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CLINICAL LIVER

Efficacy of an Interferon- and Ribavirin-Free Regimen of Daclatasvir, Asunaprevir, and BMS-791325 in Treatment-Naive Patients With HCV Genotype 1 Infection

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BACKGROUND & AIMS: The combination of peginterferon and ribavirin with telaprevir or boceprevir is the standard treatment of hepatitis C virus (HCV) genotype 1 infection. However, these drugs are not well tolerated because of their side effects and suboptimal virologic responses. In a phase 2a, open-label study, we examined the safety and efficacy of an interferon-free, ribavirin-free regimen of direct-acting antivirals, comprising daclatasvir (an NS5A replication complex inhibitor), asunaprevir (an NS3 protease inhibitor), and BMS-791325 (a non-nucleoside NS5B inhibitor), in patients with chronic HCV infection. **METHODS:** We analyzed data from 66 treatment-naive patients with HCV genotype 1 infection without cirrhosis who were assigned randomly to groups given daclatasvir (60 mg, once daily), asunaprevir (200 mg, twice daily), and BMS-791325 (75 or 150 mg, twice daily) for 12 or 24 weeks. The primary end point was an HCV-RNA level less than 25 IU/mL at 12 weeks after treatment (sustained virologic response at 12 weeks [SVR_{12}]). **RESULTS:** In 64 patients, HCV-RNA levels were less than 25 IU/mL by week 4 of treatment (including 48 of 49 patients with HCV genotype 1a infection and 45 of 46 patients with the non-CC interleukin *28B* genotype). Sixty-one patients (92%) achieved SVR_{12}, based on a modified intention-to-treat analysis. Virologic responses were similar between 12 and 24 weeks of treatment. During the study, 2 patients experienced viral breakthrough and 1 patient relapsed. There were no grade 3–4 increases in levels of alanine or aspartate aminotransferases or bilirubin; there were no deaths or discontinuations resulting from serious adverse events or adverse events related to the treatment regimen. The most common adverse events were headache, asthenia, and gastrointestinal symptoms. **CONCLUSIONS:** In a phase 2a study, the all-oral, interferon-free, and ribavirin-free regimen of daclatasvir, asunaprevir, and BMS-791325 was well tolerated and achieved high rates of SVR_{12} in patients with HCV genotype 1 infection. Further studies of this regimen are warranted. ClinicalTrials.gov, number NCT01455090.

Keywords: Liver Disease; Therapy; DAA; Drug Combination.

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The current standard of care for the treatment of patients chronically infected with hepatitis C virus (HCV) genotype (GT) 1 is a 3-drug regimen, with peginterferon alfa and ribavirin plus telaprevir or boceprevir. Sustained virologic response (SVR) rates with 3-drug therapy are approximately 70% in treatment-naive patients, a significant improvement over the SVR of approximately 40% for peginterferon/ribavirin alone.[1–4] Despite improvement in SVR, these regimens are poorly tolerated. The most common side effects of peginterferon alfa/ribavirin are flu-like symptoms, depression, and hematologic toxicity.[5] Addition of boceprevir or telaprevir to peginterferon alfa/ribavirin increases the severity of anemia and adds

Abbreviations used in this paper: **EC_{50}, median effective concentration; GT, genotype; HCV, hepatitis C virus; IL, interleukin; SVR, sustained virologic response; SVR_{24}, sustained virologic response at 12 weeks.**

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additional side effects, such as rash, which can be life-threatening.[3,4] In addition, these regimens require 24 to 48 weeks of weekly injections of peginterferon, up to 3 pills twice daily of ribavirin, and administration of 3 or 4 pills of telaprevir or boceprevir with a meal 3 times a day. An interferon-free, ribavirin-free regimen with improved tolerability and less-frequent dosing for improved adherence, while achieving high rates of SVR, is desirable.

Several antivirals with different mechanisms of action that directly inhibit HCV replication are currently in clinical development.[6] Lok et al[7] showed that SVR was possible with an interferon-free, ribavirin-free regimen combining multiple direct-acting antivirals, each having a different mechanism of action. In this study, daclatasvir, an NS5A replication complex inhibitor,[8] was combined with asunaprevir, an NS3 protease inhibitor,[9] to treat patients with HCV GT 1 who were null responders to prior treatment with peginterferon/ribavirin.[10] This dual combination achieved SVR at 24 weeks after end of treatment (SVR_{24}) in 36% of the patients (2 of 9 patients with GT 1a and 2 of 2 patients with GT 1b).[7] In subsequent studies this dual regimen achieved SVR_{24} of 83%–91% in HCV GT 1b null responders,[11–13] but a more potent regimen is required for HCV GT 1a. Addition of ribavirin to this dual combination did not improve response rates in GT 1a null responder patients,[11] thus it was hypothesized that addition of a third direct-acting antiviral agent may enhance antiviral potency.

The addition of the selective non-nucleoside polymerase inhibitor, BMS-791325, to daclatasvir and asunaprevir achieves inhibition of 3 distinct viral targets that are responsible for HCV replication. Potentially, this strategy would increase the SVR rate and protect against the emergence of viral resistance. Avoiding interferon and ribavirin also would improve tolerability, perhaps increasing compliance, resulting in more effective therapy. The study presented here describes outcomes from 12 or 24 weeks of treatment with an interferon-free, ribavirin-free combination of daclatasvir, asunaprevir, and BMS-791325 in treatment-naive patients with HCV GT 1 infection.

Methods

Study Design

This open-label, randomized, phase 2a study recruited patients from 13 centers in the United States and France. Patients were enrolled and completed treatment from November 17, 2011, to March 5, 2013. The study was approved by appropriate institutional review boards and/or independent ethics committees, and was performed in accordance with the Declaration of Helsinki and Good Clinical Practice as defined by the International Conference on Harmonization and ethical principles of local regulatory requirements. All patients provided written informed consent. All authors had access to the study data and reviewed and approved the final manuscript.

