active and 2 placebo subjects; 50 mg twice daily (BID) and 100 mg QD cohorts each had 6 active and 1 placebo subjects. Thirty subjects with HCV genotype 2, 3 or 4 were randomized to receive samatasvir 50 mg BID (n = 12), 100 mg QD (n = 12) or placebo (n = 6) in an active-to-placebo ratio of 4:1 (ClinicalTrials.gov Identifier: NCT01508156). Treatment was assigned via a computer-generated randomization code and kept blinded to subjects and clinical investigators. Subjects were admitted to one of the 8 clinical sites in the United States between January 3, 2012 and July 9, 2012 and were required to stay in the clinical facility from day −1 to study discharge on day 10 or upon early termination. Samatasvir oral suspension or matching placebo was administered under fasting conditions. Cohorts were dosed in parallel without dose escalation.

Written informed consent was obtained from all patients. This study was approved by the institutional review boards of the trial centers and conducted in accordance with Good Clinical Practice procedures and the principles of the Declaration of Helsinki, with authorization from the United States Food and Drug Administration.

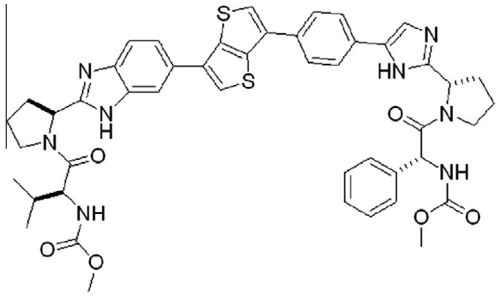

Fig. 1. Chemical structure of samatasvir.

The sample size of this study was calculated primarily based on safety endpoints. With a sample size of 4, 6 or 8 subjects per cohort to receive active samatasvir, the estimated probabilities of observing a particular adverse event (AE) with an expected rate of 20% were 0.59, 0.74, and 0.83, respectively. It was assumed that for this short-term study safety risk would be independent of HCV genotypes or dosing regimen (BID and QD) for the same daily dose. When pooled together across genotypes, the sample size for subjects receiving active samatasvir 50 mg BID or 100 mg QD was 36 leading to an estimated chance of 98% to observe a particular AE with an expected incidence rate of at least 20%.

Subjects

Major inclusion criteria included: male or female subjects 18–65 years old inclusive, with a body mass index (BMI) of 18–35 kg/m^2; documented clinical history compatible with chronic HCV, including positive anti-HCV antibody, presence of HCV RNA in the plasma for at least six months or liver biopsy within 24 months with histology consistent with chronic HCV infection; HCV genotype 1, 2, 3 or 4; plasma HCV RNA $\geqslant 5$ $\log_{10}$ IU/ml; all patients agreed to use double-barrier birth control (such as a condom plus spermicide) from screening through at least 90 days following the last dose of the study drug.

Major exclusion criteria included: pregnancy or breastfeeding; co-infection with HBV or HIV; history or evidence of decompensated liver disease; prior clinical or histological evidence of cirrhosis; alanine aminotransferase or aspartate aminotransferase level >3.0 × upper limit of normal; history of hepatocellular carcinoma (HCC) or findings suggestive of possible HCC; one or more additional known primary or secondary causes of liver disease, other than HCV; previous antiviral treatment for HCV; current abuse of alcohol or illicit drugs; or other clinically significant diseases that, in the opinion of the investigator, would jeopardize the safety of the patient or impact the validity of the study results.

Safety assessments

At specific time points throughout the study, blood and urine samples were collected for clinical laboratory analysis including hematology, blood chemistry and urinalysis. Vital signs, 12-lead electrocardiogram (ECG) and physical examinations were performed at predefined time intervals. Safety assessments were based on observed/reported AEs and serious adverse events (SAEs) as well as results from clinical laboratory tests, vital sign measurements, physical examination and ECGs.

Pharmacokinetics

For QD dosing, serial intensive blood samples for PK analysis were collected over 24 h on day 1 and over 120 h after the last dose on day 3 at the following time points: predose and 0.5, 1, 2, 3, 4, 6, 8, 12, 16, 20, 24 h postdose on day 1 and day 3, and 36, 48, 72, 96, and 120 h post the day-3 dose. For BID dosing, blood samples were obtained predose in the morning (AM) and evening (PM) and at 0.5, 1, 2, 3, 4, 6, and 8 h postdose on day 1 and day 3. In addition, blood samples were obtained at 12, 24, 36, 60, 84, and 108 h post the day-3 PM dose. PK parameters derived from non-compartmental analysis included maximum drug concentration (C_{max}), time to C_{max} (T_{max}), predose trough concentration (C_τ) at 24 h post QD dose or 12 h post BID dose, area under the plasma concentration-time curve over 24 h for the total daily dose (AUC_{24h}), and observed half-life ($t_{1/2}$) calculated following the last dose. Plasma concentrations of samatasvir were measured using a validated liquid chromatography/tandem mass spectrometry methodology. All samples were analyzed within the established stability of the analyte.