Research Article

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Potency, safety, and pharmacokinetics of the NS3/4A protease inhibitor BI201335 in patients with chronic HCV genotype-1 infection

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Background & Aims: BI201335 is a highly specific and potent HCV protease inhibitor. This multiple rising dose trial evaluated antiviral activity and safety in chronic HCV genotype-1 patients.
Methods: Thirty-four treatment-naïve patients were randomized to monotherapy with placebo or BI201335 at 20–240 mg once-daily for 14 days, followed by combination with pegylated interferon alfa/ribavirin (PegIFN/RBV) through Day 28. Nineteen treatment-experienced patients received 48–240 mg BI201335 once-daily with PegIFN/RBV for 28 days. HCV-RNA was measured with Roche COBAS TaqMan.
Results: In treatment-naïve patients, median maximal viral load (VL) reductions during 14-day monotherapy were −3.0, −3.6, −3.7, and −4.2 $\log_{10}$ for the 20, 48, 120, and 240 mg groups. VL breakthroughs ($\geqslant$1 $\log_{10}$ from nadir) were seen in most patients on monotherapy and were caused by NS3/4A variants (R155K, D168V) conferring *in vitro* resistance to BI201335. Adding PegIFN/RBV at Days 15–28 led to continuous viral load reductions in most patients. In treatment-experienced patients, treatment with BI201335 and PegIFN/RBV achieved VL <25 IU/ml at Day 28 in 3/6, 4/7, and 5/6 patients in the 48, 120, and 240 mg dose groups. VL breakthroughs were observed during triple combination in only 3/19 patients. BI201335 was generally well tolerated. Mild rash or photosensitivity was detected in four patients. Mild unconjugated hyperbilirubinemia was the only dose-dependent laboratory abnormality of BI201335. BI201335 elimination half-life supports once-daily dosing.
Conclusions: BI201335 combined with PegIFN/RBV was well tolerated and induced strong antiviral responses. These results support further development of BI201335 in HCV genotype-1 patients.

Keywords: Hepatitis C virus; Antiviral; Pegylated interferon; Ribavirin; Resistant variants.
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Abbreviations: HCV, hepatitis C virus; GT-1, genotype-1; SOC, standard-of-care; PegIFN, pegylated interferon alfa; RBV, ribavirin; SVR, sustained virologic response; HIV, human immunodeficiency virus; qd, once-daily; TN, treatment-naïve; TE, treatment-experienced; HBV, hepatitis B virus; LLOQ, lower limit of quantification; LLOD, lower limit of detection; VL, viral load; AEs, adverse events; ALT, alanine aminotransferase; AST, aspartate transaminase; CV%, coefficient of variation.

Introduction

Hepatitis C virus (HCV) infection is a leading cause of chronic hepatitis, liver cirrhosis, and liver cancer worldwide. Genotype-1 (GT-1) represents the most prevalent and also most difficult-to-treat HCV subtype. Treatment with the current standard-of-care (SOC) i.e. 48 weeks of pegylated interferon alfa (PegIFN) and ribavirin (RBV), leads to a sustained virologic response (SVR) in 40–50% of patients, indicative of the resolution of the infection [1–5]. However, more than 50% of those treated do not respond or relapse. In addition, many patients cannot tolerate interferon and/or RBV-based therapies. Furthermore, SVR rates are much lower for so-called difficult-to-treat populations like patients with decompensated liver cirrhosis, human immunodeficiency virus (HIV)-HCV co-infected patients, post-transplant HCV infection, hemodialysis patients, and others [3].

The HCV protease inhibitor BILN 2061 was the first agent in man to specifically target HCV replication and thus provided the proof-of-concept for directly inhibiting HCV NS3/NS4A protease as a means of suppressing viral replication [6]. This agent showed 3–4 $\log_{10}$ reductions in HCV RNA within 48 h of treat-

ment in HCV GT-1 patients [7], as well as a smaller but significant activity against GT-2/3 infection [8]. However, the development of BILN 2061 was halted due to ultrastructural cardiotoxic changes observed in animals treated with supratherapeutic doses. The encouraging viral response to this first protease inhibitor, however, has led to the development of several follow-up agents, many of which are now in different stages of drug development. Two developmental protease inhibitors, telaprevir and boceprevir, are now being tested in phase III trials, after successful completion of phase II studies [9–11]. In these trials, telaprevir is given for 8 or 12 weeks on a background of 24 or 48 weeks of PegIFN/RBV, while boceprevir is given for 24 or 44 weeks in combination with 28 to 48 weeks of PegIFN/RBV. However, both drugs are being given every 8 h or three times per day and have significant side effects including skin rash, anemia, and gastrointestinal symptoms. Therefore, HCV protease inhibitors with improved pharmacokinetics, safety, and tolerability are needed.

Protease inhibitors given as monotherapy also show rapid selection of drug-resistant variants. These variants result from the poor replication fidelity of the virus and are selected due to the pressure placed on the virus. Consequently, combination of a HCV protease inhibitor with PegIFN/RBV is currently required in order to minimize the risk of virologic breakthrough due to resistance [9–11].

BI201335 is a peptidomimetic HCV-specific protease inhibitor with high *in vitro* activity against GT-1a and -1b subtypes, with EC_{50} values of 6.5 and 3.1 nM, respectively. Preclinical and preliminary human pharmacokinetic studies suggested that BI201335 maintains sufficient plasma concentrations at steady-state to allow once-daily (qd) dosing (Boehringer Ingelheim, unpublished data). A multiple rising dose study in volunteers indicated that BI201335 was safe and well tolerated at doses of 20–240 mg qd for 21–28 days (Boehringer Ingelheim, unpublished data). These observations allowed progression to the phase Ib trial (1220.2) reported here.

