CLINICAL—LIVER

Efficacy of the Protease Inhibitor BI 201335, Polymerase Inhibitor BI 207127, and Ribavirin in Patients With Chronic HCV Infection

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This article has an accompanying continuing education activity on page e14. Learning Objective: Upon completion of this CME activity, successful learners will be able to explain details on the design and results of the SOUND-C1 clinical trial (BI 201335, BI 207127 and RBV in treatment-naïve patients with chronic HCV GT-1 infection) and assess the potential for this regimen as a future treatment for this patient population.

Podcast interview: www.gastro.org/gastropodcast. Also available on iTunes; see editorial on page 1963.

BACKGROUND & AIMS: Therapeutic regimens are being developed for patients with hepatitis C virus (HCV) infection that do not include the combination of peginterferon alfa and ribavirin. We investigated the antiviral effect and safety of BI 201335 (an inhibitor of the NS3/4A protease) and BI 207127 (an inhibitor of the NS5B nonnucleoside polymerase) with ribavirin. **METHODS:** Thirty-two treatment-naïve patients with chronic HCV genotype 1 infection were randomly assigned to groups that were given 400 mg or 600 mg BI 207127 3 times daily plus 120 mg BI 201335 once daily and 1000 to 1200 mg/day ribavirin for 4 weeks. The primary efficacy end point was virologic response (HCV RNA level <25 IU/mL at week 4). Thirty-two patients received treatment; 31 completed all 4 weeks of assigned combination therapy. **RESULTS:** In the group given BI 207127 400 mg 3 times daily, the rates of virologic response were 47%, 67%, and 73% at days 15, 22, and 29; a higher rate of response was observed in patients with genotype-1b compared with genotype-1a infections. In the group given BI 207127 600 mg 3 times daily, the rates of virologic response were 82%, 100%, and 100%, respectively, and did not differ among genotypes. One patient in the group given 400 mg 3 times daily had virologic breakthrough ($\geq$1 $\log_{10}$ rebound in HCV RNA) at day 22. The most frequent adverse events were mild gastrointestinal disorders, rash, and photosensitivity. There were no severe or serious adverse events; no patients discontinued therapy prematurely. **CONCLUSIONS: The combination of the protease inhibitor BI 201335, the polymerase inhibitor BI 207127, and ribavirin has rapid and strong activity against HCV genotype-1 and did not cause serious or severe adverse events.**

Keywords: Drug; Clinical Trial; Direct-Acting Antivirals; Peginterferon-Free.

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Chronic hepatitis C virus (HCV) infection represents a major public health problem worldwide. With current standard therapy for patients infected with HCV genotype (GT)-1 consisting of peginterferon alfa (PegIFN) and ribavirin (RBV) for 48 weeks, ~60% of patients fail to achieve a sustained virologic response (SVR), defined as undetectable plasma HCV RNA 24 weeks after the completion of therapy. Even lower SVR rates are reported for several populations such as HCV/human immunodeficiency virus–coinfected patients or patients with decompensated liver cirrhosis.

Abbreviations used in this paper: AE, adverse event; DAA, direct-acting antiviral agent; GT, genotype; LLOD, lower limit of detection; LLOQ, lower limit of quantification; NI, nucleoside polymerase inhibitor; NNI, non-nucleoside inhibitor; PegIFN, peginterferon alfa; RBV, ribavirin; SVR, sustained virologic response.

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Due to the side effect profile of both drugs, many patients are ineligible for interferon-based treatment. Furthermore, of patients starting therapy with PegIFN and RBV, a substantial proportion is unable to tolerate these side effects and prematurely discontinue treatment.

With the development of HCV NS3/4A serine protease inhibitors, SVR rates for HCV GT-1–infected patients have substantially increased. However, rapid selection of resistance-associated variants is observed when these HCV protease inhibitors are administered as monotherapy. Thus, HCV protease inhibitors must be combined with PegIFN and RBV to reduce treatment failure due to the selection of resistant viral variants. With such triple combinations, SVR rates for treatment-naïve GT-1 patients have been increased to 65% to 84%.[1–4] However, limitations associated with PegIFN and RBV, including multiple organ system toxicities, intolerability, convenience, and contraindications, will remain until PegIFN/RBV-free treatments become available.

In vitro and in vivo studies suggest that combinations of direct-acting antiviral agents (DAAs) with different modes of action and nonoverlapping resistance profiles can reduce the selection of resistant variants and thus may improve response to antiviral therapy.[5,6] The first clinical phase 1b trial investigating a PegIFN/RBV-free combination therapy, comprising the NS3/4A protease inhibitor danoprevir (RG7227) and the NS5B nucleoside polymerase inhibitor (NI) mericitabine (RG7128) in HCV-infected GT-1 patients, showed significant antiviral potency and suppressed viral rebound for up to 14 days.[7]