Inclusion criteria were age 18–70 years, chronic HCV GT 1 infection with RNA level of 10^5 IU/mL or greater, no previous HCV therapy (treatment-naive), and no evidence of cirrhosis (as documented by markers of cirrhosis, FibroTest [BioPredictive, Paris, France] score $\leq$0.72 and aspartate aminotransferase:platelet ratio $\leq$2, or liver biopsy). Patients with a FibroTest or aspartate aminotransferase:platelet ratio score exceeding the threshold for study inclusion were required to have a liver biopsy documenting the absence of cirrhosis. METAVIR category for each patient was derived from the FibroTest result based on the conversion on the manufacturer's website.

Exclusion criteria included an alanine aminotransferase level that was 5$\times$ or more the upper limit of normal, total bilirubin level of 2 mg/dL or greater, direct bilirubin level greater than the upper limit of normal, international normalized ratio of 1.7 or greater, albumin level of 3.2 g/dL or less, hemoglobin level less than 11 g/dL for women and less than 12 g/dL for men, absolute neutrophil count less than 1.5×10^9 cells/L (or $<1.2 \times 10^9$ cells/L for African American individuals), platelet count less than 90×10^9 cells/L, creatinine clearance less than 50 mL/min, and ineligibility for peginterferon alfa 2a or ribavirin if needed for treatment intensification (see later). Women of child-bearing potential were required to use at least 2 contraception methods.

All randomized patients received daclatasvir (60 mg, orally, once daily), asunaprevir (200 mg, orally, twice daily), and BMS-791325 orally at either 75 or 150 mg twice daily. The dose selection of BMS-791325 was based on phase 1 antiviral activity and safety. Initially, eligible patients were assigned randomly to group 1 (BMS-791325 75 mg twice daily for 24 weeks) or group 2 (BMS-791325 75 mg twice daily for 12 weeks) using a computer-generated randomization scheme. After 4 weeks of observation, a second cohort was assigned randomly to group 3 (BMS-791325 150 mg twice daily for 24 weeks) or group 4 (BMS-791325 150 mg twice daily for 12 weeks). Patients were stratified by genotype 1a/1b, with 1b patient enrollment targeted between 25% and 38% or less of the total number of patients in each group. The primary end point was an HCV-RNA level less than 25 IU/mL at SVR_{12}. Other end points included analysis of HCV RNA at various time points during and after treatment, rates of viral breakthrough and relapse, and assessment of safety and tolerability. In the event of viral breakthrough (defined as confirmed increase in HCV-RNA level $\geq$1 $\log_{10}$ from nadir or confirmed HCV RNA level $\geq$25 IU/mL on or after week 8), patients were eligible to receive treatment intensification, defined as peginterferon alfa-2a (180 μg subcutaneously, once weekly) and ribavirin (1000 mg orally per day if patient weighed $<$75 kg, or 1200 mg orally per day if patient weighed $>$75 kg) in addition to continuation of the direct-acting antivirals for up to an additional 48 weeks.

Laboratory Assessment

Blood samples were drawn at baseline, days 1–7, days 9, 11, 14, 21, 28, every week through week 8, then every 2 weeks until the end of treatment, and post-treatment weeks 4, 12, 24, 36, and 48. HCV-RNA level was determined at a central laboratory using the COBAS TaqMan v2 assay (Roche Molecular Diagnostics, Pleasanton, CA), with a lower limit of quantitation of 25 IU/mL and a lower limit of detection of approximately 10 IU/mL. HCV genotypes were determined by polymerase chain reaction amplification and sequencing using the VERSANT HCV Amplification 2.0 Kit (LiPA) (Siemens, Munich, Germany). The host interleukin (*IL)28B* genotype (rs12979860 single-nucleotide polymorphism) was determined by Monogram Biosciences (South San Francisco, CA) using a real-time polymerase chain

reaction assay. All baseline samples were analyzed for polymorphisms in HCV NS3, NS5A, and NS5B associated with drug resistance using population sequencing (sensitivity, ≈20%).

Safety Assessments

Safety and tolerability were measured by serious adverse events, treatment-emergent adverse events, discontinuations owing to adverse events, severity grade 3/4 adverse events, and severity grade 3/4 laboratory abnormalities. Vital sign and electrocardiographic measurements, physical examinations, and clinical laboratory results were assessed throughout the study.

Statistical Analysis

Binary antiviral activity end points were assessed using modified intent-to-treat methodology. Patients prescribed a different treatment as assigned for the whole treatment duration were analyzed based on actual treatment (as treated). The numerator was based on treated patients meeting response criteria (defined as an HCV-RNA level <25 IU/mL; patients with missing HCV-RNA values were considered failures for that visit; however, failure owing to a missed value does not carry forward, in that, the patient can return later and be counted in future analyses of sustained response). The denominator was based on all treated patients.

Results

Disposition, Demographics, and Baseline Characteristics

Sixty-six patients were randomized. In addition to daclatasvir and asunaprevir, patients in groups 1 (N = 16) and 2 (N = 16) received BMS-791325 75 mg twice daily for 24 or 12 weeks, respectively, and patients in groups 3 (N = 16) and 4 (N = 18) received BMS-791325 150 mg twice daily for 24 or 12 weeks, respectively. In group 1, 2 patients discontinued treatment before week 24, 1 patient withdrew consent at week 9, and the other patient was discontinued at week 14 by the investigator because of inability to comply with study procedures. In groups 2 and 3, 1 patient discontinued treatment at week 11 because of poor compliance and 1 patient voluntarily discontinued treatment at week 18 of the 24-week regimen for reasons unrelated to the study. All group 4 patients (N = 18) completed the study. All groups were similar in age, race, and baseline HCV-RNA viral load (Table 1). Seventy-four percent of all patients were infected with HCV GT 1a, 70% of all patients had *IL28B* non-CC genotype, and more than 50% of all patients had FibroTest-derived METAVIR scores of F2 or greater; patients with FibroTest scores suggestive of cirrhosis (>0.72) were enrolled based on biopsy results showing an absence of cirrhosis (Table 1). Eighteen percent of patients were African American (Table 1).

