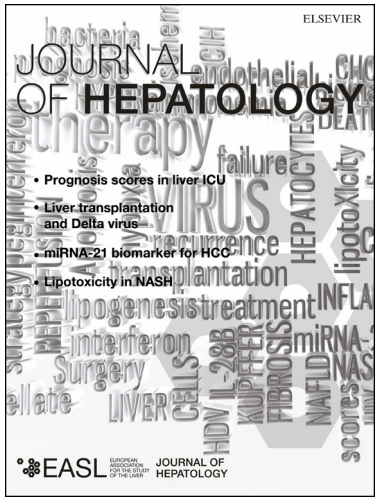
Accepted Manuscript



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

Bradley Vince, John M. Hill, Eric J. Lawitz, William O'Riordan, Lynn R. Webster, Daniel M. Gruener, Ricky S. Mofsen, Abel Murillo, Eileen Donovan, Jie Chen, Joseph F. McCarville, John Z. Sullivan-Bólyai, Douglas Mayers, Xiao-Jian Zhou

PII: S0168-8278(14)00014-2
DOI: http://dx.doi.org/10.1016/j.jhep.2014.01.003
Reference: JHEPAT 4997

To appear in: *Journal of Hepatology*

Received Date: 8 October 2013
Revised Date: 29 December 2013
Accepted Date: 6 January 2014

Please cite this article as: Vince, B., Hill, J.M., Lawitz, E.J., O'Riordan, W., Webster, L.R., Gruener, D.M., Mofsen, R.S., Murillo, A., Donovan, E., Chen, J., McCarville, J.F., Sullivan-Bólyai, J.Z., Mayers, D., Zhou, X-J., 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, *Journal of Hepatology* (2014), doi: http://dx.doi.org/10.1016/j.jhep.2014.01.003

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

Bradley Vince[1], John M. Hill[2], Eric J. Lawitz[3], William O'Riordan[4], Lynn R. Webster[5], Daniel M. Gruener[6], Ricky S. Mofsen[7], Abel Murillo[8], Eileen Donovan[9], Jie Chen[9], Joseph F. McCarville[9], John Z. Sullivan-Bólyai[9], Douglas Mayers[9], Xiao-Jian Zhou[9]

[1]Vince and Associates Clinical Research, Overland Park, KS; [2]Avail Clinical Research, DeLand, FL; [3]Alamo Medical Research, San Antonio, TX; [4]eStudySite, San Diego, CA; [5]CRI Lifetree Clinical Research, Salt Lake City, UT; [6]CRI Worldwide, Philadelphia, PA; [7]St. Louis Clinical Trials, St. Louis, MO; [8]AMPM Research Clinic, Miami, FL; [9]Idenix Pharmaceuticals, Inc., Cambridge, MA

Contact information: Xiao-Jian Zhou, Idenix Pharmaceuticals Inc., 320 Bent street, 4th floor, Cambridge MA, USA 02141-2025. Tel. +1 617 995 9805; Fax: +1 617 995 9817; E-mail address: zhou.xj@idenix.com

Keywords: Samatasvir; NS5A inhibitor; hepatitis C virus; pan-genotypic anti-HCV activity

Electronic word count: 6007

Number of figures and tables: 4 Figures; 4 Tables

Trial registration number: ClinicalTrials.gov Identifier: NCT01508156

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

Conflict of interest:

B.V., J.M.H., E.J.L., W.O'R., L.R.W., D.M.G., R.S, A.M. were clinical investigators contracted by Idenix Pharmaceuticals, Inc to conduct the reported study. E.D., J.C., J.F.M., J.Z.S.B, D.M., X.J.Z. are employees of Idenix Pharmaceuticals, Inc.

Financial support: This study was sponsored by Idenix Pharmaceuticals, Inc., Cambridge, MA, USA.

Abstract

Background & Aims: Samatasvir is a pan-genotypic inhibitor of the hepatitis C (HCV) nonstructural 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 hours 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. (*265 words*)

Keywords: Samatasvir, IDX719, NS5A, chronic hepatitis C, pan-genotypic antiviral activity, direct-acting antiviral agents, pharmacokinetics

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, nonstructural 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 hours 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's highly desirable for a DAA to possess pan-genotypic antiviral activity [6]. In that context,

several newer NS5A inhibitors 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.

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 ≥ 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 hours on day 1 and over 120 hours 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 hours postdose on day 1 and day 3, and 36, 48, 72, 96 and 120 hours 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 hours postdose on day 1 and day 3. In addition, blood samples were obtained at 12, 24, 36, 60, 84 and 108 hours post the day-3 PM dose. PK parameters derived from noncompartmental analysis included maximum drug concentration (C_{max}), time to C_{max} (T_{max}), predose trough concentration (C_{τ}) at 24 hours post QD dose or 12 hours post BID dose, area under the plasma concentration-time curve over 24 hours 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. Briefly, internal standard $^{13}C_5$-^{15}N-samatasvir was added to calibration standards (0.1 to 100 mg/mL), quality control samples (0.3-80 ng/mL) and unknown samples. The mixture was subject to liquid-liquid extraction with a recovery of 87.1% and 96.3% for samatasvir and the internal standard respectively. Chromatography was performed on a ZORBAX 300-SCX column (50 mm × 3 mm; particle size, 5 µm, Agilent Technologies, Santa Clara, CA). Elution was carried out isocratically at a constant flow rate of 1 mL/min with a mobile phase of 80:20 (v/v) acetonitrile : ammonium formate (25 mM, pH 2.5). Under these conditions, the retention time was approximately 0.96 min for samatasvir and internal standard. Mass

spectrometry data were acquired using an AB Sciex API 5500 triple quadrupole mass analyzer (Framingham, MA) at mass transition of 443.3→659.2 m/z and 446.3→659.2 m/z for samatasvir and $^{13}C_5$-^{15}N-samatasvir respectively. The mass analyzer was operated under positive ion mode using turbo ion spray ionization. This assay has a lower limit of quantitation of 0.1 ng/ml. The intra- and inter-day precisions (coefficient of variation) and accuracies (percent deviation) were from 2.0 to 5.1% and -9.1 to -2.7%, respectively.