BI 201335 is a second-generation HCV NS3/4A protease inhibitor with highly potent in vitro activity against GT-1a/1b subtypes (median effective concentration values of 6.5 and 3.1 mol/L) and improved pharmacokinetics suitable for once-daily dosing. BI 201335 is currently in phase 3 of development.[4,8,9] BI 207127 is an orally bioavailable, reversible, thumb pocket 1 non-nucleoside inhibitor (NNI) of the HCV NS5B polymerase with potent and specific antiviral activity in vitro (HCV replicon median effective concentration values of 23 and 11 nmol/L against GT-1a and GT-1b, respectively). BI 207127 is currently in phase 2 of development.[10] Drug resistance studies in cell culture show that BI 201335 and BI 207127 have different resistance profiles, and previous observations using NS3/4A protease inhibitors and NS5B thumb pocket 1 NNI compounds have shown that 2-drug combinations profoundly reduce the selection of drug-resistant variants.[11]

Here we report the results of a clinical phase 1b trial (SOUND-C1: Safety and antiviral effect of Oral combinations withoUt iNterferon in patients Diagnosed with chronic hepatitis C) investigating the safety, antiviral effect, and pharmacokinetics of BI 207127 in combination with BI 201335 and RBV for 4 weeks in treatment-naïve patients with chronic HCV GT-1 infection.

Patients and Methods

Patients

Patients were enrolled in May 2010 in Australia, France, Germany, New Zealand, and Switzerland. Eligible patients were 18 to 75 years of age with chronic HCV GT-1 infection and were therapy naïve to interferon, PegIFN, RBV, or any DAA for acute or chronic hepatitis C infection. Patients had plasma HCV RNA levels ≥100,000 IU/mL at screening and a liver biopsy within 2 years, or a FibroScan (ECHOSENS, Paris, France) within 6 months, before screening that excluded cirrhosis. Patients with HCV of mixed GT, hepatitis B virus, human immunodeficiency virus, previous or ongoing rash or photosensitivity, decompensated liver disease, or hyperbilirubinemia (>1.5× the upper limit of normal) were excluded (patients with Gilbert's polymorphism were accepted). All patients provided written informed consent before participation in the trial.

Study Design

This was a phase 1b, multicenter, open-label, randomized trial. Thirty-four treatment-naïve patients with chronic HCV GT-1 infection were randomized 1:1 to either 400 mg or 600 mg BI 207127 3 times daily plus 120 mg BI 201335 once daily and 1000 mg (body wt <75 kg) or 1200 mg (body wt ≥75 kg) of RBV per day for 4 weeks (Figure 1). BI 201335, BI 207127, and RBV were dosed with food. Use of erythropoietin and dose reductions of RBV was permitted at the discretion of the investigator. Thirty-two patients started treatment. Randomization was stratified by baseline plasma HCV RNA level (<800,000 and

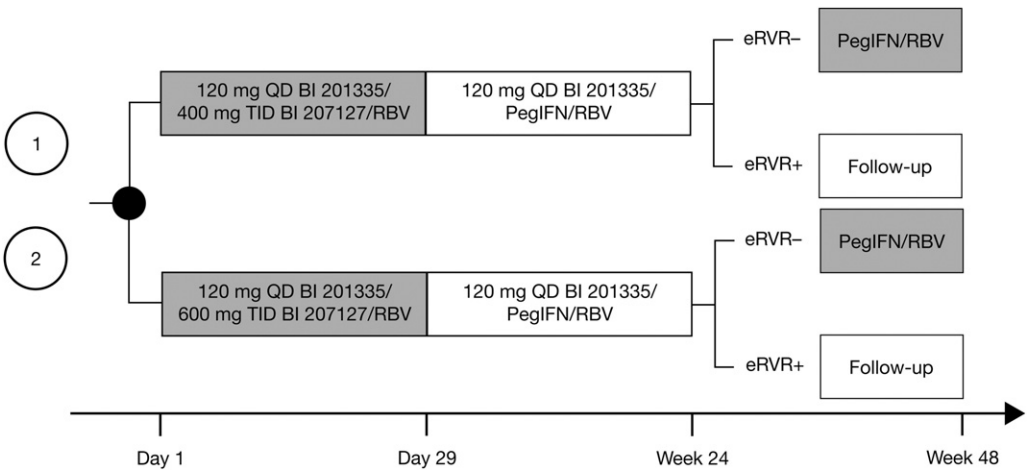

Figure 1. Trial schema. eRVR, extended rapid virologic response (HCV RNA level ≤25 IU/mL at week 4, and HCV RNA undetectable from weeks 5 to 18); QD, once daily; TID, 3 times daily.

≥800,000 IU/mL) and by HCV subtype (1a and 1b). A loading dose of 240 mg BI 201335 was given on the first day of BI 201335 administration, followed by 120 mg once daily starting on day 2. An induction dose of 1200 mg BI 207127 was given on the first day of BI 207127 administration, aiming to achieve early and profound viral load reduction,[10] followed by the assigned dose. Concomitant treatment with the following substrates of P-gp, UGT1A1, CYP3A4, or 2C9, with a narrow therapeutic range, was not permitted: alfentanil, astemizole, cisapride, mosapride, di-/ergotamine, cyclosporine, everolimus, sirolimus, tacrolimus, fentanyl, irinotecan, pimozide, quinidine, and terfenadine.

