ORIGINAL ARTICLE

Daclatasvir plus Sofosbuvir for Previously Treated or Untreated Chronic HCV Infection

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ABSTRACT

BACKGROUND

All-oral combination therapy is desirable for patients with chronic hepatitis C virus (HCV) infection. We evaluated daclatasvir (an HCV NS5A replication complex inhibitor) plus sofosbuvir (a nucleotide analogue HCV NS5B polymerase inhibitor) in patients infected with HCV genotype 1, 2, or 3.

METHODS

In this open-label study, we initially randomly assigned 44 previously untreated patients with HCV genotype 1 infection and 44 patients infected with HCV genotype 2 or 3 to daclatasvir at a dose of 60 mg orally once daily plus sofosbuvir at a dose of 400 mg orally once daily, with or without ribavirin, for 24 weeks. The study was expanded to include 123 additional patients with genotype 1 infection who were randomly assigned to daclatasvir plus sofosbuvir, with or without ribavirin, for 12 weeks (82 previously untreated patients) or 24 weeks (41 patients who had previous virologic failure with telaprevir or boceprevir plus peginterferon alfa–ribavirin). The primary end point was a sustained virologic response (an HCV RNA level of <25 IU per milliliter) at week 12 after the end of therapy.

RESULTS

Overall, 211 patients received treatment. Among patients with genotype 1 infection, 98% of 126 previously untreated patients and 98% of 41 patients who did not have a sustained virologic response with HCV protease inhibitors had a sustained virologic response at week 12 after the end of therapy. A total of 92% of 26 patients with genotype 2 infection and 89% of 18 patients with genotype 3 infection had a sustained virologic response at week 12. High rates of sustained virologic response at week 12 were observed among patients with HCV subtypes 1a and 1b (98% and 100%, respectively) and those with CC and non-CC IL28B genotypes (93% and 98%, respectively), as well as among patients who received ribavirin and those who did not (94% and 98%, respectively). The most common adverse events were fatigue, headache, and nausea.

CONCLUSIONS

Once-daily oral daclatasvir plus sofosbuvir was associated with high rates of sustained virologic response among patients infected with HCV genotype 1, 2, or 3, including patients with no response to prior therapy with telaprevir or boceprevir. (Funded by Bristol-Myers Squibb and Pharmasset (Gilead); A1444040 ClinicalTrials.gov number, NCT01359644.)

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Chronic infection with hepatitis C virus (HCV) affects approximately 170 million people worldwide and is a major cause of cirrhosis and hepatocellular carcinoma.[1,2] HCV-related morbidity and mortality are increasing; since 2007, HCV-related deaths in the United States have exceeded those from human immunodeficiency virus (HIV) infection.[3,4] HCV is classified into six major genotypes.[5,6] Genotypes 1, 2, and 3 are found worldwide, with subtype 1a predominating in the United States and subtype 1b predominating in Europe, Japan, and China.[5,7,8]

Peginterferon alfa–ribavirin treatment for chronic HCV infection is associated with a sustained virologic response (undetectable HCV RNA level after treatment) in approximately 40% of patients with genotype 1 infection and 75% of patients infected with genotype 2 or 3.[9,10] Adding boceprevir or telaprevir has been shown to improve the response in patients with genotype 1 infection.[11,12] However, the addition of boceprevir or telaprevir is limited to HCV genotype 1 and is associated with adverse events, complicated dose regimens, and viral resistance.[11-14] Currently, patients who have virologic failure (no sustained virologic response) with telaprevir or boceprevir plus peginterferon alfa–ribavirin have no other treatment options.

Daclatasvir is a first-in-class HCV NS5A replication complex inhibitor, and sofosbuvir is a nucleotide analogue HCV NS5B polymerase inhibitor.[15,16] Both have potent antiviral activity and broad genotypic coverage and are administered orally once daily.[15,17,18] Each is effective in patients infected with genotype 1, 2, or 3 when this treatment is combined with peginterferon alfa–ribavirin,[19-22] and sofosbuvir plus ribavirin is effective in patients infected with genotype 1, 2, or 3 in the absence of treatment with peginterferon alfa–ribavirin.[23] We evaluated daclatasvir plus sofosbuvir, with or without ribavirin, in previously untreated patients infected with genotype 1, 2, or 3, and in patients with genotype 1 infection who had not had a response to previous treatment with telaprevir or boceprevir.