Virologic Response

Groups 1 and 2, BMS-791325 75 mg twice daily. After treatment initiation, HCV-RNA levels rapidly decreased in both groups (Figure 1*A* and *B*). By week 4, all patients (N = 32) had achieved an HCV-RNA level less than 25 IU/mL and 97% (31 of 32) maintained an HCV-RNA level less than 25 IU/mL through the end of treatment. One patient (group 1) had an HCV-RNA level of 118 IU/mL at the last on-treatment visit but had an HCV-RNA level less than 25 IU/mL at 2, 4, and 12 weeks after treatment, suggesting a possible laboratory error. By using the modified intent-to-treat analysis (Table 2), 94% (30 of 32) of patients achieved SVR_4 and SVR_{12}. Ninety-one percent (29 of 32) of patients achieved SVR_{24} and no patient experienced viral breakthrough or post-treatment relapse.

Two patients missed their post-treatment week 4 and 12 visits and were counted as failures at these time points (Table 2): 1 patient (group 1) withdrew consent but showed undetectable HCV-RNA levels at treatment discontinuation (on-treatment week 9), and 1 patient (group 2) missed SVR_4 and SVR_{12} but achieved SVR_{24}. Two patients, 1 patient from each group, missed post-treatment week 24 visits but both had achieved SVR_4 and SVR_{12}.

Groups 3 and 4, BMS-791325 150 mg twice daily. After treatment initiation, HCV-RNA levels also rapidly decreased in both groups (Figure 1*C* and *D*). By week 4, all patients in group 3 (N = 16) achieved HCV-RNA levels less than 25 IU/mL and 94% (15 of 16) maintained HCV-RNA levels less than 25 IU/mL through the end of treatment. All but 2 patients in group 4 achieved an HCV-RNA level less than 25 IU/mL at week 4: 1 patient had an unconfirmed HCV-RNA level of 43 IU/mL and another patient missed the week 4 visit, but both achieved SVR_{12} and SVR_{24}. Ninety-four percent of patients (32 of 34) receiving BMS-791325 150 mg achieved an HCV-RNA level less than 25 IU/mL at the last on-treatment visit. By using modified intent-to-treat analysis (Table 2), 91% (31 of 34) of patients receiving BMS-791325 150 mg achieved SVR_4 and SVR_{12}.

Three patients overall experienced virologic failure: 1 patient each in groups 3 and 4 experienced viral breakthrough, and 1 patient in group 4 experienced relapse at follow-up week 4. The patient in group 3 with viral breakthrough was a 61-year-old black woman, randomized as GT 1b, however, further sequencing suggested a GT1 non-1a or 1b subtype analysis was ongoing. The patient further showed *IL28B* TT genotype, a FibroTest score of 0.62 (derived METAVIR F3), and a baseline HCV-RNA level of 5.1 $\log_{10}$ IU/mL. The patient achieved an HCV-RNA level less than 25 IU/mL at week 3 and experienced viral breakthrough at week 6. The group 4 patient was a 32-year-old white man with GT 1a, *IL28B* TT genotype, FibroTest score of 0.11 (derived METAVIR F0), and a baseline HCV-RNA level of 7.1 $\log_{10}$ IU/mL. The patient achieved an HCV-RNA level of approximately 10 IU/mL (undetectable) on week 4 and experienced viral breakthrough at week 8. Both patients intensified treatment by adding peginterferon alfa/ribavirin to the direct-acting antivirals. Sixteen weeks after starting treatment intensification, the group 3 patient discontinued all treatment because of a serious adverse event (cerebral vasoconstriction related to peginterferon alfa/ribavirin) and subsequently relapsed. The patient from group 4 achieved an HCV-RNA level less than 25 IU/mL 6 weeks after the addition of peginterferon alfa/ribavirin, and in preliminary data has achieved SVR_4. One patient (group 4) relapsed between the end of treatment and

Table 1. Demographics and Baseline Disease Characteristics

Group	1	2	3	4	Total
BMS-791325 dose, mg	75	75	150	150	
Treatment duration, wk	24	12	24	12	
N	16	16	16	18	66
Median age, y (range)	49 (44–61)	47 (24–67)	55 (25–67)	49 (29–68)	50 (24–68)
Male, n (%)	10 (63)	7 (44)	9 (56)	13 (72)	39 (59)
Race, n (%)					
White	11 (69)	13 (81)	12 (75)	16 (89)	52 (79)
African American	5 (31)	3 (19)	3 (19)	1 (6)	12 (18)
Asian other	0	0	1 (6)	1 (6)	2 (3)
Body mass index, n (%)					
<25, kg/m^2	6 (38)	4 (25)	7 (44)	7 (39)	24 (36)
25 to <30, kg/m^2	6 (38)	5 (31)	9 (56)	7 (39)	27 (41)
≥30, kg/m^2	4 (25)	7 (44)	0	4 (22)	15 (23)
HCV-RNA level					
Mean $\log_{10}$ IU/mL (SD)	6.3 (0.55)	6.2 (0.76)	6.4 (0.67)	6.3 (0.63)	6.3 (0.65)
Distribution, n (%)					
<800,000	4 (25)	6 (38)	3 (19)	4 (22)	17 (26)
≥800,000	12 (75)	10 (63)	13 (81)	14 (78)	49 (74)
HCV genotype, n (%)					
1a	12 (75)	12 (75)	12 (75)	13 (72)	49 (74)
1b	4 (25)	4 (25)	4 (25)	5 (28)	17 (26)
IL28B genotype (rs12979860), n (%)					
CC	4 (25)	5 (31)	5 (31)	6 (33)	20 (30)
CT	10 (63)	11 (69)	7 (44)	10 (56)	38 (58)
TT	2 (13)	0	4 (25)	2 (11)	8 (12)
FibroTest score, median (range)[a]	0.51 (0.09–0.78)	0.27 (0.03–0.91)	0.38 (0.05–0.66)	0.42 (0.06–0.80)	0.36 (0.03–0.91)
METAVIR scores derived from FibroTest[b]					
F0–F1	5 (31)	11 (69)	5 (31)	7 (39)	28 (42)
F2–F3	10 (63)	4 (25)	10 (63)	9 (50)	33 (50)
>F3[c]	1 (6)	1 (6)	0	2 (11)	4 (6)
Not reported	0	0	1 (6)[d]	0	1 (2)[d]