At day 29, all patients were switched from their assigned treatment to 120 mg BI 201335 once daily, PegIFN alfa-2a, and RBV until week 24 or 48, depending on the achievement of extended rapid virologic response (HCV RNA level ≤25 IU/mL at week 4 and HCV RNA undetectable from weeks 5 to 18). Patients with virologic breakthrough before day 29, defined as plasma HCV RNA rebound ≥1 $\log_{10}$ from a quantifiable nadir during BI 207127/BI 201335/RBV treatment, and confirmed by a second, consecutive plasma HCV RNA measurement, were immediately switched to treatment with PegIFN alfa-2a and RBV alone for 48 weeks.

Our study was conducted in accordance with the principles of Good Clinical Practice and was approved by the appropriate institutional review boards and regulatory agencies (NCT01132313).

Efficacy Assessments

Plasma samples for HCV RNA measurements were obtained at the time of screening and at days 1, 2, 4, 8, 10, 15, 22, and 29. Samples were processed by a central laboratory using the Roche COBAS TaqMan HCV/HPS assay (version 2.0; Roche, Basel, Switzerland) with a lower limit of quantification (LLOQ) of 25 IU/mL and a lower limit of detection (LLOD) of 17 IU/mL.

HCV GT for screening and randomization was determined using the Trugene HCV assay (Bayer, Leverkusen, Germany); due to the technical limitations of this genotyping assay, definitive HCV GTs used for all analyses were based on complete NS5B sequencing and phylogenetic analyses for all randomized patients. Samples for sequencing the HCV NS3/4A protease and NS5B polymerase were collected at screening and all patient visits. Viral genotyping was performed for all patients at baseline and for all patients in which plasma HCV RNA level plateaued above ~1000 IU/mL, or plasma HCV RNA values indicated breakthrough, during the trial period.

Viral RNA was isolated from plasma using the QiaAmp Viral RNA Extraction Kit (Qiagen, Hilden, Germany). Complementary DNA was synthesized using the SuperScript III One-Step RT-PCR System with Platinum Taq DNA Polymerase using GT-specific primers (Qiagen). The LLOD of the reverse-transcription polymerase chain reaction amplification method restricted the analysis to patient samples with HCV RNA levels ≥1000 IU/mL. The NS3/4A protease and NS5B polymerase nucleotide sequences were obtained by direct DNA sequencing of the amplified product using the ABI 3730 Genetic Analyzer Detection System (Applied Biosystems, San Francisco, CA). The base calling with ABI's software allowed for the detection of variants present at ≥30% of the total population.

Safety Assessments

All adverse events (AEs) occurring during the course of the trial were documented and reported by the investigator to the sponsor. As defined in a rash management plan, the intensity of rash was graded as mild (localized), moderate (diffuse, 30% to <70% body surface area), or severe (diffuse generalized, mucous membrane involvement, organ dysfunction, signs of anaphylaxis, or life threatening). The intensity of all other AEs was judged based on patient tolerability of the event as mild (easy to tolerate), moderate (interference with usual activity), or severe (incapacitating or causing inability to work or to perform usual activities). Electrocardiograms, vital signs, and routine laboratory parameters were also evaluated. The study included regular

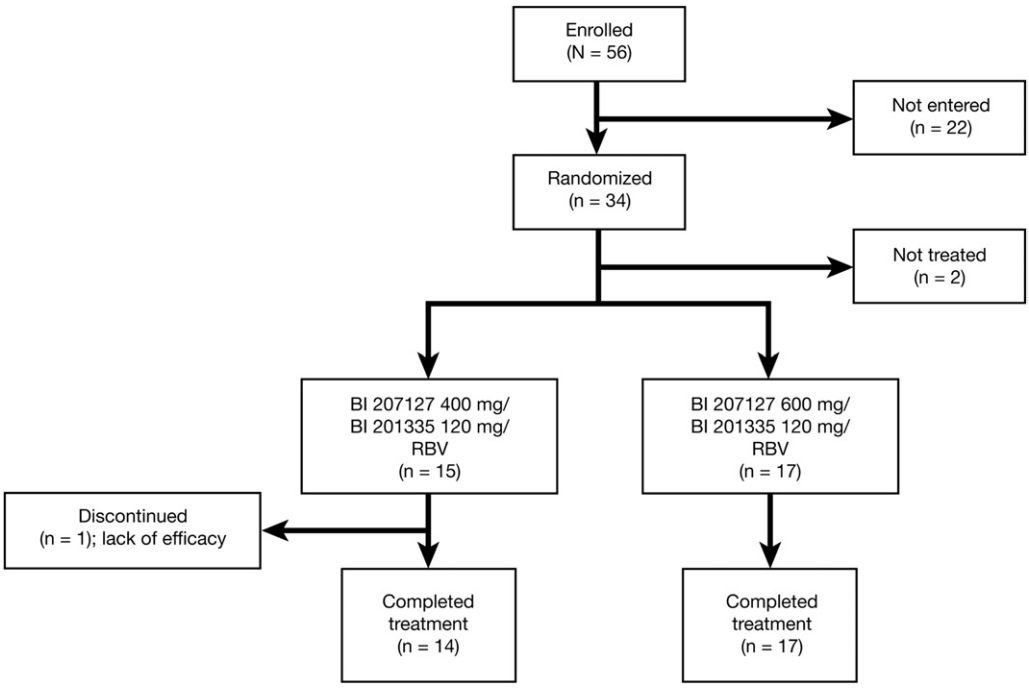

Figure 2. Patient disposition.