METHODS

PATIENTS

Eligible patients were 18 to 70 years of age and had chronic HCV genotype 1, 2, or 3 infection with an HCV RNA level of 100,000 IU per milliliter or higher. Patients did not have evidence of cirrhosis as documented by means of either liver biopsy within the previous 24 months or noninvasive assessment of serum markers of fibrosis (a FibroTest score of ≤0.72, on a scale of 0 to 1, with higher scores indicating more severe fibrosis, and an aspartate aminotransferase:platelet ratio index of ≤2, with higher scores indicating a greater likelihood of extensive fibrosis) at screening.[24,25]

Patients who had received prior treatment had confirmed virologic failure during or after treatment with telaprevir (at a dose of 750 mg three times daily) or boceprevir (at a dose of 800 mg three times daily) plus peginterferon alfa–ribavirin. Virologic failure was defined as a nonresponse (detectable HCV RNA levels at the end of the treatment period), breakthrough (>1 $\log_{10}$ increase from the nadir in the HCV RNA level or a quantifiable HCV RNA level in a patient with an undetectable level during the treatment period), or relapse (a quantifiable HCV RNA level during follow-up in a patient with an undetectable level at the end of the treatment period).

Patients who had discontinued telaprevir or boceprevir because of adverse events were excluded. Other exclusion criteria were chronic liver disease other than HCV infection and coinfection with HIV or hepatitis B virus. All patients provided written informed consent.

STUDY OVERSIGHT

The study was conducted in accordance with Good Clinical Practice guidelines and was approved by the institutional review board or independent ethics committee at each site. Bristol-Myers Squibb (the sponsor) and Pharmasset (now Gilead Sciences), which provided the study drug, designed the study; the sponsor conducted the study in collaboration with the principal investigators, collected the data, monitored the conduct of the study, and performed the statistical analyses. The first draft of the manuscript was prepared by the academic and industry authors, with assistance from a medical writer paid by the sponsor. The academic authors vouch for the completeness and accuracy of the data and data analyses and for the fidelity of the study to the protocol (available with the full text of this article at NEJM.org).

STUDY DESIGN

In this open-label study, untreated patients were randomly assigned, in a 1:1:1 ratio, to receive sofosbuvir for 1 week, then daclatasvir and sofosbuvir for 23 weeks (groups A and B); daclatasvir and sofosbuvir for 24 weeks (groups C and D); or daclatasvir, sofosbuvir, and ribavirin for 24 weeks (groups E and F). Patients with genotype 1 infection were assigned to group A, C, or E, and patients infected with genotype 2 or 3 were assigned to group B, D, or F (Fig. S1a in the Supplementary Appendix, available at NEJM.org).

The purpose of the lead-in period of therapy with sofosbuvir was to determine whether initial HCV suppression with sofosbuvir would reduce the emergence of daclatasvir-resistant variants. In accordance with a protocol amendment, 123 additional patients with genotype 1 infection were randomly assigned in a 1:1 ratio to daclatasvir plus sofosbuvir, with or without ribavirin, for 12 weeks (82 untreated patients, assigned to group G or H) or 24 weeks (41 patients who did not have a response to prior treatment with HCV protease inhibitors, assigned to group I or J) (Fig. S1b and S1c in the Supplementary Appendix).

Daclatasvir and sofosbuvir were administered orally at a dose of 60 mg once daily and 400 mg once daily, respectively. Ribavirin was administered orally twice daily at a dose of 1000 to 1200 mg per day, according to body weight (1000 mg in patients with a body weight of <75 kg, and 1200 mg in patients with a body weight ≥75 kg), in patients with genotype 1 infection, and at a dose of 800 mg per day in patients infected with genotype 2 or 3. A reduction in the dose of ribavirin to 600 mg daily was permitted if the hemoglobin level decreased to below 10 g per deciliter.

EFFICACY AND SAFETY MONITORING

Serum HCV RNA levels were assayed centrally with the use of the COBAS TaqMan HCV test, version 2.0 (Roche Molecular Systems), with a lower limit of quantification of 25 IU per milliliter and a lower limit of detection of 10 IU per milliliter. HCV RNA levels were measured at baseline; on treatment days 1 through 7, 9, 11, 14, and 21; every 2 weeks from treatment week 4 through week 24; and at weeks 4, 12, and 24 after the end of the treatment period.