NOTE. Treatment consisted of daclatasvir (60 mg, orally, once daily), asunaprevir (200 mg, orally, twice daily), and BMS-791325 (75 or 150 mg orally, twice daily) for 24 or 12 weeks.
[a]Data calculated at ICON Central Laboratories.
[b]METAVIR category for each patient was derived from the FibroTest (BioPredictive) score, conversion was based on the manufacturer's website. F0–F1, METAVIR categories F0, F0–F1, and F1; F2–F3, METAVIR categories F1–F2, F2, and F3; >F3, METAVIR categories F3–F4 and F4.
[c]Patients with a FibroTest score exceeding the threshold for inclusion in the study (≤0.72 and an aspartate aminotransferase:platelet ratio ≤2) were required to have a liver biopsy to show the absence of cirrhosis.
[d]One patient was not able to have the FibroTest result calculated and instead had a biopsy performed to exclude cirrhosis.

post-treatment week 4. This patient was a 61-year-old white man, with GT 1a, *IL28B* CT genotype, FibroTest score of 0.66 (derived METAVIR F3), and baseline HCV-RNA level of 6.8 $\log_{10}$ IU/mL. The patient achieved an HCV-RNA level of approximately 10 IU/mL (undetectable) at week 3, which continued through the end of treatment. Resistance-associated variants detected at baseline and at the time of virologic failure are summarized for the 3 patients who experienced virologic failure in Supplementary Table 1.

Sustained virologic response by HCV subtype and *IL28B* host genotype are presented in Table 3.

Resistance

Polymorphisms at amino acid positions associated with resistance to one or more of the direct-acting antivirals were detected at baseline (Table 4).[14–17] One patient from group 4 infected with HCV GT 1a had NS5A-L31M at baseline and experienced viral breakthrough at week 8; this polymorphism has been shown to yield a 250-fold change in the in vitro median effective concentration (EC_{50}) of daclatasvir.[15] NS5A-Q30R-L31M, NS3-R155K, and NS5B-P495L were detected at viral breakthrough. Another HCV GT 1a–infected patient (group 4) who experienced a relapse 4 weeks after treatment had NS5A-H58P and NS3-V36M polymorphisms at baseline, and NS5A-M28A-Q30R-H58P, NS3-V36M-R155K, and NS5B-P495L at post-treatment weeks 4 and 12. Sequence polymorphism data at baseline and virologic failure for the patient in group 3 who experienced viral breakthrough at week 6 currently are unavailable owing to poor sequence amplification despite multiple methodologies.