Patients and methods

Study design

This was a randomized, multi-center, multiple rising-dose trial, performed between December 2007 and June 2008 at 16 sites in France, Germany, Spain, and USA. The trial was double-blind and placebo-controlled for groups with treatment-naïve (TN) patients. The trial was conducted in full compliance with the Guidelines of Good Clinical Practice and the Declaration of Helsinki and approved by all competent institutional review boards, ethical committees, and national authorities. All patients gave written informed consent. An overview of the trial design is provided in Fig. 1.

Study treatments

TN patients were randomized to receive, in successive cohorts, either BI201335 monotherapy (20, 48, 120, and 240 mg qd) for 14 days, or matching placebo in a 3:1 ratio within each cohort. The data from each cohort was reviewed by a Data Monitoring Committee prior to enrolling the next dose cohort. In patients with a HCV RNA decrease $\geqslant 1 \log_{10}$ from baseline (on Day 10), BI201335 treatment was combined with PegIFN alfa 2a (180 μg/week) and RBV (1000 or 1200 mg/day for body weight $\leqslant$ or >75 kg) from Days 14 to 28. Treatment-experienced (TE) patients received triple combination treatment for 28 days: BI201335 at 48, 120, or 240 mg qd, combined with PegIFN alfa 2a and RBV as above. The data from each cohort were reviewed by a Data Monitoring Committee prior to enrolling the next dose cohort. All patients were offered to extend SOC to Week 48, with an additional 24 weeks of follow-up, at the discretion of the investigator.

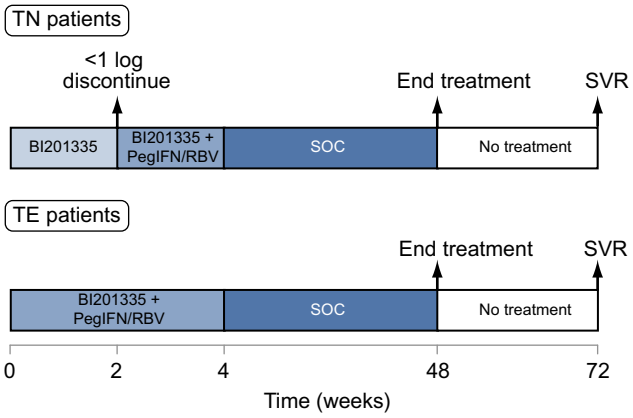

Fig. 1. Trial design.

Inclusion and exclusion criteria

Patients with chronic HCV infection of GT-1 were recruited to the study, if they were TN (no prior therapy with interferon, PegIFN, or RBV) or TE (virologic failure during or after treatment with an approved dose of PegIFN combined with RBV), had HCV RNA $\geqslant$100,000 IU/ml and were aged 18 years or older. Patients with liver cirrhosis, hyperbilirubinemia (>1.5× upper limit of normal; patients with Gilbert's disease were accepted), HIV, or hepatitis B virus (HBV) co-infection were excluded. Furthermore, patients who had previously received any treatment with a protease inhibitor and females of child-bearing potential not agreeing or able to use medically accepted contraception throughout the study were excluded.

Pre-treatment response as reported by the investigator was classified in accordance with recent FDA definitions [12]; *relapse:* re-appearance of HCV RNA in serum after treatment discontinuation; *break-through:* re-appearance of HCV RNA during treatment; *null response:* maximum reduction in HCV RNA <2 $\log_{10}$ at Week 12 of treatment; *partial response:* maximal reduction in HCV RNA at Week 12 >2 $\log_{10}$ but HCV RNA never undetectable. All patients provided written informed consent before study participation.

Assessments

Analysis of HCV RNA and genotype

Plasma samples (10 ml) for HCV RNA analysis were obtained from all patients at for Days 1, 2, 3, 4, 6, 10, and 14. Additionally, for TN patients, for Days 15, 16, 17, 18, and 28, samples were taken from those having achieved an HCV RNA reduction by $\geqslant 1 \log_{10}$ at Day 10. Similarly, for TE patients with a $\geqslant 1 \log_{10}$ HCV RNA decline at Day 10, additional samples were taken on Days 21 and 28. Plasma samples were processed in a central laboratory using the Roche COBAS TaqMan HCV/HPS assay (Roche Diagnostics, Indianapolis, IN). The lower limit of quantification (LLOQ) was 25 IU/ml; the lower limit of detection (LLOD) approximates 10 IU/ml, although not validated by the manufacturer. HCV genotype was determined using the Trugene HCV assay (Bayer, Leverkusen, Germany). Viral load (VL) breakthrough was defined as HCV RNA rebound $\geqslant 1 \log_{10}$ from the HCV RNA nadir or to $\geqslant$1000 IU/ml if HCV RNA was previously undetectable.