Adverse events were recorded throughout the study. Clinical laboratory tests, physical examinations, and electrocardiographic monitoring were performed at screening, at baseline, and at scheduled visits throughout treatment.

VIROLOGIC BREAKTHROUGH, RELAPSE, AND RESISTANCE MONITORING

Virologic breakthrough during the treatment period was defined as a confirmed increase from the nadir in the HCV RNA level of at least 1 $\log_{10}$ IU per milliliter or a confirmed HCV RNA level of 25 IU per milliliter or higher at or after week 8. In groups A through F, breakthrough also included detectable HCV RNA of less than 25 IU per milliliter at or after week 8; results from the first cohort showed that detectable but unquantifiable HCV RNA was not associated with the clinical outcome, so this definition was later removed. For patients with virologic breakthrough, peginterferon alfa and ribavirin could be added as rescue therapy (unless they were already receiving ribavirin). Virologic relapse was defined as a confirmed HCV RNA level of 25 IU per milliliter or higher in patients with an HCV RNA level that was less than 25 IU per milliliter at the end of treatment.

At baseline, the HCV NS5A and NS5B regions from all samples and the NS3 region from samples in groups I and J were analyzed by means of population sequencing (sensitivity, approximately 20%). Samples from patients with virologic breakthrough or relapse were analyzed by means of population sequencing if the HCV RNA level was at least 1000 IU per milliliter. After viral RNA was isolated from plasma, the HCV NS3, NS5A, and NS5B regions were amplified by means of polymerase chain reaction and sequenced. Consensus sequences in samples obtained from the patients were compared with the appropriate reference sequences (GT1a [H77], GT1b [Con1], GT2 [JFH1], and GT3 [S52]) by means of the Basic Local Alignment Search Tool.

END POINTS

The primary efficacy end point was the proportion of patients with a sustained virologic response (an HCV RNA level of less than 25 IU per milliliter) at week 12 after the end of treatment. Secondary efficacy end points included a sustained virologic response at 4 weeks after treat-

ment and at 24 weeks after treatment. Safety end points included adverse events, discontinuation of a study drug due to adverse events, and grade 3 or 4 laboratory abnormalities.

STATISTICAL ANALYSIS

With sample sizes of 14, 20, and 40 patients, the probability of observing at least one safety event occurring at an incidence rate of 10% was 0.771, 0.878, and 0.985, respectively. With these three sample sizes, the two-sided 80% exact confidence intervals for a sustained virologic response at week 12 after treatment were, respectively, 58 to 92% if the observed rate was 79% (11 of 14 patients with an event), 59 to 87% if the observed rate was 75% (15 of 20 patients with an event), and 64 to 84% if the observed rate was 75% (30 of 40 patients with an event). For efficacy end points, the analyses included all patients who received at least one dose of study medication (modified intention-to-treat population). Patients for whom data were missing were classified as not having had a response at that visit but could be classified as having a response at future visits if the lack of response was solely due to the missing HCV RNA measurement. Patients who required rescue therapy were classified as not having had a response at the time of rescue and at all subsequent visits.

RESULTS

CHARACTERISTICS OF THE PATIENTS

A total of 211 patients received treatment at 18 centers in the United States between June 2011 and November 2012. A total of 44 were infected with HCV genotype 2 or 3, and 167 had genotype 1 infection (126 untreated patients and 41 patients who did not have a response to prior treatment with protease inhibitors) (Fig. S1 in the Supplementary Appendix). Among patients with genotype 1 infection, the median age ranged from 54 to 59 years, and most had fibrosis of Metavir stage 2 or higher (on a scale from F0 to F4, with higher stages indicating a greater degree of fibrosis) (Table 1, and Table S1 in the Supplementary Appendix). Of the 41 patients who did not have a response to prior treatment with protease inhibitors, 19 (46%) had NS3 polymorphisms conferring resistance to telaprevir or boceprevir (Tables S2 and S3 in the Supplementary Appendix).