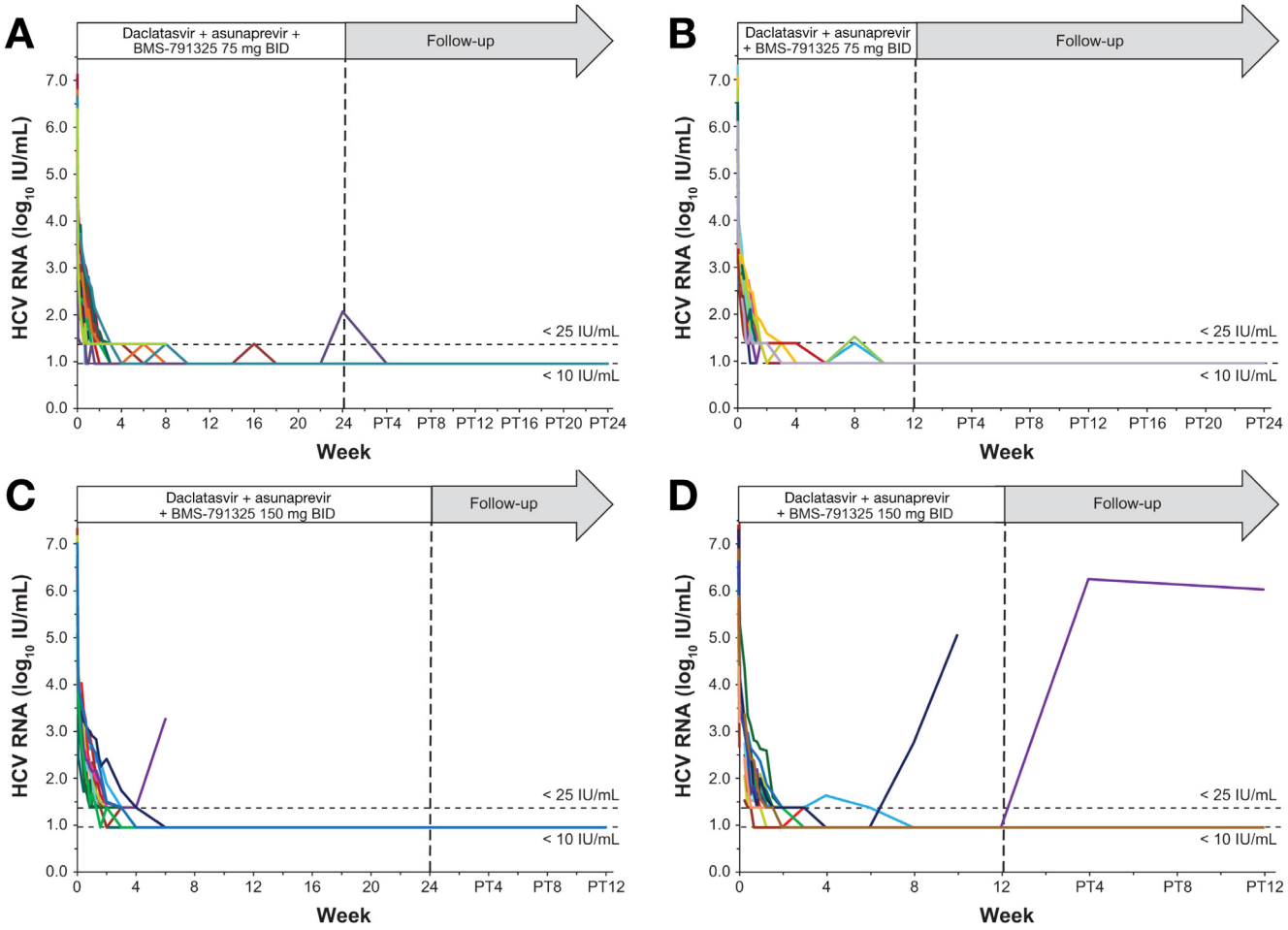

Figure 1. (*A–D*) HCV-RNA levels over time for individual patients in groups 1–4. Patients were treated with daclatasvir (60 mg, orally, once daily), asunaprevir (200 mg, orally, twice daily), plus (*A*) BMS-791325 75 mg twice daily for 24 weeks, (*B*) BMS-791325 75 mg twice daily for 12 weeks, (*C*) BMS-791325 150 mg twice daily for 24 weeks, (*D*) or BMS-791325 150 mg twice daily for 12 weeks. N equals 16 each for groups 1 through 3, and 18 for group 4. PT, post-treatment.

Safety

One serious adverse event of ureteral calculus (group 2) occurred on treatment day 24 and was considered by the investigator to be unrelated to study therapy (Table 5). No deaths or adverse events leading to discontinuation occurred during the study on the direct-acting antiviral regimen alone (Table 5). One patient (group 2) had a grade 3 headache that resolved after 7 days with continuation of study treatment. The most common adverse events (≥10% of patients) included headache, asthenia, diarrhea, nausea, and abdominal pain, all were mild or moderate in intensity. One patient (group 2) experienced grade 4 lymphopenia on day 14 concomitant with influenza infection, which started on day 12 (Table 5). All subsequent lymphocyte results were within the normal range. During treatment intensification, 1 patient (group 3) experienced grade 3 neutropenia and a serious adverse event of cerebral vasoconstriction (grade 3) leading to treatment discontinuation, both considered by the investigator to be related to peginterferon alfa/ribavirin and not to the direct-acting antiviral regimen. There were no grade 3–4 laboratory events on the direct-acting antiviral regimen alone specific to alanine aminotransferase, aspartate aminotransferase, bilirubin, hemoglobin, leukocytes, absolute neutrophil count, or platelet count. Importantly, no clinically meaningful change in hemoglobin values were observed during treatment, although modest mean hemoglobin changes of -0.42 to -0.92 g/dL were observed up to treatment week 4 (Supplementary Table 2). These decreases were not dose-dependent and improved during the course of treatment, thus likely reflecting the intense safety, efficacy, and pharmacokinetic phlebotomy requirements during the first 28 days of this study.

Discussion

Currently approved treatment regimens for HCV GT 1–infected patients include a protease inhibitor combined with peginterferon/ribavirin and have modest antiviral activity, poor tolerability, and long treatment durations.[18–20] For these reasons, interferon-free treatment regimens with multiple direct-acting antivirals are in clinical development. Two direct-acting antivirals, daclatasvir and asunaprevir, without interferon or ribavirin, were able to achieve high SVR rates in GT 1b–infected patients, but a high rate of viral

Table 2. Virologic Response During and After Treatment

Group	1	2	3	4
BMS-791325 dose, mg	75	75	150	150
Treatment duration, wk	24	12	24	12
N	16	16	16	18
% < LOQ on-treatment				
Week 4	16/16 (100)	16/16 (100)	16/16 (100)	16/18 (89)[a]
End of treatment (includes early discontinuations)	15/16 (94)[b]	16/16 (100)	15/16 (94)	17/18 (94)
Post-treatment SVRs				
Week 4	15/16 (94)[c]	15/16 (94)[d]	15/16 (94)	16/18 (89)
Week 12	15/16 (94)[c]	15/16 (94)[d]	15/16 (94)	16/18 (89)
Week 24	14/16 (88)[d,e]	15/16 (94)[f]	In progress	In progress
Virologic failure				
Viral breakthrough	0/16	0/16	1/16 (6)	1/18 (6)
Viral relapse	0/16	0/16	0/16	1/18 (6)

NOTE. Data are shown as n (%). Virologic response was defined as an HCV-RNA level <25 IU/mL. The analyses were performed using modified intent-to-treat population (all patients who received at least 1 dose of study medication). Treatment consisted of daclatasvir (60 mg, orally, once daily), asunaprevir (200 mg, orally, twice daily), and BMS-791325 (75 or 150 mg orally, twice daily) for 24 or 12 weeks.
LOQ, limit of quantitation.
[a]Includes 1 patient with unconfirmed HCV-RNA level of 43 IU/mL and 1 patient who missed a visit.
[b]Includes 1 patient with an HCV-RNA level of 118 (IU/mL) at last on-treatment visit but <25 IU/mL at 2, 4, and 12 weeks post-treatment.
[c]Includes 1 patient who withdrew consent before week 12.
[d]Includes 1 patient who achieved SVR_{24} but missed the SVR_4 and SVR_{12} visits.
[e]Includes a patient who achieved SVR_4 and SVR_{12} but missed the week 24 visit.
[f]Includes a patient who achieved SVR_4 and SVR_{12} but missed the week 24 visit.

breakthrough occurred in patients infected with GT 1a.[7,11–13] This finding is consistent with modeling that predicts successful interferon-free, direct-acting antiviral combinations must impose a high genetic barrier to 4 or more HCV variants to prevent emergence of resistance.[21]

Two clinical paradigms have emerged to enhance the genetic barrier of interferon-free regimens. First, treatment with a combination of 2 direct-acting antivirals, including a nucleotide polymerase inhibitor with a high barrier to viral resistance such as sofosbuvir, combined with a direct-acting