Table 1. Summary of Baseline Characteristics

	BI 207127 400 mg 3 times daily + BI 201335 120 mg once daily + RBV (n = 15)	BI 207127 600 mg 3 times daily + BI 201335 120 mg once daily + RBV (n = 17)
Age (*y*)		
Mean	51	51
SD	10.0	11.5
Body mass index (kg/m^2)		
Mean	23	24
SD	2.9	3.8
Sex, n (%)		
Male	8 (53.3)	10 (58.8)
Female	7 (46.7)	7 (41.2)
Ethnicity, n (%)		
Asian	0 (0.0)	1 (5.9)
White	15 (100.0)	16 (94.1)
HCV RNA ($log_{10}IU/mL$)		
Mean	6.45	6.51
SD	0.51	0.66
Genotype, n (%)		
1a	10 (66.7)	8 (47.1)
1b	5 (33.3)	8 (47.1)
Other	0	1[a] (5.9)

[a]Retrospective GT analysis based on NS5B sequence identified 1 GT-1 sample as GT-6e.

reviews of data by a data monitoring committee external to the sponsor.

Statistical Assessments

The primary efficacy end point of this study was rapid virologic response, defined as HCV RNA level below the LLOQ (<25 IU/mL) at week 4. Safety end points included vital signs, physical examination, discontinuation due to AEs, and laboratory abnormalities. Descriptive statistics for efficacy, safety, and pharmacokinetic end points were calculated. One interim analysis was planned after all patients had finished day 29.

Results

Patient Disposition and Baseline Characteristics

Fifty-six patients were enrolled into the trial, of which 34 were randomized and 24 did not start treatment due to withdrawn consent (n = 3), not meeting inclusion/exclusion criteria (n = 9), or missing the predefined randomization cut due to very fast enrollment (n = 12) (Figure 2). The latter 12 patients were offered randomization into the second part of the trial, which is ongoing. Therefore, 32 patients received at least one dose of assigned treatment (Figure 2). Of these 32 patients, 31 completed all 4 weeks of assigned treatment; one patient with confirmed virologic breakthrough was switched to PegIFN and RBV at day 29 as defined in the protocol.

Patients were evenly distributed over both dose groups with regard to baseline HCV RNA level, ethnicity, age, sex, body mass index, and subtype (Table 1). Mean age was 51 ± 11 years, mean body mass index was 23.8 ± 3.5 kg/m^2, and mean HCV RNA level was 6.48 log_{10} IU/mL. A slightly higher number of patients had GT-1a infection, and all but one patient were white.

Efficacy

During the first 2 days of treatment, all patients showed a rapid and steep decline in HCV RNA levels. This was followed by a slower second phase decline until day 29 in all patients receiving BI 207127 600 mg 3 times daily and in all but 2 patients receiving 400 mg 3 times daily (Figure 3). In the group receiving 600 mg 3 times daily, the declines in HCV RNA levels were slightly steeper and the HCV RNA curves clustered more tightly, and a higher proportion of patients achieved HCV RNA below LLOQ or LLOD by the end of oral treatment compared with the group receiving 400 mg 3 times daily.

Virologic response rates (HCV RNA level <25 IU/mL) in the group receiving BI 207127 400 mg 3 times daily were 27%, 47%, 67%, and 73% at days 8, 15, 22, and 29, respectively; higher response rates were observed in GT-1b–infected patients compared with GT-1a–infected patients (Table 2). In contrast, corresponding response rates in the group receiving BI 207127 600 mg 3 times were 18%, 82%, 100%, and 100%, without subtype differences (Table 2). Most patients in the group receiving 600 mg 3 times daily even had undetectable HCV RNA at days 22 and 29 (53% and 71%, respectively), whereas these rates did not exceed 20% in the group receiving 400 mg 3 times daily.

One virologic breakthrough was observed during treatment at day 22 and confirmed at day 29, when the patient was switched per protocol to PegIFN plus RBV; the patient showed a good virologic response 4 weeks later (HCV RNA decrease 2 log_{10}). One other patient showed an increase in HCV RNA level from a nadir of 0.7 log_{10} IU/mL. HCV RNA plateaued at this level until the patient was switched to BI 201335/PegIFN/RBV triple therapy at day 29; after 10 days of triple therapy, HCV RNA level decreased to <100 IU/mL. Both patients were GT-1a, had never achieved undetectable HCV RNA, and were treated with the lower dose of BI 207127 at 400 mg 3 times daily.

Safety

Overall, the safety and tolerability profile of oral treatment was favorable. Most patients had mild gastrointestinal disorders (diarrhea, nausea, vomiting), and at the higher dose, 42% of patients had a mild rash or photosensitivity reaction (Table 3). Four patients in the group receiving 600 mg 3 times daily experienced transient and mild paraesthesias of very different localizations and underlying conditions (2 were associated in time and localization with photosensitivity or rash). Due to overlap in the safety profiles, these AEs cannot be assigned to one particular compound. However, there were no severe AEs, serious AEs, or AE-related premature treatment discontinuations within the 4-week study period.