VIROLOGIC RESPONSE

During the initial 48 hours, the slope of the mean viral decline was steeper in groups receiving daclatasvir and sofosbuvir than in those receiving sofosbuvir alone (P<0.001) (Fig. S2 in the Supplementary Appendix). This difference did not persist; all patients had an HCV RNA level of less than 25 IU per milliliter by week 4.

All patients infected with genotype 2 or 3 had an undetectable HCV RNA level during the treatment period. One patient with genotype 3 infection who was treated without ribavirin had a detectable HCV RNA level of less than 25 IU per milliliter at weeks 8 and 10, which, per protocol, was defined as a virologic breakthrough (Table 2). However, before the initiation of rescue therapy at week 12, HCV RNA was undetectable; the patient had a sustained virologic response at 24 weeks after rescue therapy (Table S4 in the Supplementary Appendix). Overall, 91% of the patients infected with genotype 2 or 3 had a sustained virologic response 12 weeks after treatment and 93% had a sustained virologic response 24 weeks after treatment (Table 2). Rates of sustained virologic response 12 weeks after treatment were 92% among patients with genotype 2 infection (24 of 26 patients) and 89% among patients with genotype 3 infection (16 of 18).

None of the patients with genotype 1 infection had a virologic breakthrough, and all had an HCV RNA level of less than 25 IU per milliliter at the end of the treatment period (Table 2). After the treatment period, no patient had a virologic relapse. Overall, 164 of 167 patients with genotype 1 infection (98%) had a sustained virologic response at week 12 after treatment, including 84 of 85 patients who received treatment for 24 weeks (all 44 patients who had not received previous treatment and 40 of 41 patients who had received a protease inhibitor) and 80 of 82 patients who received treatment for 12 weeks. Of the 3 patients who were classified as not having a sustained virologic response 12 weeks after treatment, 2 missed the assessment visit at 12 weeks but had a sustained virologic response at week 24 after treatment, and 1 was lost to follow-up (Table 2, and Tables S2 and S4 in the Supplementary Appendix).

Rates of sustained virologic response 12 weeks after treatment were similar in subgroups defined according to viral subtype (genotype 1a, 98% [129 of 132 patients]; genotype 1b, 100% [35 of

Table 1. Baseline Demographic Characteristics of the Patients and Characteristics of the Disease.*

Characteristic	Genotype 2 or 3, Previously Untreated			Genotype 1, Previously Untreated					Genotype 1, Previously Treated	
	Group B: SOF for 7 days, then SOF and DCV for 23 wk (N=16)	Group D: DCV and SOF for 24 wk (N=14)	Group F: DCV and SOF plus RBV for 24 wk (N=14)	Group A: SOF for 7 days, then SOF and DCV for 23 wk (N=15)	Group C: DCV and SOF for 24 wk (N=14)	Group E: DCV and SOF plus RBV for 24 wk (N=15)	Group G: DCV and SOF for 12 wk (N=41)	Group H: DCV and SOF plus RBV for 12 wk (N=41)	Group I: DCV and SOF for 24 wk (N=21)	Group J: DCV and SOF plus RBV for 24 wk (N=20)
Median age — yr	51	50	52	56	54	54	55	54	59	57
Male sex — no. (%)	11 (69)	6 (43)	5 (36)	7 (47)	9 (64)	7 (47)	20 (49)	21 (51)	13 (62)	12 (60)
Race — no. (%)†										
White	16 (100)	10 (71)	12 (86)	11 (73)	11 (79)	12 (80)	33 (80)	33 (80)	19 (90)	18 (90)
Black	0	2 (14)	0	4 (27)	3 (21)	2 (13)	5 (12)	7 (17)	2 (10)	1 (5)
Other	0	2 (14)	2 (14)	0	0	1 (7)	3 (7)	1 (2)	0	1 (5)
HCV RNA — $\log_{10}$ IU/ml‡	6.5±0.7	6.8±0.5	6.6±0.6	6.5±0.5	6.6±0.3	6.7±0.6	6.2±0.5	6.4±0.6	6.3±0.4	6.3±0.4
HCV genotype — no. (%)§										
1a	0	0	0	11 (73)	10 (71)	11 (73)	34 (83)	33 (80)	16 (76)	17 (85)
1b	0	0	0	4 (27)	4 (29)	4 (27)	7 (17)	8 (20)	5 (24)	3 (15)
2	9 (56)	8 (57)	9 (64)	0	0	0	0	0	0	0
3	7 (44)	6 (43)	5 (36)	0	0	0	0	0	0	0
IL28B genotype CC — no. (%)	8 (50)	5 (36)	7 (50)	4 (27)	8 (57)	4 (27)	9 (22)	15 (37)	1 (5)	0
Metavir score for fibrosis — no. (%)¶										
F0 or F1: none or minimal	6 (38)	6 (43)	6 (43)	4 (27)	6 (43)	6 (40)	15 (37)	13 (32)	2 (10)	3 (15)
F2 or F3: moderate	7 (44)	7 (50)	6 (43)	8 (53)	7 (50)	6 (40)	19 (46)	22 (54)	14 (67)	11 (55)
F4: clinically significant	3 (19)	1 (7)	2 (14)	3 (20)	1 (7)	2 (13)	6 (15)	5 (12)	3 (14)	6 (30)