Antiviral activity

Serial blood samples for measuring plasma HCV RNA were obtained during screening, on day -1, during dosing from day 1 to day 3 (day 1 predose and postdose at 4, 8, 12, 16, 24, 48 and 72 hours) and during follow-up from day 4 to day 9 or 10 (post day 1 dose at 96, 120, 144, 168, 192 and 216 or 240 hours). Plasma HCV RNA was determined by a validated real-time polymerase chain reaction assay (COBAS® AmpliPrep/COBAS® Taqman HCV Test, Roche, Pleasanton, CA) with a lower limit of quantitation of 25 IU/mL.

NS5A sequence analysis

Plasma samples were collected predose on day 1 as well as on day 4 (1 day after the last dose) and day 10 (1 week post the last dose). Samples with viral load > 1000 IU/mL were subjected to population sequencing of the NS5A region of the virus at DDL Diagnostic Laboratory (Rijswijk, The Netherlands).

205

206 ***Statistical analysis***

207 Antiviral activity was measured as the changes on $\log_{10}$ scale from baseline in plasma
208 HCV RNA. The primary endpoint of antiviral activity was the $\log_{10}$ change from baseline
209 to day 4. Secondary endpoints included individual maximum viral load reduction and
210 corresponding time. Antiviral activity data were summarized by dose for each genotype
211 and/or sub-genotype. Additional exploratory analyses by stepwise and logistic regression
212 were performed to identify PK and baseline predictors of viral response. The baseline
213 characteristics included gender, weight, BMI, race, pretreatment HCV RNA, HCV
214 genotype and IL28B genotype.

215 PK parameters were summarized by dose regardless of HCV genotypes. AEs
216 were tabulated by system organ class, preferred term and dose. Other safety data
217 including vital signs, ECG and clinical laboratory results were summarized by dose for
218 each scheduled measurement.

219 All statistical analyses were performed using SAS (Version 9.2, SAS Institute Inc.,
220 Cary, NC).

221 **Results**

222 ***Baseline characteristics***

223 In total, 64 treatment-naïve subjects with genotype 1, 2, 3 or 4 chronic HCV infection
224 were enrolled and completed the 3-day treatment. Of the 64 subjects, 34 had genotype 1

HCV (mostly 1a; 1a/1b:29/5), and 10 each had genotype 2, genotype 3 or genotype 4. One placebo subject typed as being infected with genotype 2b HCV at baseline was subsequently determined by direct sequencing to be infected with genotype 2b/1a chimeric virus. Most (approximately two-thirds) of the subjects with genotype 1 (23/34) or genotype 2-4 (21/30) HCV had IL28B genotype CT or TT. Subjects were predominantly male and Caucasian. Approximately one-third of the subjects were female. Baseline and demographic characteristics were comparable across dose groups (Table 1).

Antiviral activity

Mean changes over time of plasma HCV RNA from baseline are shown in Fig. 2 for subjects with genotype 1, 3 or 4 HCV. Fig 3 depicts the individual and mean changes over time of plasma HCV RNA from baseline for subjects with genotype 2 HCV. The mean change in $\log_{10}$ HCV RNA from baseline to 24 h and 72 h, the mean maximum change from baseline and the corresponding time were summarized in Table 2. Samatasvir dosed QD or BID for 3 days produced substantial pan-genotypic antiviral activity. The greatest antiviral activity was achieved in subjects having genotype 1b HCV with mean maximum decrease in HCV RNA of 3.0-4.3 $\log_{10}$, followed by 3.6-3.9 $\log_{10}$ in genotype 4, 3.2-3.6 $\log_{10}$ in genotype 1a and 3.2-3.4 $\log_{10}$ in genotype 3 HCV (see below for antiviral response in subjects with genotype 2 HCV). Maximum decrease in HCV RNA typically occurred upon completion of dosing. Antiviral activity was not observed in subjects receiving placebo. After completion of samatasvir dosing, plasma HCV RNA slowly returned towards baseline but did not attain pre-treatment level within the follow-up period of up to 10 days.

Antiviral response to samatasvir in subjects with genotype 2 HCV was determined by a single polymorphism at the NS5A amino acid position 31. Among the 8 subjects with genotype 2 HCV who received samatasvir, high antiviral activity with maximum decrease in HCV RNA of 4.0 and 4.1 $\log_{10}$ was achieved in 2 subjects (Fig. 3, 002-006 and 004-019) who had L31 at baseline with no detectable M31 on day 4. In 2 subjects who had L31 at baseline but emergence of M31on day 4, robust antiviral activity was retained with maximum decrease in HCV RNA of 2.5 and 3.2 $\log_{10}$ (Fig. 3, 001-147 and 001-163). In 1 subject who had an L/M31 mixture at baseline but M31 on day 4, a much reduced antiviral activity (a decrease of 0.8 $\log_{10}$) was obtained (Fig. 3, 007-004). Virtually no antiviral activity (0.3-0.5 $\log_{10}$ reduction) was observed in the 3 subjects with pre-existing M31 who received samatasvir (Fig. 3, 001-188, 002-017 and 002-016). In contrast, despite all genotype 4 -infected subjects carrying an NS5A M31 at baseline, all responded well to samatasvir treatment (Fig. 2). Additional details on sequence analyses of other studied HCV genotypes will be reported elsewhere.

Pharmacokinetics

Fig. 4 depicts the mean day-3 plasma concentration vs. time profiles over the first 24h after dosing, corresponding to the intended QD dosing interval. Table 3 summarizes PK parameters of samatasvir.