Table 3. Virologic Response by HCV and Host *IL28B* Genotype

	HCV genotype, n/N (%)				*IL28B* genotype, n/N (%)			
	1a		1b		CC		Non-CC	
BMS-791325 dose, mg	75	150	75	150	75	150	75	150
Week 4	24/24 (100)	24/25 (96)[a]	8/8 (100)	8/9 (89)[b]	9/9 (100)	10/11 (91)[a]	23/23 (100)	22/23 (96)[b]
End of treatment	23/24 (96)[c]	24/25 (96)[d]	8/8 (100)	8/9 (89)[d]	9/9 (100)	11/11 (100)	22/23 (96)[c]	21/23 (91)[d]
SVR_4	23/24 (96)[e]	23/25 (92)[d,f]	7/8 (88)[g]	8/9 (89)[d]	9/9 (100)	11/11 (100)	21/23 (91)[e,g]	20/23 (87)[d,f]
SVR_{12}	23/24 (96)[e]	23/25 (92)[d,f]	7/8 (88)[g]	8/9 (89)	9/9 (100)	6/6 (100)	21/23 (91)[e,g]	10/12 (83) [d,f]
SVR_{24}	21/24 (88)[e,h]		8/8 (100)		9/9 (100)		20/23 (87)[e,h]	

NOTE. Virologic response was defined as an HCV-RNA level of <25 IU/mL. The analyses were performed in a modified intention-to-treat population (all patients who received at least 1 dose of study medication). Treatment consisted of daclatasvir (60 mg, orally, once daily), asunaprevir (200 mg, orally, twice daily), and BMS-791325 (75 or 150 mg orally, twice daily, as indicated) for 24 or 12 weeks.
[a]Includes 1 patient with an unconfirmed HCV-RNA level of 43 IU/mL.
[b]Includes 1 patient who missed a visit.
[c]Includes 1 patient with an HCV-RNA level of 118 IU/mL at the last on-treatment visit but an HCV-RNA level <25 IU/mL at 2, 4, and 12 weeks after treatment.
[d]Viral breakthrough.
[e]Includes 1 patient who withdrew consent before week 12.
[f]Patient relapsed.
[g]Includes 1 patient who achieved SVR_{24} but missed the SVR_4 and SVR_{12} visits.
[h]Includes 2 patients who achieved SVR_4 and SVR_{12} but missed the SVR_{24} visit.

Table 4. Polymorphisms Observed at Baseline Known to Decrease Susceptibility to Daclatasvir, Asunaprevir, or BMS-791325

Protein	Genotype	Polymorphisms	Number of patients
NS3	1a	V36M	2
	1a	V55A/I	3
	1a	Q80K	12
NS5A	1a	L31M	2
	1b	L31M	1
	1b	Y93H	2
NS5B	1a	L392I	1
	1a	A421V	11
	1b	V499A	5

NOTE. Some polymorphisms were detected as minor variants or as the only variant at that position by population sequencing. NS5A and NS5B polymorphisms conferred ≥2-fold loss in potency to daclatasvir and BMS-791325 in vitro, respectively. No polymorphisms were identified that conferred ≥3-fold loss in potency to asunaprevir in vitro.

antiviral with a different mechanism of action with high potency such as the NS5A inhibitor daclatasvir or the NS3 protease inhibitor simeprevir, can achieve sustained response without viral breakthrough.[22,23] An alternative strategy involves combining 2 or more non-nucleotide direct-acting antivirals with or without ribavirin to improve the genetic barrier to resistance.[24–27] An interferon-free combination of 3 direct-acting antivirals (an NS5B non-nucleoside inhibitor, a ritonavir-boosted protease inhibitor, and an NS5A inhibitor) plus ribavirin showed high SVR rates, but treatment success was reduced when ribavirin or any single direct-acting antiviral was omitted.[27] In this study, combining 3 direct-acting antivirals without interferon or ribavirin showed a high SVR rate after 12 weeks of treatment in HCV GT 1–infected, treatment-naive patients.

This all-oral, interferon-free, ribavirin-free treatment consisting of daclatasvir, asunaprevir, and BMS-791325 75 or 150 mg twice daily achieved up to 94% SVR_{12} after 24 or 12 weeks of treatment. Sustained response was achieved in both HCV GT 1a–infected and HCV GT 1b–infected patients, including patients with reduced interferon responsiveness predicted by *IL28B* non-CC genotypes. No viral breakthrough or relapse was observed in patients treated with the 75 mg twice-daily dose of BMS-791325. There was 100% concordance between SVR_4 and subsequent SVR time points in all patients with available data.

An important aspect to this study is that SVR was achieved without inclusion of ribavirin. Ribavirin contributes to anemia and it is teratogenic; thus, effective treatments without ribavirin are desirable. This interferon- and ribavirin-free regimen did not alter hemoglobin levels in a clinically meaningfully manner as evidenced by no grade 1 or higher hemoglobin reductions and no adverse events of anemia. The mechanism of action of ribavirin is not clear, and its contribution to clinical efficacy varies by regimen. Ribavirin clearly improves SVR rates in interferon-based therapies, including telaprevir-based regimens.[28] Among interferon-free regimens, the benefit of ribavirin remains unclear. The combination of the ritonavir-boosted protease inhibitor ABT-450, the NS5B non-nucleoside inhibitor ABT-333, and the NS5A inhibitor ABT-267 with or without ribavirin showed lower SVR rates without ribavirin.[27] However, the combination of daclatasvir and sofosbuvir did not require ribavirin to achieve high SVR rates in patients with unfavorable characteristics, including HCV genotypes 1a and 3, and host *IL28B* non-CC genotypes.[22] The regimen presented here provides additional evidence that combinations of potent direct-acting antivirals do not always require ribavirin to achieve a sustained response.