Laboratory studies indicated a continuous decline in alanine aminotransferase level in all patients (Table 4),

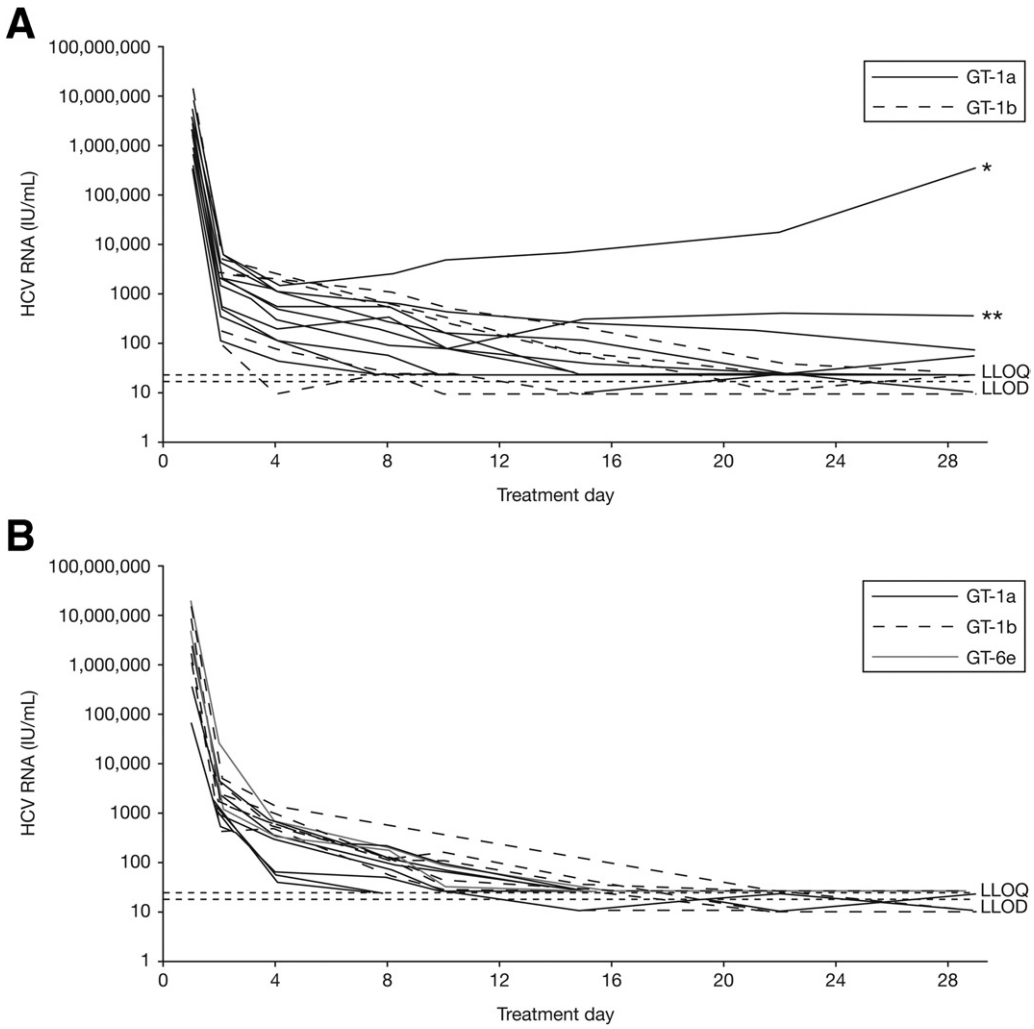

Figure 3. Absolute HCV RNA level from baseline to day 29 for individual patients in (*A*) the BI 207127 400 mg dose group and (*B*) the BI 207127 600 mg dose group. *Patient with virologic breakthrough observed during treatment at day 22. **Patient with an increase in HCV RNA from nadir of 0.7 $\log_{10}$ IU/mL. LLOD, 17 IU/mL; LLOQ, 25 IU/mL.

reflecting the rapid and effective suppression of HCV replication.[12] There was a slight dose-dependent decrease in hemoglobin levels that was in line with the effect of RBV on red blood cells.[13] The dose of RBV had to be reduced for hematologic reasons in one patient in the 400 mg group after 24 days, and no patient required the use of erythropoietin. After the protocol-defined switch to BI 201335, PegIFN, and RBV at day 29, the median hemoglobin level decreased further (median change from baseline at day 36, −3.0 g/dL), most likely in part due to the added effect of PegIFN. A mild increase in platelet count was found in both dose groups, which was rapidly reversible after stopping treatment or switching patients to PegIFN/RBV therapy (median change from baseline at day

Table 2. Frequency of Patients Attaining Virologic Response (HCV RNA Level Less Than LLOQ at Day 29) by Duration of Treatment and Genotype

	HCV RNA level less than LLOQ, n/N (%)					
	BI 207127 400 mg 3 times daily + BI 201335 120 mg once daily + RBV (n = 15)			BI 207127 600 mg 3 times daily + BI 201335 120 mg once daily + RBV (n = 17)		
Day	All (n = 15)	GT-1a (n = 10)	GT-1b (n = 5)	All (n = 17)	GT-1a (n = 8[a])	GT-1b (n = 8[a])
8	4 (27)	2 (20)	2 (40)	3 (18)	2 (25)	1 (13)
15	7 (47)	5 (50)	2 (40)	14 (82)	8 (100)	5 (63)
22	10 (67)	6 (60)	4 (80)	17 (100)	8 (100)	8 (100)
29	11 (73)	6 (60)	5 (100)	17 (100)	8 (100)	8 (100)

NOTE. LLOQ was 25 IU/mL.