Following oral administration in HCV-infected subjects at daily doses of 25, 50 and 100 mg, samatasvir exhibited dose-related plasma exposures. Peak exposures were reached with a median time of 3-4 h postdose. With a half-life of approximately 20 h, plasma samatasvir increased over time with a mean accumulation ratio of approximately

50% based on trough exposures for QD dosing. For the same total daily dose, samatasvir 50 mg BID achieved higher trough exposures than did the 100 mg QD dose although no marked differences in antiviral activity were noted between the two regimens. Both 100 mg QD and 50 mg BID reached trough concentrations that were at least 7 fold above the protein-binding adjusted 90% effective concentration (EC_{90}) of samatasvir against the least susceptible HCV genotype (genotype 2a, EC_{90}=2.3 ng/mL), while plasma concentrations of samatasvir remained above the EC_{90} over the entire dosing interval after multiple dosing for all doses/regimens (Fig. 4).

Predictors of antiviral response

Among various baseline characteristics and dose examined using regression analysis, only genotype and dose were significant predictors of viral response ($P < 0.0001$). Genotype 1b was the most susceptible following by 1a, 3 and 4 which responded comparably to samatasvir; genotype 2 was the least susceptible due to high prevalence of the pre-existing M31 or M/L31 polymorphisms which virtually did not respond to samatasvir.

Safety and tolerability

Samatasvir was well-tolerated in all subjects. There were no treatment-emergent deaths, SAEs or safety-related discontinuations. AEs were reported at a similar frequency in samatasvir-treated subjects (20 of 48 or 41.7%) and placebo (6 of 12 or 50.0%). As summarized in Table 4, the most frequent AEs reported were constipation, headache, and nausea. All AEs were mild or moderate in intensity and did not appear to be dose related.

There were no apparent dose-related or other patterns of newly occurring or worsening graded hematology, chemistry, or urinalysis abnormalities. There were no clinically significant treatment-emergent changes in vital sign measurements, physical examination findings or ECG parameters.

Discussion

NS5A replication complex inhibitors are among the most potent DAAs: as a class, these agents at low doses are capable of producing multi-log reductions in plasma HCV RNA within hours of dosing [4,5]. Other common features of NS5A inhibitors including good safety/tolerability, lack of cross-resistance with other classes of DAAs and once daily dosing make these agents ideal candidates for all oral combination therapy for HCV [3]. While clinical stage NS5A inhibitors all demonstrated antiviral activity against genotype 1a/1b HCV as a single agent in their respective proof-of-concept studies, clinical data on activity against other HCV genotypes are scarce [3]. To our knowledge, as monotherapy, samatasvir was the first to demonstrate potent pan-genotypic activity in HCV-infected subjects [5]. In an initial exploratory phase of the current study, single doses of 25-100 mg samatasvir afforded a maximum reduction in plasma HCV RNA of up to 3.7 $\log_{10}$ in subjects with genotype 2 or 3 HCV, similar to genotype 1 [5].

The samatasvir doses for the 3-day proof-of-concept phase were selected based on the single-dose antiviral activity in HCV-infected subjects as well as *in vitro* antiviral activity against various HCV genotypes [7,8]. While a single low dose of 1 mg samatasvir was able to produce over 3 $\log_{10}$ reduction in plasma HCV RNA, dose-response analyses using data from the single-dose phase suggested that doses of 25 mg

and above might achieve more consistent antiviral effect [5]. Therefore, doses of 25-100 mg/day were selected for the 3-day dosing in subjects with genotype 1 HCV. Samatasvir exhibits potent and slightly differential *in vitro* antiviral activity against the more sensitive genotype 1a/1b and genotype 4a replicons (EC_{50}: 2.0-6.2 pM) and the less sensitive genotype 2 (EC_{50}=24 pM) and genotype 3 (EC_{50}=17 pM) [7,8]. These *in vitro* data in conjunction with the single-dose anti-HCV activity observed in patients with genotype 2 or 3 in part 1 of the study favored the 100 mg/day dose in subjects with these genotypes in the 3-day dosing phase.

Results from the current 3-day proof-of-concept part of the study confirmed the pan-genotypic antiviral activity observed during the exploratory single-dose phase, but showed more profound and persistent virologic response due to continuous suppressive pressure resulting from multiple doses. Subjects with genotype 1 HCV achieved mean maximum reduction of plasma HCV RNA of 3.0-4.3 $\log_{10}$, which is numerically in the upper 2.3-4.0 $\log_{10}$ range of virologic response obtained with other clinical stage NS5A inhibitors as a single agent dosed for 1 to 14 days (median 3 days) [9-17] At the tested doses administered for 3 days in subjects with genotype 3 or 4 HCV, virologic responses were comparable with those observed in genotype 1 subjects with a mean maximum reduction of 3.2-3.9 $\log_{10}$. A similar degree of viral suppression was also achieved in subjects with genotype 2 HCV who had no pre-existing M31 or L/M31 polymorphism. Pre-existing M31 polymorphism in genotype 2 subjects predicts lack of virologic response, and emerging M31 is associated with reduced antiviral activity to samatasvir monotherapy.

While all current clinical-stage NS5A inhibitors are able to induce substantial early viral response, this class of HCV DAA is, however, prone to rapidly select viral variants as a single agent [3]. Indeed, viruses in the current study underwent treatment-emergent selection of NS5A variants associated with *in vitro* resistance (primarily at positions 93, 28, 30 and 31, details to be presented elsewhere) although no subject experienced an on-treatment rebound defined as a 0.5 $\log_{10}$ increase above nadir with samatasvir as a single agent dosed for 3 days. During a 14-day monotherapy with daclatasvir, viral rebounds occurred early (generally before 7 days) and were associated with emergence of resistant variants [4]. The lack of viral breakthrough while on samatasvir in the current study contrasts with the observed rapid selection of resistance-associated variants. These conflicting observations might be a consequence of the short duration of treatment, i.e., the treatment-selected variants may not have had sufficient time to rebound from their suppressed levels in the presence of drug. The low barrier to resistance with NS5A inhibitors as monotherapy appears to have little clinical relevance when these drugs are used in combination with other classes of DAAs including nucleotide, non-nucleotide NS5B and protease inhibitors. In fact, the majority of the best sustained virologic response data (>90%) obtained to date are from experimental all-oral combination regimens involving NS5A inhibitors [18-21].