* Plus–minus values are means ±SD. Previously treated patients were those who had virologic failure during or after treatment with telaprevir or boceprevir. The doses of medication were as follows: daclatasvir (DCV), 60 mg once daily; sofosbuvir (SOF), 400 mg once daily; and ribavirin (RBV), administered twice daily, at a dose of 1000 to 1200 mg per day according to weight in patients with genotype 1 infection and 800 mg per day in patients infected with genotype 2 or 3. Percentages may not total 100 because of rounding. None of the characteristics differed significantly among the randomized groups. HCV denotes hepatitis C virus.

† Race was self-reported.

‡ The HCV RNA assay had a limit of detection 10 IU per milliliter.

§ Genotypes 2 and 3 are indicated for groups B, D, and F, and genotypes 1a and 1b are indicated for groups A, C, E, and G through J.

¶ The Metavir score (on a scale from F0 to F4, with higher scores indicating a greater degree of fibrosis) was derived from a FibroTest score and classified according to information on the FibroTest manufacturer's website (www.biopredictive.com). Patients with a score of F4 were required to have no evidence of cirrhosis on the basis of a liver biopsy. Data were not available for one patient in group E, one patient in group G, one patient in group H, and two patients in group I. For additional clinical findings according to the fibrosis score, see Table S1 in the Supplementary Appendix.

Table 2. Virologic Response during and after Treatment.

Virologic Response	Genotype 2 or 3, Previously Untreated			
	Group B: SOF for 7 days, then SOF and DCV for 23 wk (N=16)	Group D: DCV and SOF for 24 wk (N=14)	Group F: DCV and SOF plus RBV for 24 wk (N=14)	Total: Groups B, D, and F (N=44)
	number of study participants (percent)			
During treatment				
Week 2				
HCV RNA <25 IU/ml	13 (81)	12 (86)	12 (86)	37 (84)
HCV RNA undetectable	5 (31)	4 (29)	4 (29)	13 (30)
Week 4				
HCV RNA <25 IU/ml	16 (100)	14 (100)	14 (100)	44 (100)
HCV RNA undetectable	14 (88)	11 (79)	9 (64)	34 (77)
End of treatment‡				
HCV RNA <25 IU/ml	15 (94)§	14 (100)	14 (100)	43 (98)
HCV RNA undetectable	15 (94)§	13 (93)	14 (100)	42 (95)
After treatment				
Week 4				
HCV RNA <25 IU/ml	14 (88)¶	14 (100)	12 (86)‖**	40 (91)
HCV RNA undetectable	14 (88)¶	14 (100)	11 (79)‖**	39 (89)
Week 12				
HCV RNA <25 IU/ml	14 (88)	14 (100)	12 (86)**	40 (91)
HCV RNA undetectable	14 (88)	13 (93)	12 (86)**	39 (89)
Week 24				
HCV RNA <25 IU/ml	14 (88)	14 (100)	13 (93)	41 (93)
HCV RNA undetectable	14 (88)	14 (100)	13 (93)	41 (93)