Genotypic and phenotypic resistance monitoring

Viral RNA was isolated from the plasma of HCV-infected subjects, at baseline and indicated time-points, using the QiaAmp Viral RNA extraction kit (Qiagen, Hilden, Germany). A DNA fragment containing the complete NS3/NS4A region was synthesized using Superscript III one step reverse transcription polymerase chain reaction system and two gene-specific primers. Two second-round, semi-nested polymerase chain reaction products spanning either the entire NS3/NS4A region or the NS3 protease domain were generated using Novagen KOD Hot Start DNA polymerase (Merck, Darmstadt, Germany). The LLOD of the reverse transcription polymerase chain reaction amplification method restricted the analysis to patient samples with a VL $\geqslant$1000 IU/ml. The NS3/NS4A nucleotide sequence was obtained by direct DNA sequencing of the amplified product using Big Dye Terminator V3.1 and the ABI 3100 Genetic Analyzer (Applied Biosystems, CA, USA) detection system in combination with 3100 Genetic Analyzer Data collection software V1.1.

For phenotypic resistance testing, polymerase chain reaction products from HCV-infected patient plasma samples were used to amplify DNA fragments containing the NS3 protease domain. NS3 amplicons were ligated into a HCV replicon shuttle vector (pIT2) containing a luciferase reporter gene, and reconstituted plasmid DNA was used to generate HCV subgenomic replicon RNA transcripts (T7 Ribomax kit; Promega, WI, USA). The *in vitro* transcribed RNA was electroporated into Huh-7.5 cells which were then seeded in 96-well plates for 24 h and treated with a range of BI201335 (or interferon alfa) concentrations for a period of 72 h. At the end of the incubation, luciferase activity was measured with Bright-Glo substrate, as a marker for HCV RNA replication, and luminescence quantified. The concentration of BI201335 giving 50% inhibition of HCV RNA replication (EC_{50}) was determined.

Safety assessments

Adverse events (AEs) were recorded descriptively for all patients receiving study drugs and coded using the Medical Dictionary for Drug Regulatory Affairs. Vital signs (blood pressure, pulse rate), electrocardiogram and safety laboratory parameters were also evaluated.

Assessment of drug plasma concentrations

Plasma samples were collected and stored at −20 °C at various times during the course of the study. Plasma concentrations of BI201335 were determined by a validated high performance liquid chromatography and tandem mass spectrometry assay method with a LLOQ of 0.2 ng/ml. Pharmacokinetic parameters calculated from the plasma concentration–time data on Day 28 following the last BI201335 dose administration were obtained by non-compartmental analysis.

Statistical assessments

Activity and safety
Due to the exploratory multiple rising dose design of this trial, sample sizes per cohort sufficient and typical for early phase Ib trials were chosen. Frequency tables for categorical endpoints and summary statistics including mean, median, quartiles, and minimum and maximum for continuous endpoints are presented.

Results

Patient disposition and baseline characteristics

In 2007 and 2008, a total of 171 patients were screened: 53 patients were randomized to treatment in this study; 75 patients did not meet the inclusion criteria, 43 patients were entered into later cohorts that will be reported in a separate manuscript. The rate of study completion was high; 52 out of 53 patients randomized to treatment completed the study. The reason for the discontinuation of one subject was the diagnosis of an unexpected pregnancy of his partner representing an exclusion criterion for treatment with RBV. Baseline demographics were similar across all cohorts (Table 1).

Antiviral response

TN patients
With the exception of one patient in the 20 mg treatment group, all patients receiving BI201335 ($n = 25$, 96.2%) achieved the primary efficacy endpoint of $\geqslant 2\ \log_{10}$ plasma VL reduction at any time up to Day 14. No significant change in VL was observed in any patients in the placebo-control groups. Maximum changes from baseline to Day 14 (for naïve patients) were dose-dependent and are summarized in Table 2A and median VL changes from baseline are shown in Fig. 2A.

In the active treatment groups, HCV RNA decline was rapid, with a nadir that typically reached 2–3 days after starting monotherapy (Fig. 2B). However, in the majority of patients in all dose groups, virologic breakthrough during treatment was seen in the first 14 days of monotherapy (Fig. 2). The proportion of patients with viral breakthrough by Day 14 was 83.3%, 71.4%, 71.4%, and 83.3% with 20, 48, 120, and 240 mg qd of BI201335, respectively.

Following 2 weeks of monotherapy, SOC (PegIFN/RBV) treatment was added in patients with a $\geqslant 1\ \log_{10}$ reduction in VL at Days 6 or 10. This criterion was met in none of the placebo patients, but in all patients on BI201335. After start of SOC treatment, 4/6, 4/7, 6/7, and 6/6 patients treated with 20, 48, 120, or 240 mg BI201335, respectively, showed VL reductions by $\geqslant 1$ log from Days 15 to 28.

TE patients
In all TE triple combination groups, rapid, strong VL reductions were observed from Days 1 to 28 during triple-combination therapy (Fig. 3A). All TE patients achieved the primary efficacy endpoint of $\geqslant 2\ \log_{10}$ reduction in VL from baseline during treatment. As shown in Fig. 3A, median VL reduction appeared to be dose-dependent. The numbers of patients in the 120 and 240 mg dose groups attaining rapid viral response at Day 28 showed a trend to decreased breakthroughs and increased rapid viral response rates compared to the 48 mg group, as shown in Table 2B.

In contrast to monotherapy, VL breakthrough was rare under triple therapy with BI201335 and PegIFN/RBV, despite enrollment of pre-treatment failures including null-responders, as only three patients with VL breakthrough were observed: two in the 48 mg and one in the 120 mg dose groups (two previous null-responders, one partial responder). There were no breakthroughs at the highest dose level of 240 mg qd (Fig. 3b).

Across all dose groups, 3 of 6 null-responders, 4 of 7 partial responders, 0 of 1 breakthrough and 5 of 5 relapsers to previous PegIFN/RBV treatment experienced a RVR (VL <25 IU/ml at Day 28) during re-treatment with BI201335 plus PegIFN/RBV.