Samatasvir exhibited a consistent and long plasma half-life of 20 h across doses/regimens. Its long half-life is in the range of 12-16 h and 13-50 h reported respectively for daclatasvir and ledipasvir, the most advanced NS5A inhibitors in clinical development [9,10]. Long half-life results in sustained plasma exposures and supports QD dosing of samatasvir. Despite being able to achieve higher trough concentrations, for

the same total daily dose of 100 mg, samatasvir dosed BID did not produce more virologic response than QD dosing, presumably due to the fact that both regimens resulted in troughs largely surpassing the protein-binding adjusted 90% effective concentration (EC_{90}) of the drug against the tested HCV genotypes. Albeit limited number of subjects for each (sub)-genotype per cohort and rather comparable viral declines across most genotypes, a multivariate analysis was able to identify HCV genotype and dose as the only predictors of antiviral response. In this 3-day trial with samatasvir as a single agent, subjects with genotype 1b HCV had the best response followed by genotype 1a, 3 or 4 with similar responses. Subjects with genotype-2 HCV having emerging M31, pre-existing M31 or M/L31 polymorphisms had reduced to no response to samatasvir. Therefore, the relative clinical potencies of samatasvir observed in the present study against various HCV genotypes were in good agreement with in vitro data [7,8]. Traditional predictors of viral response including baseline viral load and IL28B polymorphism were not significant predictors.

During this short-term proof-of-concept study, samatasvir was well tolerated with no dose-related safety findings. While its safety and tolerability will need to be further defined in longer-term/larger trials, clinical data to date have shown satisfactory safety profiles for NS5A inhibitors as a class [3].

In conclusion, at the tested doses of 25-100 mg/day for 3 days, samatasvir was safe and demonstrated substantial pan-genotypic antiviral activity in treatment-naïve patients infected with genotype 1, 2, 3 or 4 HCV. The pharmacokinetic and antiviral profiles of samatasvir make it a desirable component in all-oral DAA combination

regimens. A phase II study of once-daily samatasvir in combination with simeprevir in treatment-naïve patients with genotype 1b or 4 HCV is ongoing.

Acknowledgement

The authors wish to thank the HCV-infected subjects who generously agreed to participate in this clinical study. We also thank the study coordinators, nurses, and staff at clinical study sites.

References

[1]. Shah N, Pierce T, Kowdley KV. Review of direct-acting antiviral agents for the treatment of chronic hepatitis C. Expert Opin Investig Drugs 2013; 22:1107-1121.

[2]. Belda O, Targett-Adams P. Small molecule inhibitors of the hepatitis C virus-encoded NS5A protein. Virus Res 2012; 170:1-14.

[3]. Pawlotsky JM. NS5A inhibitors in the treatment of hepatitis B. J Hepatol 2013; 59:375-382.

[4]. Gao M, Nettles RE, Belema M, Snyder LB, Nguyen VN, Fridell RA, et al. Chemical genetics strategy identifies an HCV NS5A inhibitor with a potent clinical effect. Nature 2010; 465:96-100.

[5]. Zhou XJ, Vince B, Dubuc-Patrick G, Chen J, Pietropaolo P, Sullivan-Bolyai J, et al. Safety, pharmacokinetic and antiviral activity results from a first-in-human study of IDX719, a pan-genotypic HCV NS5A inhibitor. 7th International Workshop on Clinical Pharmacology of hepatitis Therapy. Cambridge, MA, USA, June 27, 2012.

[6]. Hnatyszyn HJ. Chronic hepatitis C and genotyping: the clinical significance of determining HCV genotypes. Antivir Ther 2005; 10:1-11.

[7]. Bilello JP, Dousson CB, La Colla M, Chapron C, Bhadresa S, Camire M, et al. Antiviral and preclinical profiles of HCV NS5A inhibitors IDX380 and IDX719. 18th International Symposium on Hepatitis C Virus and Related Viruses. Seattle, WA, USA, September 8-12, 2011.

[8]. Bilello JP, La Colla M, Chapron C, Lallos LB, Serra I, Gillum JM, et al. The preclinical profile of the pan-genotypic HCV NS5A inhibitor IDX719 demonstrates its potential for combination therapy. 7th International Workshop on Hepatitis C-Resistance and New Compounds. 2012, Cambridge, MA, USA, June 28, 2012.

[9]. Nettles RE, Gao M, Bifano M, Chung E, Persson A, Marbury TC, et al. Multiple ascending dose study of BMS-790052, a nonstructural protein 5A replication complex inhibitor, in patients infected with hepatitis C virus genotype 1. Hepatology 2011; 54:1956-65

[10]. Lawitz EJ, Gruener D, Hill JM, Marbury T, Moorehead L, Mathias A, et al. A phase 1, randomized, placebo-controlled, 3-day, dose-ranging study of GS-5885, an NS5A inhibitor, in patients with genotype 1 hepatitis C. J Hepatol 2012; 57:24-31.

[11]. Lalezari JP, Agarwal K, Dusheiko GM, Brown AS, Weis N, Christensen PB, et al. Dose-ranging study of trial of PPI-461, a potent new pan-genotypic HCV NS5A inhibitor, in patients with HCV genotype-1 infection. Hepatology 2011; 54:400A.

[12]. Nettles R, Wang X, Quadri S, Wu Y, Gao M, Belema M, et al. BMS-824393 is a potent hepatitis C virus NS5A inhibitor with substantial antiviral activity when given as monotherapy in subjects with chronic G1 HCV infection. Hepatology 2010; 52:1203A.