* Data on HCV RNA levels were missing for two patients at week 4; both had an HCV RNA level of less than 25 IU per milliliter at week 3 and again at week 6.
† Data on the HCV RNA level were missing for one patient.
‡ The end-of-treatment analysis (week 24 for groups A through F and week 12 for groups G and H) included patients who discontinued treatment early, for whom the last HCV RNA measurement was considered "end of treatment."
§ One patient had a protocol-defined virologic breakthrough (detectable HCV RNA level of <25 IU/ml).
¶ One patient had a relapse.
‖ One patient was lost to follow-up.
** Data on the HCV RNA level were missing for one patient, who had a sustained virologic response at week 24 after the end of treatment.
†† Data on the HCV RNA level were missing for one patient, who had a sustained virologic response at week 12 after the end of treatment.
‡‡ One patient with an unconfirmed HCV RNA level of 54 IU per milliliter at week 4 after treatment had a sustained virologic response at week 12 after the end of treatment.
§§ Data on the HCV RNA level were missing for one patient, who had a sustained virologic response at week 24 after the end of treatment, and one patient was lost to follow-up.
¶¶ Data on the HCV RNA level were missing for one patient, who had a sustained virologic response at week 24 after the end of treatment.
‖‖ One patient probably had reinfection.
*** Data on the HCV RNA level were missing for two patients, both of whom had a sustained virologic response at week 36 after the end of treatment.
††† Data on the HCV RNA level were missing for two patients, both of whom had a sustained virologic response at week 36 after the end of treatment, and one patient was lost to follow-up.

Genotype 1, Previously Untreated						Genotype 1, Previously Treated	
Group A: SOF for 7 days, then SOF and DCV for 23 wk (N=15)	Group C: DCV and SOF for 24 wk (N=14)	Group E: DCV and SOF plus RBV for 24 wk (N=15)	Group G : DCV and SOF for 12 wk (N=41)	Group H: DCV and SOF plus RBV for 12 wk (N=41)	Total: Groups A, C, E, G, and H (N=126)	Group I: DCV and SOF for 24 wk (N=21)	Group J: DCV and SOF plus RBV for 24 wk (N=20)
number of study participants (percent)							
10 (67)	11 (79)	10 (67)	36 (88)	34 (83)	101 (80)	19 (90)	16 (80)
7 (47)	2 (14)	3 (20)	12 (29)	13 (32)	37 (29)	3 (14)	3 (15)
15 (100)	14 (100)	15 (100)	39 (95)*	41 (100)	124 (98)	21 (100)	19 (95)†
13 (87)	13 (93)	11 (73)	31 (76)*	32 (78)	100 (79)	17 (81)	14 (70)†
15 (100)	14 (100)	15 (100)	41 (100)	41 (100)	126 (100)	21 (100)	20 (100)
15 (100)	14 (100)	15 (100)	41 (100)	41 (100)	126 (100)	19 (90)	19 (95)
15 (100)	14 (100)	15 (100)	40 (98)††	39 (95)†††‡‡	123 (98)	21 (100)	20 (100)
15 (100)	14 (100)	15 (100)	40 (98)††	39 (95)†††‡‡	123 (98)	21 (100)	19 (95)
15 (100)	14 (100)	15 (100)	41 (100)	39 (95)§§	124 (98)	21 (100)	19 (95)¶¶
15 (100)	14 (100)	15 (100)	41 (100)	39 (95)§§	124 (98)	21 (100)	19 (95)¶¶
14 (93)‖‖	14 (100)	15 (100)	39 (95)***	38 (93)†††	120 (95)		
14 (93)‖‖	14 (100)	15 (100)	39 (95)***	38 (93)†††	120 (95)		

35 patients]), IL28B genotype (CC, 93% [57 of 61 patients]; non-CC, 98% [147 of 150 patients]), race (white, 97% [170 of 175 patients]; black, 96% [25 of 26 patients]; and other race, 90% [9 of 10 patients]), ribavirin status (ribavirin, 94% [85 of 90 patients]; no ribavirin, 98% [119 of 121 patients]), and history of treatment failure with protease inhibitors (98% [40 of 41 patients]).

Of 126 patients with previously untreated genotype 1 infection, 120 (95%) had a sustained virologic response at week 24 after treatment (Table 2). Of the 6 patients who were classified as not having a sustained virologic response at week 24 after treatment, 4 missed the assessment visit at week 24 but were classified as having a sustained virologic response at week 36 after treatment, and 1 was lost to follow-up. The remaining patient, whose history included injection-drug use, had a high level of viremia (HCV RNA level, 670,772 IU per milliliter), and viral sequences at week 24 after treatment that differed from the sequences at baseline suggested a new HCV infection. Furthermore, no daclatasvir-resistant or sofosbuvir-resistant variants were detected (Fig. S3 in the Supplementary Appendix).