[a]Genotype corrected by HCV NS5B sequencing and phylogenetic analysis; numbers do not add up to 100% of patients treated in the BI 207127 600 mg arm due to the exclusion of one patient with GT-6e.

Table 3. Most Frequent (>20%) Adverse Events

	BI 207127 400 mg 3 times daily + BI 201335 120 mg once daily + RBV (n = 15)			BI 207127 600 mg 3 times daily + BI 201335 120 mg once daily + RBV (n = 17)		
	All AEs	Mild	Moderate	All AEs	Mild	Moderate
Headache	5 (33)	3 (20)	2 (13)	2 (12)	2 (12)	0 (0)
Paraesthesias	0 (0)	0 (0)	0 (0)	4 (24)	4 (24)	0 (0)
Nausea	4 (27)	3 (20)	1 (7)	11 (65)	9 (53)	2 (12)
Vomiting	4 (27)	4 (27)	0 (0)	8 (47)	6 (35)	2 (12)
Diarrhea	4 (27)	3 (20)	1 (7)	3 (18)	3 (18)	0 (0)
Jaundice[a]	4 (27)	3 (20)	1 (7)	3 (18)	3 (18)	0 (0)
Pruritus	3 (20)	3 (20)	0 (0)	6 (35)	5 (29)	1 (6)
Photosensitivity reaction	3 (20)	3 (20)	0 (0)	3 (18)	3 (18)	0 (0)
Rash	1 (7)	1 (7)	0 (0)	4 (24)	4 (24)	0 (0)
Asthenia	5 (33)	4 (27)	1 (7)	4 (24)	2 (12)	2 (12)
Fatigue	3 (20)	2 (13)	1 (7)	4 (24)	4 (24)	0 (0)
Anemia	1 (6.7)	1 (6.7)	0 (0)	1 (5.9)	0 (0)	1 (5.9)

NOTE. All values are expressed as n (%). There were no severe AEs, serious AEs, or AE-related discontinuations.
[a]Clinical symptom as reported by the investigator, including scleral icterus.

36, -7.7×10^9/L). Finally, a dose-dependent increase in unconjugated bilirubin (median +5.3 and +7.8 μmol/L; direct/total bilirubin ratio <0.5 in all cases) was observed, which was not associated with signs of liver dysfunction. A total of 3 patients each had total bilirubin elevation >3 to 5 times the upper limit of normal or >5 to 10 times the upper limit of normal (peaks of 129, 131, and 199 μmol/L), with levels peaking between days 10 and 14 of treatment. However, none of these patients had simultaneously elevated direct bilirubin (10, 5, and 9 μmol/L), alanine aminotransferase (19, 41, and 45 U/L), or hemoglobin values <10 g/dL, and only 3 of these patients had jaundice (all with peak bilirubin level >5 times the upper limit of normal). Other safety laboratory parameters showed no relevant changes from baseline (Table 4).

Resistance

No known resistance mutations to either the NS3/4A protease inhibitor BI 201335 or the NS5B NNI polymerase inhibitor BI 207127 were detected at baseline by population sequencing; a detailed phenotypic and genotypic analysis will be reported elsewhere. Nucleic acid sequencing of virus isolated at day 30 (and compared with the sequence obtained at baseline) from the one patient with confirmed breakthrough identified R155K in NS3 and P495L in NS5B as major changes in amino acid sequence that represented the selection of double mutant virus conferring resistance to BI 201335 and BI 207127. Comprehensive NS3/4A and NS5B genotyping could not be performed on samples from the patient with the increase of 0.7 $\log_{10}$ IU/mL, because the HCV RNA level was less than 1000 IU/mL and too low to generate full-length amplicons. However, we obtained partial sequence of the NS3 protease domain from virus isolated on day 17 and, relative to the baseline sequence, identified a R155K amino acid change in NS3; an amplicon spanning the 3′ end of the NS5B region from the day 17 virus did not detect any canonical changes in P495 or P496 residues that are known to confer resistance to the thumb pocket 1 class of NNI.