[13]. Spreen W, Wilfret D, Bechtel D, Adkinson K, Lou Y, Jones LH, et al. GSK2336805 HCV NS5A inhibitor demonstrates potent antiviral activity in chronic hepatitis C (CHC) genotype 1 infection: results from a first time in human (FITH) single and repeat dose study. Hepatology 2011; 54:400A-1A.

[14]. Lawitz E. Safety and antiviral activity of ABT-267, a novel NS5A inhibitor, during 3-day monotherapy: first study in HCV genotype-1 (GT1)-infected treatment-naive subjects. J Hepatol 2012; 56:S469–S470.

[15]. Vince B, Lawitz E, Searle S, Marbury T, Robison H, Robarge L, et al. Novel NS5A inhibitor ACH-2928 phase 1 results in healthy volunteers and HCV GT-1 patients. J Hepatol 2012; 56:S480–S481.

[16]. Lalezari JP, Farrell GC, Shah PS, Schwab C, Walsh D, Vig P, et al. PPI-668, a potent new pan-genotypic HCV NS5A inhibitor: phase 1 efficacy and safety. Hepatology 2012; 56:1065A-66A.

[17]. Muir A, Hill J, Lawitz E, Marbury T, Robarge L, Robison H, et al. ACH-3102, a second generation NS5A inhibitor, demonstrates potent antiviral activity in patients with genotype 1a HCV infection despite the presence of baseline NS5A-resistant variants. J Hepatol 2013; 58:S360.

[18]. Kowdley KV, Lawitz E, Poordad F, Cohen DE, Nelson D, Zeuzem S, et al. safety and efficacy of interferon-free regimens of ABT-450/r, ABT-267, ABT-333 +/-

ribavirin in patients with chronic HCV GT1 infection: results from the AVIATOR study. J Hepatol 2013; 58:S2.

[19]. Everson GT, Sims KD, Rodriguez-Torres M, Hezode C, Lawitz E, et al. Interim analysis of an interferon (IFN) - and ribavirin (RBV) - free regimen of daclatasvir (DCV), asunaprevir (ASV), and BMS-791325 in treatment-naïve, hepatitis C virus genotype 1-infected patients. J hepatol 2013; 58:S573.

[20]. Sulkowski M, Gardiner D, Lawitz E, Hinestrosa F, Nelson D, Thuluvath P, et al. Potent viral suppression with all-oral combination of daclatasvir (NS5A inhibitor) and GS-7977 (NS5B inhibitor), +/-ribavirin, intreatment-naïve patients with chronic hepatitis C GT 1, 2, or 3. J hepatol 2012; 56:S560.

[21]. Gane EJ, Stedman CA, Hyland RH, Ding X, Svarovskaia ES, Pang PS, et al. Once Daily Sofosbuvir/Ledipasvir Fixed Dose Combination with or without Ribavirin: the ELECTRON trial. Hepatology 2013; 58:243A.

458

459 Table 1. Demographics and baseline characteristics

460

	QD			BID	Placebo
	25 mg n=8	50 mg n=8	100 mg n=18	50 mg n=18	n=12
Mean age, yr (range)	45.1 (4.2)	48.8 (3.2)	41.9 (2.1)	46.3 (2.4)	44.7 (3.2)
Male, n (%)	5 (62.5)	7 (87.5)	14 (77.8)	12 (66.7)	8 (66.7)
Race, n (%)					
White	7 (87.5)	7 (87.5)	15 (83.3)	13 (72.2)	11 (91.7)
African American	1 (12.5)	1 (12.5)	3 (16.7)	4 (22.2)	1 (8.3)
Other	0	0	0	1 (5.6)	0
Mean BMI, kg/m^2 (range)	26.1 (1.7)	26.9 (1.1)	26.2 (0.8)	25.6 (0.7)	28.3 (0.9)
Mean baseline HCV RNA, log_{10} IU/mL	6.6 (0.18)	5.9 (0.12)	6.5 (0.13)	6.3 (0.11)	6.3 (0.18)
HCV genotype					
1a	5	6	6	6	6
1b	3	2	0	0	0
2	0	0	1	1	1
2b	0	0	3	3	1
3a	0	0	4	4	2
4	0	0	4	4	2
IL28B genotype, n					
CC	3	3	6	4	4
CT	4	3	9	11	8
TT	1	2	3	3	0

461 QD, once daily; BID, twice daily; BMI, body mass index

462 Table 2. Summary antiviral activity of samatasvir in subjects with HCV genotype 1, 2, 3
463 or 4

Endpoint	GT	Placebo	QD			BID
			25 mg	50 mg	100 mg	50 mg
Mean (min,max; n) change from baseline to 24 h in HCV RNA, log_{10}	1a	0.1 (-0.1,0.3; 6)	3.1 (2.4,3.5; 6)	3.2 (2.9,3.3; 6)	3.2 (2.6,3.8; 6)	2.7 (1.2,3.7; 6)
	1b	NA	2.6 (2.0,3.7; 3)	3.4 (3.3,3.5; 2)	-	-
	2	0.3 (0.1,0.2; 2)	-	-	1.8 (0.1,3.6; 4)	1.7 (0.2,3.4; 4)
	3	-0.1 (-0.5,0.3; 2)	-	-	2.9 (2.4,3.6; 4)	2.7 (2.0,3.7; 4)
	4	0.0 (0.0-0.13; 2)	-	-	3.2 (2.4,3.7; 4)	2.9 (1.6,3.7; 4)
Mean (min,max) change from baseline to 72 h in HCV RNA, log_{10}	1a	0.1 (-0.1,0.2)	3.1 (2.5,3.6)	3.6 (3.3,3.9)	3.1 (2.2,4.2)	2.9 (2.3,3.6)
	1b	-	2.8 (1.6,4.1)	4.1 (4.0,4.2)	-	-
	2	0.2 (0.1,0.2)	-	-	1.8 (0.1,3.6)	1.7 (0.2,3.4)
	3	0.1 (0 ,0.3)	-	-	3.2 (2.8,3.9)	3.0 (2.1,4.3)
	4	0.1 (-0.3,0.5)	-	-	3.8 (2.2,4.6)	3.6 (2.6,4.5)
Mean (min,max) maximum change from baseline in HCV RNA, log_{10}	1a	0.4 (0.2-0.6)	3.3 (2.9,3.7)	3.6 (3.3,3.9)	3.5 (2.6,4.3)	3.2 (2.7,3.8)
	1b	-	3.0 (2.0,4.3)	4.3 (4.1,4.5)	-	-
	2	0.4 (0.3-0.5)	-	-	2.0 (0.3,4.1)	2.0 (0.5,4.0)
	3	0.5 (0.4-0.5)	-	-	3.4 (3.1,3.9)	3.2 (2.5,4.3)
	4	0.6 (0.4-0.7)	-	-	3.6 (2.4,4.6)	3.9 (3.5,4.5)
Median (min,max) time to maximum change, day	1a	-	2.0 (0.7,3.0)	2.3 (2.0,5.0)	1.5 (0.7,4.0)	3.0 (1.0,4.0)
	1b	-	2.0 (1.0,5.0)	4.0 (4.0,4.0)	-	-
	2	-	-	-	0.8 (0.3,3.0)	1.7 (0.3,4.0)
	3	-	-	-	2.0 (1.0,3.0)	2.5 (1.5,3.5)
	4	-	-	-	2.5 (1.0,3.0)	2.9 (2.5,6.0)