Safety

Monotherapy (TN patients)
During the 14-day monotherapy period, the frequency of AEs was similar with all doses of BI201335, compared with placebo. Most AEs were mild-to-moderate in intensity and not dose-dependent in frequency or intensity. There were no reports of rash or erythema during monotherapy with BI201335.

Safety laboratory analyses indicated that there was no negative impact of monotherapy on blood counts, liver enzymes, or other safety parameters in TN patients (Table 3A). Median alanine aminotransferase (ALT) and aspartate transaminase (AST) concentrations decreased in all dose groups from baseline to Day 14, accompanying the decline in HCV RNA. There was a slight and dose-dependent increase of the median unconjugated bilirubin (−0.1, +0.1, +0.3, +0.8 mg/dl in the 20, 48, 120, and 240 mg dose groups, respectively).

Table 1. Summary of baseline characteristics for (A) treatment-naïve and (B) treatment-experienced patient cohorts.

A	TN				
	Placebo (n = 8)	BI201335 20 mg qd (n = 6)	BI201335 48 mg qd (n = 7)	BI201335 120 mg qd (n = 7)	BI201335 240 mg qd (n = 6)
Gender, *n* (%)					
Male	6 (75)	6 (100)	5 (71.4)	5 (71.4)	5 (83.3)
Female	2 (25)	0 (0)	2 (28.6)	2 (28.6)	1 (16.7)
Genotype, *n* (%)					
1	1 (12.5)	0 (0)	0 (0)	2 (28.6)	1 (16.7)
1a	2 (25.0)	3 (50.0)	2 (28.6)	2 (28.6)	3 (50.0)
1b	5 (62.5)	3 (50.0)	5 (71.4)	3 (42.9)	2 (33.3)
Race, *n* (%)					
Caucasian	8 (100)	5 (83.3)	7 (100)	7 (100)	6 (100)
Asian	0 (0)	1 (16.7)	0 (0)	0 (0)	0 (0)
Age, years (SD)	49.9 (8.4)	51.0 (8.0)	47.1 (13.8)	48.6 (16.3)	47.8 (9.3)
Body mass index, kg/m^2	27.2 (6.0)	23.4 (2.2)	25.4 (5.6)	26.6 (3.0)	26.8 (3.8)
ALT, U/L (SD)	58 (24)	67 (40)	101 (60)	87 (47)	53 (11)
HCV RNA, $\log_{10}$ IU/ml	6.8	7.1	6.7	6.5	6.8
Median (range)	(6.0–7.3)	(6.4–7.7)	(4.7–7.3)	(5.8–7.0)	(6.0–7.2)

B	TE		
	BI201335 48 mg qd + PegIFN/RBV (n = 6)	BI201335 120 mg qd + PegIFN/RBV (n = 7)	BI201335 240 mg qd + PegIFN/RBV (n = 6)
Gender, n (%)			
Male	4 (66.7)	4 (57.1)	3 (50.0)
Female	2 (33.3)	3 (42.9)	3 (50.0)
Genotype, n (%)			
1	0 (0.0)	1 (14.3)	1 (16.7)
1a	3 (50.0)	4 (57.1)	4 (66.7)
1b	3 (50.0)	2 (28.6)	1 (16.7)
Race, n (%)			
Caucasian	6 (100.0)	7 (100.0)	6 (100.0)
Age, years (SD)	46.8 (11.8)	46.7 (4.8)	49.3 (10.1)
Body mass index, kg/m^2	26.4 (4.4)	25.4 (6.1)	28.0 (4.0)
ALT, U/L (SD)	74 (37)	86 (34)	89 (64)
HCV RNA, $\log_{10}$ IU/ml	7.1	7.0	6.8
Median (range)	(5.9–7.3)	(6.2–7.4)	(6.3–7.1)
Pre-treatment response, n			
Relapse	2	1	2
Break-through	1	0	0
Null-response	2	4	0
Partial response	1	2	4

Abbreviations: TN, treatment-naïve; TE, treatment-experienced; ALT, alanine aminotransferase; HCV, hepatitis C virus; SD, standard deviation; qd, once-daily; relapse [re-appearance of HCV RNA in serum after treatment discontinued], break-through [re-appearance of HCV RNA in serum during treatment], null response: <2 $\log_{10}$ maximum reduction in HCV RNA at week 12 of treatment; partial response: maximal reduction in HCV RNA at week 12 $\geqslant$2 $\log_{10}$, but never achieved HCV RNA below level of detection.

Combination therapy (TN and TE patients)

Under combination therapy of BI201335 with PegIFN/RBV, the most common AEs in both, TN or TE patients, were fatigue, nausea, headache, gastrointestinal tract disorders, and anemia. Three cases of mild rash or erythema occurred in TN patients during treatment with 20, 48, or 240 mg BI201335 and SOC, that all

Table 2. HCV RNA maximum change from (A) baseline to day 14 of BI201335 monotherapy in treatment-naïve patients and (B) baseline to day 28 of combination therapy with BI201335 and PegIFN/RBV in treatment-experienced patients; and proportion of patients achieving HCV RNA <LLOD (RNA <10 IU/ml) and <LLOQ (RNA <25 IU/ml) at Week 4 (intention-to-treat cohort).