Discussion

Many patients with chronic hepatitis C who require antiviral therapy are unable to tolerate PegIFN and account for 13% of treatment discontinuations.[14,15] Moreover, an estimated 30% to 60% of patients with HCV cannot be treated with current treatment because of med-

Table 4. Laboratory Changes During Treatment at Week 4 Compared With Baseline

Test parameter (normal range)	BI 207127 400 mg 3 times daily + BI 201335 120 mg once daily + RBV (n = 15)	BI 207127 600 mg 3 times daily + BI 201335 120 mg once daily + RBV (n = 17)
Alanine aminotransferase (0.0–35.0 U/L)	−35 (−236, −6)	−27 (−147, −5)
Bilirubin, total (5.1–17 μmol/L)	+9.9 (+2.6, +65.5)	+17.9 (+2.0, +99.2)
Bilirubin, indirect (3.4–12 μmol/L)	+4.5 (+1.2, +38.5)	+7.8 (+1.2, +59.8)
Hemoglobin[a] (12.0–17.2 g/dL)	−1.4 (−3.5, −0.7)	−2.6 (−4.7, −0.5)
Platelets (150–350 × 10^9/L)	+67 (+21, +103)	+89 (+21, +144)
White blood cells (4.5–11.0 × 10^9/L)	+0.3 (−1.1, +2.1)	+0.6 (−1.4, +2.4)

NOTE. Values are expressed as median (minimum, maximum).
[a]The percentages of patients with hemoglobin levels <10 g/dL were 6.7% (n = 1) and 5.9% (n = 1), respectively, in the groups treated with 400 mg 3 times daily and 600 mg 3 times daily, while no patient had a hemoglobin level <8.5 g/dL.

ical contraindications to PegIFN/RBV,[16,17] highlighting the high clinical unmet need for interferon-free regimens. Several PegIFN-free combinations of 2 DAAs with different modes of action, with or without RBV, are currently under clinical investigation.[7,18–21] NS3/4A protease inhibitors, NS5A inhibitors, and NS5B NNIs have a lower genetic barrier to resistance compared with NS5B NIs.[22] So far, most dual combinations of DAAs with a lower barrier to resistance have failed to maintain suppression of viral replication.[18,20] Variants with resistance to 2 DAAs seem to preexist and are selected during dual DAA combination therapy.[23] Potential strategies to overcome this problem are (1) DAA combinations including polymerase inhibitors with a high barrier to resistance, (2) triple DAA therapy, and (3) combinations of 2 DAAs with a lower genetic barrier to resistance plus RBV.

Virologic breakthrough, with the selection of resistant variants, has not yet been observed in short-term clinical studies of the NS3/4A protease inhibitor danoprevir plus the NI mericitabine,[7] suggesting that inclusion of an NI in DAA combination therapy may be an attractive strategy. However, additional efficacy (SVR) and safety data from longer-term treatments are still required. The approach of combining 3 non–cross-resistant DAAs with a lower genetic barrier to resistance (ie, an NNI plus a NS3/4A protease inhibitor and an NS5A inhibitor) is well supported by mathematical analyses. Rong et al showed that resistant variants against the 3 drug classes are unlikely to preexist before treatment initiation, and emergence is unlikely to occur during therapy.[24] However, drug-drug interaction and overlapping safety profiles remain an issue.

A third highly attractive strategy is to combine 2 DAAs with a lower genetic barrier to resistance plus RBV. A trial evaluating GS-9256 plus tegobuvir with or without RBV showed the major role of RBV in HCV RNA decline and the reduction of viral breakthroughs for DAA combinations with a low barrier to resistance.[18] In the present trial, all patients received the NS3/4A protease inhibitor BI 201335 and the NS5B NNI BI 207127 in combination with RBV. This PegIFN-free combination showed rapid, strong antiviral activity in patients chronically infected with HCV GT-1. Only one patient, treated with the lower dose of BI 207127, experienced virologic breakthrough and selected for dually resistant virus. This was a GT-1a patient in the lower dose group, reflecting the slightly reduced antiviral activity of BI 207127 against this subtype, which was also seen in monotherapy.[10] In contrast, no virologic breakthrough occurred in the group receiving BI 207127 600 mg 3 times daily, showing that the rapid and uniform selection of resistance mutations associated with NS3/4A protease inhibitor monotherapy is effectively reduced or delayed in this PegIFN-free regimen. The reason for the high antiviral activity associated with a low breakthrough rate of this combination as compared with other protease inhibitor/NNI combinations with only 2 of 32 patients developing a single or double mutant to BI 207127 and/or BI 201335[18,19] may be due to the RBV effect, which is probably caused by a weak direct antiviral activity as well as by induction of interferon-sensitive genes.[12] Importantly, BI 207127 belongs to the thumb pocket 1 class of NNIs that displace the NS5B amino-terminal λ1 finger loop from the upper thumb domain and interfere with a conformational change required to initiate RNA synthesis. In contrast to other sites that are exposed on the surface of the NS5B polymerase, the thumb pocket 1 binding site comprises an interface between functional domains that restricts sequence polymorphism and may provide for a higher resistance barrier relative to other NNIs.[11] Results from the ongoing phase 2b study comparing dose regimens of BI 201335 and BI 207127 with or without RBV will provide further insights.