This study also assessed the impact of treatment duration on SVR and the regimen showed efficacy with only 12 weeks of

Table 5. Safety Through the End of Treatment

Group	1	2	3	4	Total
BMS-791325 dose, mg	75	75	150	150	
Treatment duration, wk	24	12	24	12	
N	16	16	16	18	66
Serious adverse events	0	1 (6.3)[a]	0	0	1 (1.5)
Adverse events leading to discontinuation	0	0	0	0	0
Grade 3–4 adverse events	0	1 (6.3)[b]	0	0	1 (1.5)
Grade 3–4 laboratory abnormalities	0	1 (6.3)[c]	0	0	1 (1.5)
Adverse events in >10% of patients in combined treatment groups during treatment					
Headache	4 (25.0)	6 (37.5)	4 (25.0)	4 (22.2)	18 (27.3)
Asthenia	2 (12.5)	3 (18.8)	2 (12.5)	4 (22.2)	11 (16.7)
Diarrhea	2 (12.5)	6 (37.5)	2 (12.5)	1 (5.6)	11 (16.7)
Nausea	1 (6.3)	2 (12.5)	2 (12.5)	4 (22.2)	9 (13.6)
Abdominal pain	0	0	3 (18.8)	4 (22.2)	7 (10.6)
Abdominal pain, upper	1 (6.3)	1 (6.3)	3 (18.8)	2 (11.1)	7 (10.6)

NOTE. Data are n (%). Treatment consisted of daclatasvir (60 mg, orally, once daily), asunaprevir (200 mg, orally, twice daily) and BMS-791325 (75 or 150 mg orally, twice daily) for 24 or 12 weeks.
[a]Ureteral calculus, considered by investigator to be unrelated to study therapy.
[b]Grade 3 headache, resolved after 7 days with continued study treatment.
[c]Lymphopenia at a single study visit concomitant with influenza.

treatment. This therapeutic duration is consistent with publication of viral kinetic modeling data suggesting 10 weeks of treatment with a potent antiviral regimen may be needed to clear infected hepatocytes.[29] In contrast, current treatment for GT 1-infected patients includes peginterferon, ribavirin, and the NS3 protease inhibitors telaprevir or boceprevir for up to 48 weeks.[30] Shorter treatment durations are preferable because they may improve patient compliance. In this study, treatment periods of both 12 and 24 weeks yielded high SVR rates, suggesting no advantage for extending treatment duration to 24 weeks. The study using ABT-450 boosted with ritonavir, ABT-333, ABT-267, and ribavirin in GT 1 treatment-naive patients also showed high rates of SVR with only 12 weeks of therapy.[27] Similarly, regimens including sofosbuvir and daclatasvir with or without ribavirin also showed high rates of SVR with 12 weeks of treatment.[22,31]

In our study, virologic failure was uncommon and observed in only 3 patients in the 150 mg twice-daily dosing groups: 2 patients with viral breakthrough, and 1 patient with virologic relapse. The reasons for treatment failure in these patients remain unclear but could include baseline virus polymorphisms, host immune status, and/or reduced drug exposure or adherence. Two of these patients were infected with HCV GT 1a. One patient had a pre-existing NS5A variant with increased resistance to daclatasvir (L31M, 250-fold change in the EC_{50} of daclatasvir in vitro). The second patient had baseline resistance-associated polymorphisms to daclatasvir and asunaprevir (NS5A-H58P and NS3-V36M, respectively), which alone do not appear to alter the EC_{50} value of the direct-acting antivirals in vitro. It is possible that these variants acted in a compensatory manner by enhancing the fitness of the emergent variants. The emergent linked NS5A substitution M28A-Q30R confers high-level resistance to daclatasvir in vitro (>200,000-fold resistance). NS3-V36M enhances resistance by approximately 3-fold against asunaprevir when combined with NS3-R155K in vitro. The HCV-RNA sequences of the third patient with HCV GT 1b reported at screening have not been amplified successfully despite numerous attempts, thus the HCV GT remains unconfirmed and the presence of baseline and emergent variants is unknown. The relationship between pre-existing polymorphisms and treatment failure of interferon-free regimens is unclear because patients in this and other studies with similar findings have achieved a sustained response. Thus, larger studies are needed to clarify the impact of pre-existing polymorphisms on efficacy.[13,22,32,33] The role of *IL28B* host genotype in virologic failure in patients treated with other interferon-free regimens also is unclear. All 3 patients with virologic failure in this study were *IL28B* non-CC, but this may be a reflection of most patients enrolled in the study (70%) being *IL28B* non-CC. Recent studies have confirmed that interferon-free regimens achieve high SVR rates in patients with *IL28B* non-CC genotype, and *IL28B* non-CC genotype did not predict failure.[7,11–13,22,25] Finally, preliminary pharmacokinetic assessments of patients in this study do not suggest any clinically relevant drug–drug interactions among the 3 direct-acting antivirals in this combination, and the pharmacokinetic profiles of daclatasvir, asunaprevir, and BMS-791325 did not differ markedly in the 3 patients with virologic failure as compared with the remainder of the patients in the study.[34] The observation that virologic failure occurred only among patients receiving BMS-791325 150 mg may reflect the small sample size studied thus far and not necessarily represent a true difference in efficacy between BMS-791325 doses. Both doses remain under evaluation in the ongoing phase 2b study expansion.

In summary, the combination of daclatasvir, asunaprevir, and BMS-791325 generally was well tolerated and may represent a significant improvement over current treatments. SVR rates generally exceeded 90%, and the most common adverse events were headache, asthenia, and gastrointestinal complaints (diarrhea, nausea, and abdominal pain); and did not lead to treatment discontinuation. Furthermore, no serious adverse events related to these direct-acting antivirals were observed and only one grade 3 or 4 laboratory value was documented while on direct-acting antiviral–only therapy (reversible lymphopenia during influenza infection). These adverse events and side effects were minor in comparison with the reported rates and severity of adverse events associated with peginterferon, ribavirin, and either telaprevir or boceprevir.[35,36] Indeed, during treatment intensification with peginterferon alfa/ribavirin, 1 patient experienced a serious adverse event of cerebral vasoconstriction (cerebrovascular disorders are observed with the use of peginterferon alfa-2a [Pegasys, Genentech - Hoffmann-La Roche, South San Francisco, CA] per the package insert).[37] This tolerability profile is also similar to that observed in studies of asunaprevir and daclatasvir given as dual therapy for HCV GT 1b,[7,11–13] suggesting that the addition of BMS-791325 does not add to the adverse event profile of this regimen.