A	Placebo (n = 8)	BI201335 20 mg qd (n = 6)	BI201335 48 mg qd (n = 7)	BI201335 120 mg qd (n = 7)	BI201335 240 mg qd (n = 6)
Max. change from baseline	- 0.06	- 3.0	- 3.6	- 3.7	- 4.4
Median (min, max), log_{10} IU/ml	(- 0.7, 0.1)	(- 3.9, - 1.5)	(- 3.8, - 3.1)	(- 4.1, - 3.3)	(- 4.9, - 3.8)
RNA LLOD, n (%)	0 (0)	0 (0)	2 (28.6)	1 (14.3)	1 (16.7)
RNA LLOQ, n (%)	0 (0)	1 (16.7)	2 (28.6)	2 (28.6)	3 (50.0)

B	BI201335 48 mg qd + PegIFN/RBV (n = 6)	BI201335 120 mg qd + PegIFN/RBV (n = 7)	BI201335 240 mg qd + PegIFN/RBV (n = 6)
Max. change from baseline	- 5.0	- 5.2	- 5.3
Median (min, max), log_{10} IU/ml	(- 5.9, - 3.4)	(- 6.0, - 3.9)	(- 6.1, - 4.7)
RNA LLOD, n (%)	2 (33.3)	1 (14.3)	1 (16. 7)
RNA LLOQ, n (%)	3 (50.0)	4 (57.1)	5 (83.3)

Abbreviations: HCV, hepatitis C virus; LLOD, lower limit of detection (RNA <10 IU/ml); LLOQ, lower limit of quantification (RNA <25 IU/ml); PegIFN/RBV, pegylated interferon alfa/ribavirin; qd, once-daily.

recovered spontaneously. In addition, one case of mild photosensitivity reaction emerged in a TE patient treated with 240 mg BI201335 and SOC. Following the initiation of therapy with PegIFN/RBV, two patients experienced serious AEs (asthenia, cataract); neither were considered to be related to the study drug. There were no AEs leading to pre-term discontinuation of treatment. The only other changes were decreases of blood counts from baseline to Day 28 and a slight dose-dependent increase of unconjugated bilirubin across the three dose groups (Table 3B). The highest total bilirubin values were 4 mg/dl in one patient treated with 240 mg qd and 3.2 mg/dl in two individuals treated with 120 and 240 mg QD. Unconjugated hyperbilirubinemia was isolated, asymptomatic and reversible after completion of BI201335 in all affected patients. However, there were no reports of jaundice during treatment with BI201335 and no signs of liver toxicity or hemolysis in individuals with elevated unconjugated bilirubin.

Viral resistance

Population sequencing of viral isolates obtained at baseline revealed in one patient a variant encoding an NS3V/I170T substitution that conferred a sevenfold reduction in BI201335 sensitivity (increased EC_{50}) relative to the subtype reference; the patient harboring this variant was in the lowest dose group (20 mg TN) who had failed to achieve $\geqslant 2\ log_{10}$ VL reduction within the first 14 days. HCV subgenomic replicon based phenotyping of all other baseline-derived NS3 amplicons resulted in mean EC_{50} values for GT-1a (10 ± 8 nM) or -1b (9 ± 4 nM) that were within threefold of the reference values.

In patients experiencing viral breakthroughs, the predominant GT-1a resistance mutations in on-treatment samples encoded an R155K substitution, whereas GT-1b viruses mainly encoded changes at D168, with valine (D168V) as a predominant and glutamate (D168E) as a rare substitution. R155K variants conferred reductions in sensitivity to BI201335 with EC_{50} values of 1.8–6.5 μM, whereas the EC_{50} values for D168V variants were 3.6–15 μM (Table 4). However, all BI201335 resistant mutant replicons remained susceptible to inhibition by interferon alfa. The details of these phenotyping and genotyping studies, including longitudinal clonal sequence analyses, will be reported in a follow-up paper.

Pharmacokinetics

Steady-state pharmacokinetic parameters of BI201335 following the last dose on Day 28 are summarized in Table 5. Plasma BI201335 concentrations peaked at 2–6 h (t_{max}) and increased supra-proportionally with dose as seen for mean C_{max}, C_{min} and $AUC_{0-\tau}$. Mean elimination half-life of BI201335 was approximately 20–30 h, suggesting suitability for qd dosing. No apparent difference was found between TN and TE HCV patients in plasma concentrations and exposure. Inter-individual variability was moderately high with a coefficient of variation (CV%) for $AUC_{0-\tau}$ of up to 65%.

Discussion

This trial investigated the antiviral activity, safety and pharmacokinetics of BI201335 in 53 patients with HCV GT-1 infection treated with BI201335 ± PegIFN and RBV for 28 days.

When given qd as monotherapy in TN patients for 14 days, 48–240 mg BI201335 qd induced a rapid, dose-dependent decrease in plasma HCV RNA by $\geqslant 2\ log_{10}$ from baseline in all patients. Maximal VL declines from baseline occurred within 2–3 days of first administration. At the 240 mg qd dose, the median maximal decline from baseline was $-4.4\ log_{10}$ IU/ml. The magnitude of VL declines was similar to those recorded for other highly