GT: genotype; QD: once-daily; BID: twice-daily; -: not available

464

Table 3. Summary pharmacokinetics of samatasvir in subjects with HCV genotype 1, 2, 3 or 4

Dose (mg)/ Regimen	N	Day	C_{max} (ng/mL)	T_{max} (h)	AUC_{24h} (ng*h/mL)	C_{τ} (ng/mL)	$t_{1/2}$ (h)
25 QD	8	1	13.5±5.19	4.0 (3.0-4.0)	142±44.5	2.93±0.98 (1.76-4.91)	-
		3	20.0±6.74	4.0 (3.0-6.0)	235±79.9	5.20±2.02 (3.00-8.15)	20.8±4.06
50 QD	8	1	36.0±20.0	4.0 (3.0-4.0)	384±204	6.81±3.68 (2.78-13.0)	-
		3	32.4±8.12	4.0 (2.0-4.0)	387±115	8.20±2.75 (5.17-13.2)	23.0±3.81
100 QD	18	1	50.9±24.0	4.0 (2.0-6.0)	520±234	10.7±4.61 (6.01-23.4)	-
		3	65.3±33.0	3.0 (2.0-4.0)	728±344	15.6±6.44 (6.03-25.9)	20.4±3.32
50 BID	18	1, AM	33.9±17.6	3.0 (3.0-4.0)	-	11.7±6.95 (2.98-25.5)	-
		1, PM	21.4±7.40	4.0 (0.0-12)	438±180	15.5±6.90 (5.32-29.6)	-
		3, AM	49.4±18.3	3.0 (2.0-4.0)	-	20.8±9.31 (9.86-42.0)	-
		3, PM	27.2±10.5	3.0 (1.0-8.0)	681±259	20.8±6.65 (6.49-43.1)	19.7±4.68

Values are reported as mean ± standard deviation, except for T_{max} where medians (min-max) are reported. For C_{τ}, (min-max) is also shown; AM: morning; PM: evening; -: not applicable; C_{τ}: C_{24h} for QD and C_{12h} for BID; For BID, AUC_{24h} is the sum of the am and pm AUC_{12h} (not shown).

469

470 Table 4. Number (%) of subjects with adverse events regardless of attributability (>5% in

471 any group)

Adverse events	QD			BID	Any dose/regimen	Placebo
	25 mg (n=8)	50 mg (n=8)	100 mg (n=18)	50 mg (n=18)	(n=48)	(n=12)
Constipation	0	1 (12.5)	3 (16.7)	1 (5.6)	5 (10.4)	2 (16.7)
Headache	0	1 (12.5)	4 (22.2)	1 (5.6)	6 (12.5)	1 (8.3)
Nausea	0	0	2 (11.1)	2 (11.1)	4 (8.3)	1 (8.3)
Catheter site pruritus	1 (12.5)	0	2 (11.1)	0	2 (4.2)	0
Dyspepsia	0	0	0	2 (11.1)	2 (4.2)	1 (8.3)
Decreased appetite	1 (12.5)	0	0	0	1 (2.1)	1 (8.3)
Insomnia	0	1 (12.5)	0	0	1 (2.1)	1 (8.3)

472 QD: once-daily; BID: twice-daily

474 **Figure legends**

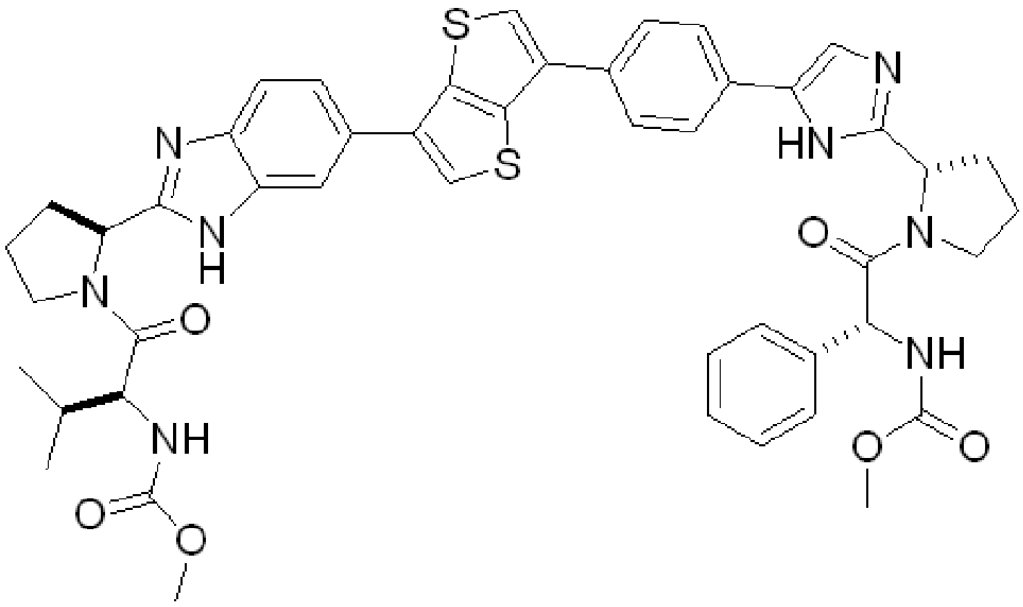
475 **Fig. 1. Chemical structure of samatasvir**