We conclude that this all-oral, interferon-free, ribavirin-free treatment of daclatasvir, asunaprevir, and BMS-791325 is a promising therapy for chronic hepatitis C that warrants additional investigation. Limitations of this pilot study included small patient numbers, restrictive inclusion and exclusion criteria, and selection of noncirrhotic, treatment-naive HCV GT 1–infected patients for initial study. This study has been expanded to examine both doses of BMS-791325 in additional HCV GT 1 treatment-naive patients, GT 1 null responders to prior peginterferon/ribavirin treatment, patients with cirrhosis, and patients infected with HCV GT 4.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at http://dx.doi.org/10.1053/j.gastro.2013.10.057.

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Conflicts of interest
These authors disclose the following: Gregory Everson has been on advisory committees or review panels for Roche/Genentech, Merck, and HepC Connection; has been a board member of HepQuant, LLC, and PSC Partners; has performed consulting for Roche/Genentech and Abbott; has received grant/research support from Roche/Genentech, Pharmassett, Vertex, GSK, Schering-Plough, Bristol-Myers Squibb, Tibotec, GlobeImmune, Pfizer, Abbott, Conatus, ZymoGenetics, and PSC Partners; has held a management position for HepQuant, LLC; and has a patent held/filed with the University of Colorado; Maribel Rodriguez–Torres has been a consultant for Akros Pharmaceutical, Bristol-Myers Squibb, Genentech, Hoffman-La Roche, Inhibitex, Janssen R&D Ireland, Merck Sharp & Dohme, Corp, Pharmasset, Santaris Pharma, and Vertex Pharmaceutical, Inc; has received grant/research support from Inhibitex, Johnson & Johnson, Merck Sharp & Dohme, Corp, Mochida Pharmaceutical, Novartis, Pfizer, Pharmasset, Santaris Pharma, A/S, Scynexis, Inc, Siemens Healthcare Diagnostics, Vertex Pharmaceutical, Inc, ZymoGenetics, Abbott Laboratories, Akros Pharmaceutical, Anadys Pharmaceutical, Beckman Coulter, Boehringer Ingelheim, Bristol-Myers Squibb, Genentech, Gilead Pharmaceuticals, Glaxo Smith Kline, Hoffman-La Roche, Human Genome Sciences, Idenix Pharmaceutical, and Idera Pharmaceutical; Christophe Hézode has been compensated for speaking and teaching by Roche, Bristol-Myers Squibb, Merck Sharp & Dohme, Corp, Janssen, Abbvie, and Gilead; Eric Lawitz had performed consulting for Theravance; has received grant/research support from Abbott Laboratories, Anadys Pharmaceuticals, Bristol-Myers Squibb, Gilead Sciences, Glaxo-SmithKline, GlobeImmune, Medtronic, Idenix Pharmaceuticals, Idera Pharmaceuticals, Medarex, Merck & Co, Novartis, Roche, Schering-Plough, Pfizer, Vertex Pharmaceuticals, ZymoGenetics, Tibotec/Johnson and Johnson, Pharmasset, Biolex, Achillion Pharmaceuticals, Biolex Therapeutics, Boehringer Ingelheim, Inhibitex Pharmaceuticals, Pharmasset, Santaris, and Scynexis Pharmaceuticals; Marc Bourlière has been a member of advisory committees or review panels for Merck; has been a board member of Gilead and Tibotec; has performed consulting for Boehringer Ingelheim, Roche, Schering-Plough, Novartis, Bristol-Myers Squibb, Abbott, and Janssen; Veronique Loustaud–Ratti has been compensated for speaking and teaching by Roche, Schering-Plough, Merck Sharp & Dohme, Corp, Janssen, Bristol-Myers Squibb, and Gilead; and has received grant/research support from Roche and Bristol-Myers Squibb; and has been a board member of Gilead and Roche; Vinod Rustgi has received grant/research support from Gilead, Bristol Myers Squibb, Abbot, Anadys, and Boehringer-Ingelheim; has been compensated for speaking and teaching by Merck, Genentech, and Vertex; Patrick Marcellin has performed consulting for Roche, Gilead, Bristol-Myers Squibb, Vertex, Novartis, Janssen-Tibotec, Merck Sharp & Dohme, Corp, Boehringer, Abbott, and Pfizer; has received grant/research support from Roche, Gilead, Janssen-Tibotec, and Merck Sharp & Dohme, Corp; has been compensated for speaking and teaching by Roche, Gilead, Bristol-Myers Squibb, Vertex, Janssen-Tibotec, Merck Sharp & Dohme, Corp, and Abbott; and is a stock shareholder in Novartis; Stanislas Pol has served as a board member of Sanofi, Bristol-Myers-Squibb, Boehringer Ingelheim, Tibotec Janssen Cilag, Vertex, Gilead, Roche, Merck Sharp & Dohme, Corp, Novartis, and Abbvie; and has been compensated for speaking and teaching by Glaxo Smith Kline; Paul Thuluvath has been a member of advisory committees or review panels for Bayer; has received grant/research support from Bayer, Bristol-Myers Squibb, Boehringer, and Novartis; and has been compensated for speaking and teaching by Bayer/Onyx, Roche, Vertex, and Gilead; Karen Sims, Timothy Eley, Xiaodong Wang, Shu–Pang Huang, Fiona McPhee, Megan Wind–Rotolo, Ellen Chung, Claudio Pasquinelli, Dennis Grasela, and David Gardiner are employees and may be shareholders of Bristol-Myers Squibb. The remaining authors have nothing to disclose.

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