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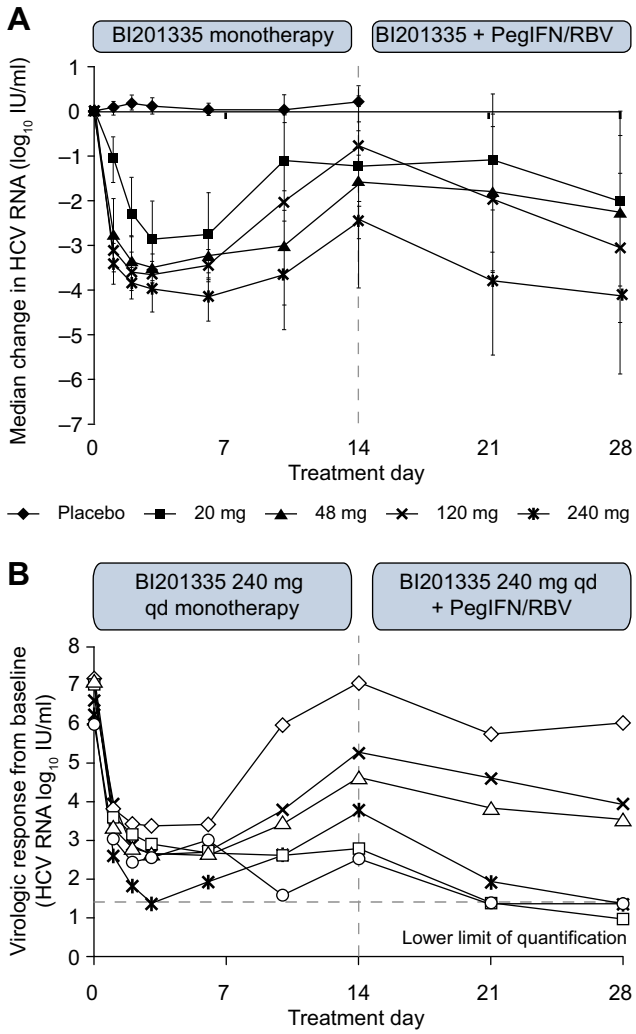

Fig. 2. Antiviral response of TN patients receiving monotherapy with BI201335 (Days 1–14) followed by combination with PegIFN and RBV (Days 15–28). (A) Median change in HCV RNA (log_{10}) from baseline to day 28 (± standard deviation) in different dose groups. (B) Individual patient HCV RNA levels from baseline to day 28 in the 240 mg cohort ($n = 6$).

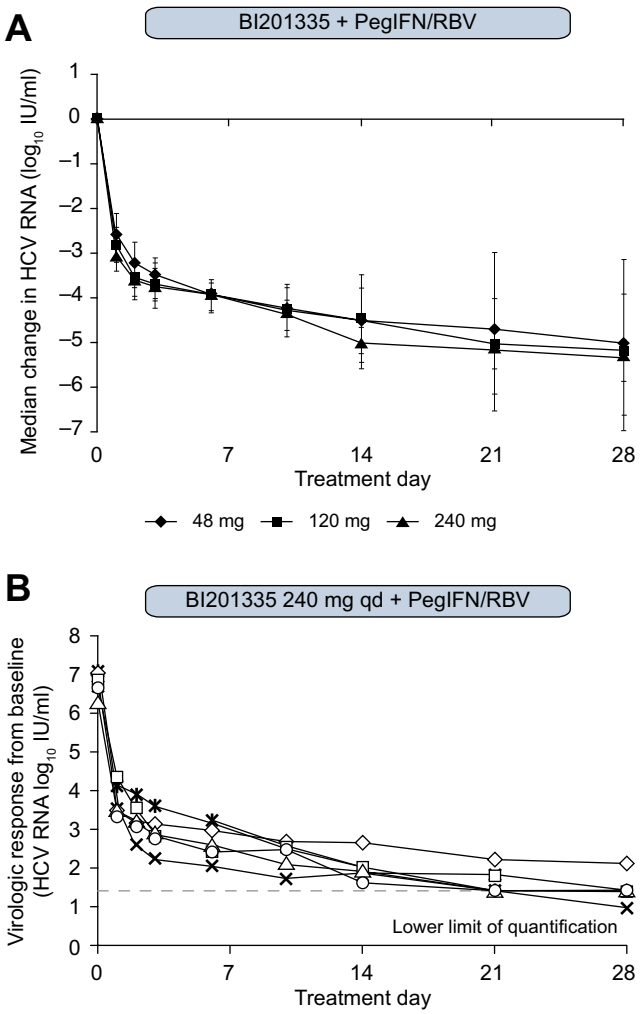

Fig. 3. Antiviral response of TE patients receiving triple therapy with BI201335/PegIFN and RBV (days 1–28). (A) Median change in HCV RNA (log_{10}) from baseline to day 28 (± standard deviation) in different dose groups. (B) Individual patient HCV RNA levels from baseline to day 28 in the 240 mg cohort ($n = 6$).

potent protease inhibitors in development (typical range −3.5 to −4.5 log_{10} IU/ml). Notably, compared with telaprevir (maximal median HCV RNA decline −4.4 log_{10} at 750 mg every 8 h for 14 days [13]) and boceprevir (maximal mean HCV RNA decline −2.06 log_{10} IU/ml at 400 mg three times per day for 14 days [14]), the virologic responses for BI201335 were achieved from single daily doses as opposed to multiple daily dosing. During BI201335 monotherapy, the initial rapid VL drop was followed by VL breakthrough in most patients.

When BI201335 was given in combination with PegIFN/RBV, a rapid and more profound dose-related reduction in HCV RNA was found even in patients failing previous PegIFN/RBV treatment. At the dose of 240 mg BI201335 qd, the decline in HCV RNA achieved by triple combination treatment was −5.3 log_{10} IU/ml. The rapid virological response rates (HCV RNA <25 IU/ml at Day 28) in TE patients increased with dose up to 5/6 (83.3%) in the 240 mg dose group. Notably, virologic breakthroughs were infrequent and confined to previous non-responders treated in the lower 48 and 120 mg dose groups (total $n = 3$). No virologic breakthrough was observed over 28 days of triple therapy in the 240 mg dose group in TE patients.