476 **Fig. 2. Plasma HCV RNA, genotypes 1, 3 and 4.** Mean (+standard error) reduction
477 from baseline in plasma HCV RNA in subjects with genotype 1, 3 or 4 HCV

478 **Fig. 3. Plasma HCV RNA, genotypes 2.** Individual and mean (+standard error)
479 reduction from baseline in plasma HCV RNA in subjects with genotype 2 HCV

480 **Fig. 4. Pharmacokinetics.** Mean (+standard deviation) day-3 plasma pharmacokinetic
481 profiles over 24 hours of samatasvir

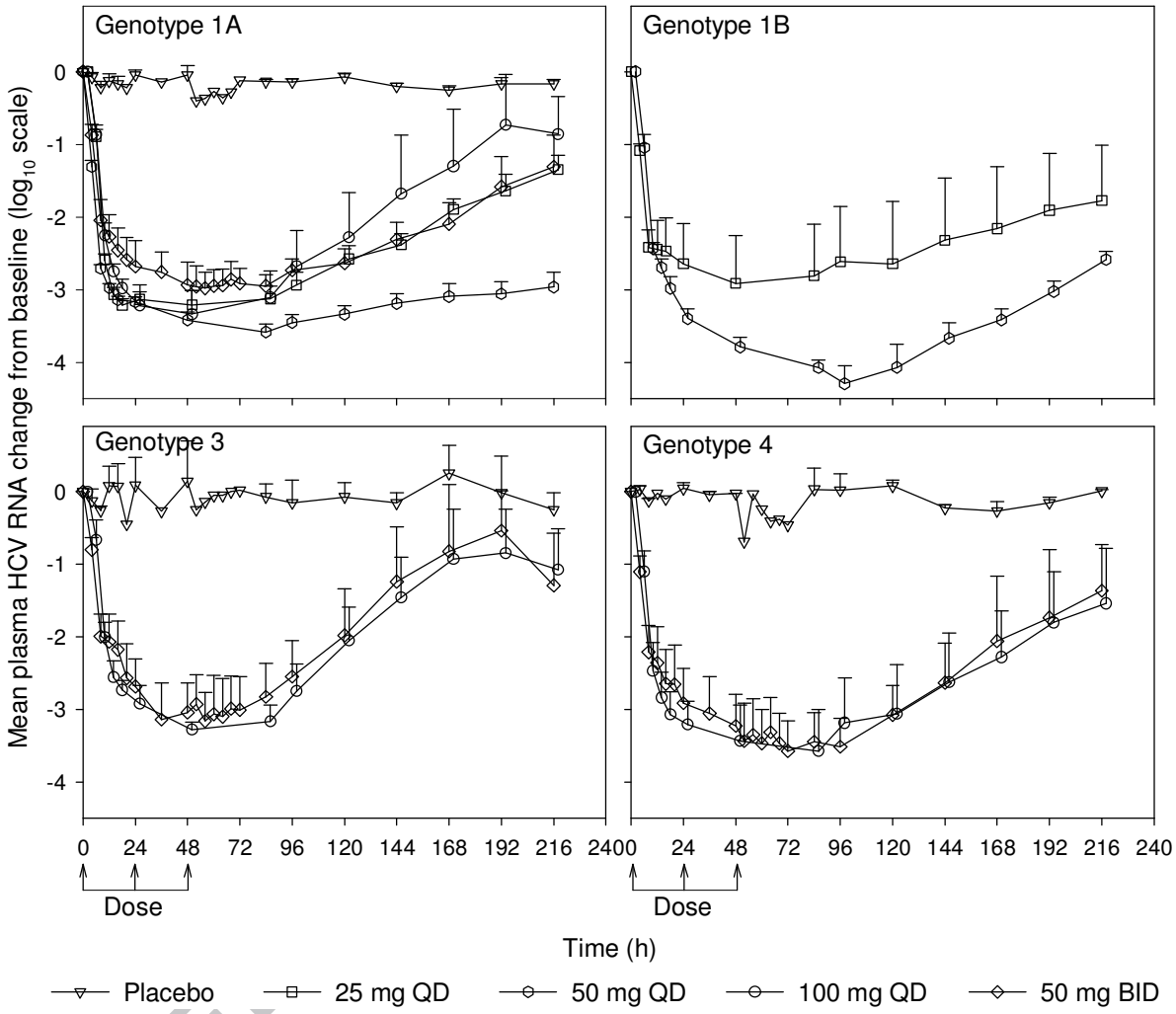
482 Figure 1



483

484

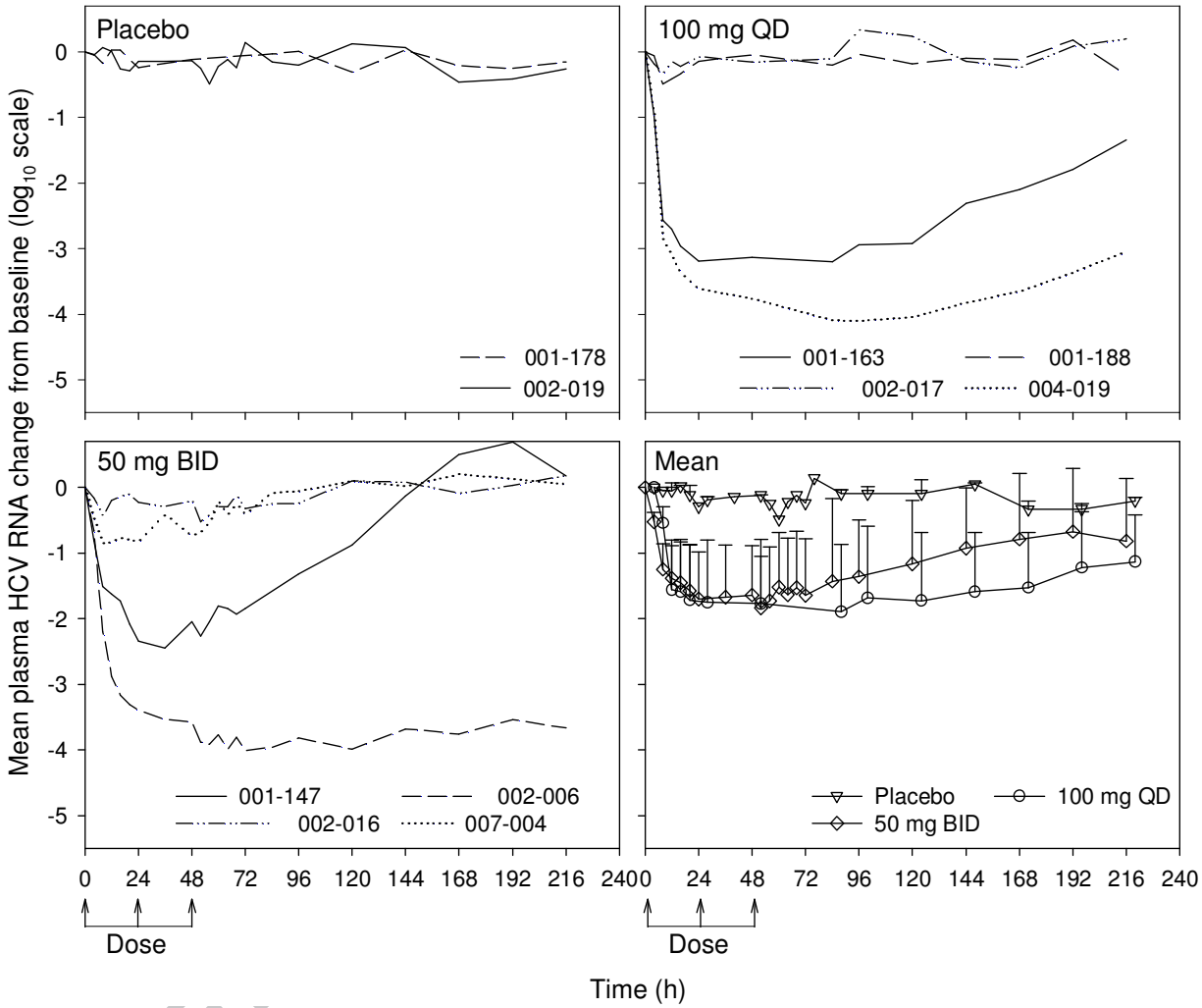
485 Figure 2



486

487

488 Figure 3

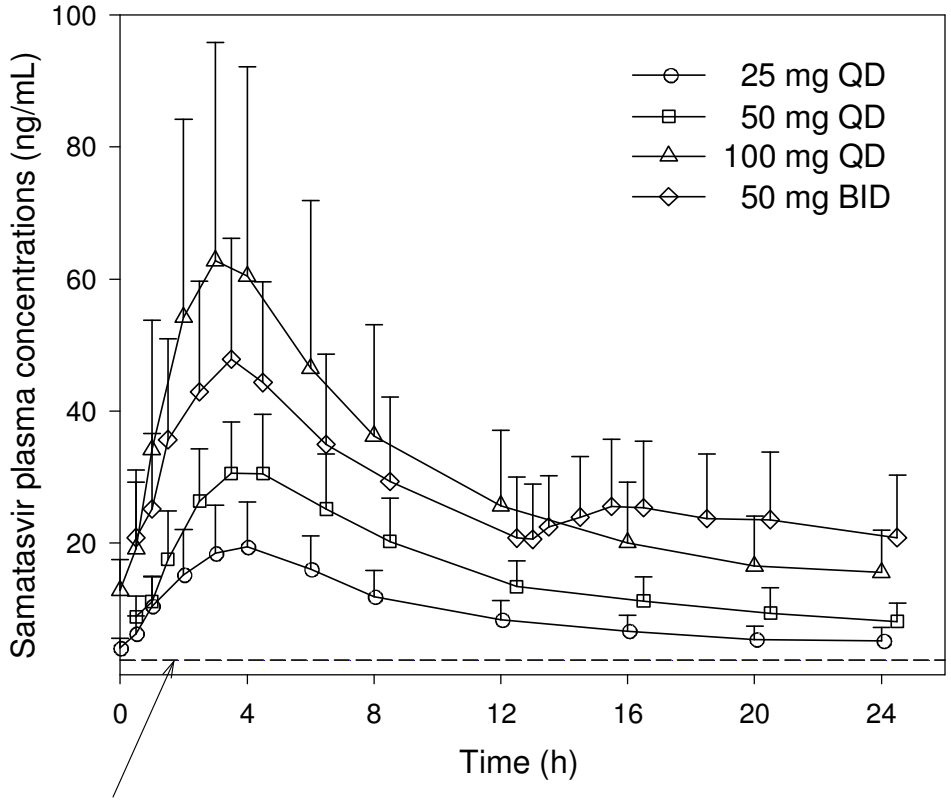


489

490

491

492 Figure 4



493