Treatment with BI 201335 and BI 207127 plus RBV showed a favorable overall safety and tolerability profile. As expected from phase 1 and 2 results of both compounds,[4,9,10] a substantial proportion of patients had mild to moderate rash, photosensitivity, or gastrointestinal symptoms, which did not impact treatment continuation. Jaundice was rather common but usually mild and always due to isolated, predominantly unconjugated hyperbilirubinemia, without signs of liver toxicity. The mechanisms involved, as proposed from extensive in vitro studies, include BI 201335-mediated inhibition of bilirubin transporters (eg, OATP1B1, MRP-2) and conjugating enzymes (eg, UGT1A1)[25] in the presence of RBV-induced hemolysis. The simultaneous slight elevation of conjugated bilirubin level found in some patients can be explained by the common diazo assay-related overestimation of direct bilirubin[26] and the inhibitory effect of BI 201335 on the bilirubin efflux transporter MRP-2.[25] Similar mechanisms of interaction with bilirubin metabolism have been described for other HCV[27] and human immunodeficiency virus protease inhibitors.[28,29] In confirmation of these reports, jaundice associated with BI 201335 was not associated with any symptoms other than jaundice in the phase 2 studies of BI 201335 in combination with PegIFN/RBV.[25] The mild elevation of platelets observed was in a similar range as that found with RBV monotherapy.[30] However, there were no thrombotic events reported in our trial and the effect was rapidly reversible with an incremental decrease in platelet count after switching patients to PegIFN/RBV. Similarly, the decrease in hemoglobin levels and the rate of anemia found after 4 weeks of HCV treatment with BI 201335, BI 207127, and RBV is likely caused by RBV-induced hemolysis. The largest RBV monotherapy study described a mean decrease in hemoglobin level after 4 weeks of RBV by >2 g/dL, with 20% of subjects experiencing a decline by ≥4 g/dL.[13] However, the ongoing phase 2b study is evaluating several longer-term dosing regimens of this oral combination, including an RBV-free dose regimen, and thus will show whether the DAA combination has an additional effect on hemoglobin or platelet counts. In summary, the general safety profile of this DAA combination is comparable with other compounds of both drug classes. Lack of serious AEs and

treatment discontinuations shows that this treatment is generally safe and side effects are manageable.

Recently, proof of concept for achievement of SVR with a PegIFN-free treatment regimen in patients with HCV was reported. The combination of an NS5A inhibitor and an NS3/4A protease inhibitor in GT-1 patients with null response to previous PegIFN/RBV treatment achieved SVR in 4 of 11 patients who were treated for 24 weeks, with only one relapse in this cohort.[20] This indicates that HCV can be eradicated in chronically infected patients with a PegIFN-free DAA combination regimen and supports investigations with various DAA combinations that may be able to improve SVR rates without the need for PegIFN.

The BI 201335 and BI 207127 plus RBV all oral regimen showed potent antiviral activity in HCV GT-1 patients that was generally safe and tolerable without the selection of resistance in the higher dose group. The crucial next step in clinical development is the evaluation of safety and SVR with longer-term treatment with this PegIFN-free combination. Moreover, eliminating not only PegIFN but also RBV from future HCV treatment would undoubtedly improve tolerability and would potentially allow for the treatment of patients with RBV contraindications. A prospective phase 2b study evaluating sustainability of virologic responses to different longer-term treatments is ongoing.

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Conflicts of interest

The authors disclose the following: S.Z. is a consultant for Abbott Laboratories, Achillion Pharmaceuticals, Anadys Pharmaceuticals, Boehringer Ingelheim, Bristol-Myers Squibb, Gilead Sciences, iTherX, Janssen/Tibotec, Merck, Novartis, Pfizer, Pharmasset, Roche/Genentech, Santaris Pharma, and Vertex Pharmaceuticals. T.A. is a consultant for Boehringer Ingelheim, Bristol-Myers Squibb, Gilead Sciences, Janssen, Merck, Novartis, and Roche. J.-P.Z. is a consultant for Boehringer Ingelheim, Bristol-Myers Squibb, Gilead Sciences, Roche, Janssen, and Merck/Schering-Plough. D.L. is a consultant for Boerhinger Ingelheim, Bristol-Myers Squibb, Roche, Janssen, Merck, and Cytheris. B.M. is a consultant for MSD, Roche, Janssen, and Gilead Sciences. M.S. is a consultant for Bristol-Myers Squibb and Roche and a speaker for Bristol-Myers Squibb, Falk, Gilead Sciences, Merck, and Roche. A.L. has participated in trials for Boehringer Ingelheim, Roche, MSD, Janssen, and Falk and has received lecture fees from MSD, Roche, and Falk. S.P. is a consultant for Bristol-Myers Squibb, Boehringer Ingelheim, Tibotec/Janssen-Cilag, Gilead Sciences, Roche, Merck/Schering-Plough, and Abbott Laboratories; is a speaker for GlaxoSmithKline, Bristol-Myers Squibb, Boehringer Ingelheim, Tibotec/Janssen-Cilag, Gilead Sciences, Roche, and Schering-Plough; and has received grants from Bristol-Myers Squibb, Gilead Sciences, Roche, and Merck/Schering-Plough. J.-P.B. is a consultant for Boehringer Ingelheim, Bristol-Myers Squibb, Gilead Sciences, GlaxoSmithKline, Janssen, Merck, Novartis, and Roche. F.Z. is a consultant for Janssen, Schering-Plough, and Vertex Pharmaceuticals. M.H. is a consultant for Novartis, MSD, Roche, Jansen, and Gilead Sciences. J.O.S., G.K., G.N., C.H., and W.O.B. are employees of Boehringer Ingelheim. The remaining authors disclose no conflicts.