All relapsers to previous PegIFN/RBV treatment achieved RVR, whereas previous null or partial responders across all dose groups had similar response and breakthrough rates. Thus, in this study relapsers had on-treatment response rates similar to TN patients, while there were no differences in RVR rates between partial and null responders. However, the small numbers for these comparisons limit strong conclusions.

In all cases, viral breakthroughs during BI201335 treatment were caused by the rapid emergence of drug-resistant viral variants, as observed with other protease inhibitors [15]. The predominant GT-1a NS3/NS4A resistance mutation in on-treatment viral breakthrough samples encoded an R155K substitution. GT-1b viruses mainly encoded changes at D168, with valine as a predominant substitution. R155K variants conferred reductions in sensitivity to BI201335 with EC_{50} values of 1.8–6.5 μM, whereas the EC_{50} for D168V variants were 3.6–15 μM. These vari-

Table 3. Laboratory changes during (A) monotherapy with BI201335 at Day 14 compared with baseline in treatment-naïve patients and (B) treatment with BI201335 in combination with PegIFN/RBV at Day 28 compared with baseline in treatment-experienced patients (intention-to-treat cohort).

A

Test Parameter	(Normal Range)	Change from Day 1 to Day 14, Median (Min, Max)				
		Placebo (n = 8)	BI201335 20 mg qd (n = 6)	BI201335 48 mg qd (n =7)	BI201335 120 mg qd (n = 7)	BI201335 240 mg qd (n = 6)
ALT	(6.0–36.0 U/L)	-4 (-77, 21)	6 (-18, 9)	-44 (-123, 10)	-46 (-93, -18)	-24 (-43, -9)
AST	(9.0–36.0 U/L)	1 (-22, 19)	-4 (-11, 8)	-26 (-91, -16)	-32 (-50, -14)	-11 (-18, -5)
AP	(31.0–131.0 U/L)	-2 (-6, 7)	-1 (-3, 10)	-4 (-26, 29)	-6 (-10, 3)	3 (-6, 13)
Bilirubin, total	(0.1–1.6 mg/dl)	-0.2 (-0.4, 0.1)	-0.1 (-0.6, 0.1)	0.1 (-0.3, 0.2)	0.3 (0, 0.5)	0.8 (0.2, 1.6)
Bilirubin, indirect	(0–1.2 mg/dl)	-0.2 (-0.3, 0.1)	-0.1 (-0.3, 0.1)	0.1 (-0.2, 0.2)	0.3 (0.1, 0.5)	0.8 (0.1, 1.3)
GGT	(4.0–60.0 U/L)	-3 (-7, 3)	-7 (-32, 0)	-12 (-34, -1)	-15 (-106, -3)	-24 (-84, -4)
Hemoglobin	(11.5–18.1 g/dl)	-0.4 (-0.7, 0.8)	-0.1 (-1.0, 1.2)	-0.1 (-0.8, 1.0)	-0.6 (-1.2, 0.6)	-0.7 (-1.6, 0.5)
Platelets	(130–400 x 10^3/UL)	6 (-28, 41)	1 (-33, 27)	10 (-67, 26)	8 (-36, 26)	-9 (-48, 68)
White blood cells	(3.8–10.7 x 10^3/UL)	-0.4 (-1.4, 0.4)	0.5 (-1.5, 2.2)	0.1 (-1.1, 1.3)	1.4 (-0.2, 4.7)	-0.9 (-3.3, 1.0)

B

Test Parameter	(Normal Range)	Change from Day 1 to Day 28, Median (Min, Max)		
		BI201335 48 mg qd + PegIFN/RBV (n = 6)	BI201335 120 mg qd + PegIFN/RBV (n = 7)	BI201335 240 mg qd + PegIFN/RBV (n = 6)
ALT	(6.0–36.0 U/L)	-30 (-90, -12)	-48 (-150, -8)	-26 (-152, -13)
AST	(9.0–36.0 U/L)	-8 (-29, -2)	-41 (-56, -11)	-13 (-52, -3)
AP	(31.0–131.0 U/L)	2 (-18, 15)	-3 (-27, 21)	10 (-6, 28)
Bilirubin, total	(0.1–1.6 mg/dl)	0.3 (0.2, 1.6)	0.3 (-0.3, 1.3)	1.0 (0.8, 3.7)
Bilirubin, indirect	(0–1.2 mg/dl)	0.2 (0.1, 1.4)	0.2 (0.1, 0.9)	0.7 (0.6, 3.6)
GGT	(4.0–60.0 U/L)	-9 (-39, -5)	-24 (-139, 4)	-24 (-92, 6)
Hemoglobin	(11.5–18.1 g/dl)	-3.1 (-4.3, -2.5)	-2.4 (-4.5, 0.3)	-1.9 (-4.1, -0.5)
Platelets	(130–400 x 10^3/UL)	-32 (-73, 27)	-59 (-178, -21)	-19 (-98, 60)
White blood cells	(3.8–10.7 x 10^3/UL)	-3 (-4.7, -1.3)	-4.1 (-6.7, 1.4)	-3.4 (-6.6, -1.6)

Abbreviations: AST, aspartate transaminase; ALT, alanine transaminase; AP, alkaline phosphatase; GGT, gamma-glutamyl-transferase; PegIFN/RBV, pegylated interferon alfa/ribavirin; qd, once-daily.

Table 4. Summary of viral resistance.

Genotype	Baseline EC_{50} Value	Major NS3 Resistant Variants	Phenotype EC_{50} Value (Range)
1a	10 ± 8 nM	R155K (n = 11)	1800–6500 nM
		D168V* (n = 1)	10,500 nM
1b	9 ± 4 nM	D168V (n = 11)	3600–15,200 nM
		D168E (n = 2)	970–1700 nM

*One genotype-1a in the 240 mg treatment-naïve dose group selected for a D168V variant; notably the R155 codon in this isolate resembled a genotype-1b subtype.

ants among others have also been observed with other protease inhibitors such as telaprevir, boceprevir, ITMN-191, and TMC-435 [15–17], and are thus likely to confer cross-resistance and to eventually cause virologic breakthrough in case of combination or sequential treatment with different compounds of this class. However, the *in vitro* sensitivity of both variants to interferon was uncompromised (Boehringer Ingelheim, unpublished data). This is consistent with the clinical findings of (i) substantial HCV RNA reductions found in patients with resistance mutations selected during monotherapy following add-on treatment with SOC; and (ii) by the rare frequency of HCV RNA breakthroughs under triple therapy with BI201335 and PegIFN/RBV even in patients failing previous PegIFN/RBV treatment. Thus, the combination of BI201335 with PegIFN/RBV successfully minimized the risk of virologic breakthrough during the treatment period. All three breakthroughs detected on triple treatment emerged at lower dose levels and in patients with previous null- or partial response to PegIFN/RBV.

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Table 5. Steady-state PK parameters of BI201335 on Day 28 in treatment-naïve and treatment-experienced patients.

Dose (mg qd)		t_{max} (h)	C_{max} (ng/ml)	C_{min} (ng/ml)	$AUC_{0-\tau}$ (ng*h/ml)	$t_{1/2}$ (h)
Treatment-naïve						
20 (n = 6)	Mean	5.33	167	75.6	2,770	28.6
	CV%	38.7	57.2	54.7	60.1	16.7
48 (n = 7)	Mean	5.57	717	229	9,570	38.7
	CV%	146	65.3	57.2	53.5	30.5
120 (n = 7)	Mean	2.83	4,340	1170	53,000	25.2
	CV%	26.6	40.0	42.9	38.9	25.3
240 (n = 6)	Mean	4.00	16,500	4,130	189,000	17.8
	CV%	61.2	41.7	22.0	22.6	12.0
Treatment-experienced						
48 (n = 6)	Mean	2.33	485	157	6,950	24.1
	CV%	44.3	17.9	38.7	19.8	20.6
120 (n = 7)	Mean	2.00	4,740	1,080	51,400	21.4
	CV%	28.9	40.3	67.4	56.1	19.9
240 (n = 5)	Mean	2.17	17,400	4,750	201,000	20.8
	CV%	45.4	53.5	67.8	64.5	29.0

Abbreviations: CV%, coefficient of variation; qd, once-daily.

BI201335 was well tolerated in both TN and TE patient groups. Typical AEs were those commonly associated with PegIFN/RBV [5]. The majority of AEs were mild-to-moderate in severity, not dose-dependent and judged by the investigators to be unrelated to BI201335. In this study, the incidence of mild rash or photosensitivity (4/34 patients) during combination treatment of BI201335 with PegIFN and RBV was similar to the 22–28% incidence of such events reported for SOC treatment alone [1,18]. However, interim analyses of ongoing phase II trials of BI201335 with PegIFN/RBV have indicated dose-dependent increases of mostly mild or moderate rash and phototoxicity at BI201335 doses of 240 mg once or twice daily, while the incidence of such events in the 120 mg dose group was similar to the SOC control [19,20]. The lower incidence in this phase I trial is likely due to the shorter treatment duration and smaller sample size. The only relevant safety laboratory effects were asymptomatic and isolated elevations in unconjugated bilirubin at higher doses of BI201335. However, this was reversible upon treatment completion and may be explained by the competitive inhibition of UGT1A1 by BI201335, which has been detected *in vitro* (Boehringer Ingelheim, unpublished data). This is supported by the finding of a strong correlation of frequency and extent of unconjugated hyperbilirubinemia with the presence of functional UGT1A1 polymorphisms, which has been observed in a healthy volunteer study (Boehringer Ingelheim, unpublished data). Importantly, no hemolysis or liver-related events of concern were reported (e.g. increase in direct bilirubin or liver enzyme markers). UGT1A1 inhibition has been identified previously as a mechanism for other antiviral drugs to cause indirect hyperbilirubinemia, such as atazanavir and indinavir [21,22]. Isolated unconjugated hyperbilirubinemia associated with these drugs is generally regarded as harmless.

Pharmacokinetic analysis shows that plasma BI201335 concentrations increase supra-proportionally with dose in TN and TE patients and are consistent with the virologic responses seen in these patient populations. Notably, BI201335 has a long elimination half-life with a steady-state achieved after 1 week of dosing. This allows for a once-daily dosing schedule without risk of suboptimal plasma concentrations between doses.

In conclusion, BI201335 is a potent and well tolerated protease inhibitor in both TN and TE HCV GT-1a and -1b patients. Its qd oral dosing schedule does not further complicate anti-HCV therapy. These data support the investigation of different treatment regimens testing doses from 120 to 480 mg daily in phase IIb trials.

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