VIROLOGIC BREAKTHROUGH, RELAPSE, AND RESISTANCE

Virologic relapse was confirmed in 1 patient with genotype 3 infection who received treatment without ribavirin; adherence to the treatment regimen was documented on the basis of pill counts, as well as plasma concentrations of daclatasvir and

major sofosbuvir metabolites that were consistent with those in other patients. Resistance analysis showed a preexisting NS5A-A30K polymorphism, associated with daclatasvir resistance, at baseline and at the time of relapse. No other resistance-associated changes were detected at the time of relapse. Of the available baseline samples, pretreatment polymorphisms, including NS5A-A30K and others known to confer loss of susceptibility to daclatasvir in vitro, were observed in 10 of 123 untreated patients with genotype 1 infection (8%), 3 of 40 patients with genotype 1 infection in whom prior treatment with protease inhibitors had failed (8%), 14 of 23 patients with genotype 2 infection (61%), and 5 of 18 patients with genotype 3 infection (28%) (Table S5 in the Supplementary Appendix). Except for the patient described above, all patients with preexisting daclatasvir resistance variants had a sustained virologic response. With respect to sofosbuvir, no preexisting NS5B-S282T polymorphisms were detected. In the only patient with protocol-defined virologic breakthrough, no baseline daclatasvir or sofosbuvir resistance-associated polymorphisms were detected, and the HCV RNA level at the time of virologic breakthrough was too low (<25 IU per milliliter) for resistance testing; the patient had a sustained virologic response after rescue therapy.

SAFETY

The most common adverse events were fatigue, headache, and nausea (Table 3, and Table S6 in the Supplementary Appendix). Two patients discontinued treatment because of adverse events (fibromyalgia in one patient and a stroke in one patient); both had a sustained virologic response (Table S7 in the Supplementary Appendix). Serious adverse events during the treatment period (Table 3, and Table S8 in the Supplementary Appendix) included single events of gastroenteritis, colitis, stroke, acute renal failure from dehydration that resolved with administration of fluids, forearm fracture, anxiety and pleuritic pain, exacerbation of psoriasis, and hypokalemia. The most common grade 3 or 4 laboratory abnormalities were low phosphorus and elevated glucose levels. The mean change in the hemoglobin level associated with regimens that contained ribavirin versus those that did not contain ribavirin was –2.2 g per deciliter versus –0.3 g per deciliter after 24 weeks of therapy and –2.8 g per deciliter versus –0.9 g per deciliter after 12 weeks of therapy. The ribavirin dose was reduced in five patients because of anemia. Additional safety findings are listed in Tables S6, S7, and S8 in the Supplementary Appendix.

DISCUSSION

We assessed daclatasvir plus sofosbuvir in untreated patients and patients in whom previous treatment with telaprevir or boceprevir had failed. Overall, most patients had a sustained virologic response, including 98% of patients with genotype 1 infection, regardless of viral subtype or failure of prior treatment with protease inhibitors, and 91% of patients infected with genotype 2 or 3. The most common adverse event was fatigue, which was reported in approximately one third of patients. Our study shows that the combination of an NS5A inhibitor and an NS5B inhibitor was associated with high cure rates in a range of HCV-infected patients, including patients who had persistent HCV variants conferring resistance to protease inhibitors after unsuccessful treatment with telaprevir or boceprevir.

Daclatasvir plus sofosbuvir was associated with high rates of sustained virologic response among patients with characteristics that were previously associated with a poor response to treatment — HCV genotypes 1a and 3, the non-CC IL28B genotype, and black race. Studies evaluating peginterferon alfa–ribavirin plus a single direct-acting antiviral agent[11,12,26,27] or different combinations of direct-acting antiviral agents[28-30] have shown that patients with genotype 1a infection have a worse response than patients with genotype 1b infection; indeed, some oral regimens are effective primarily in patients with genotype 1b infection. In our study, most of the patients had genotype 1a infection, and the rates of response were high in both these patients and those with genotype 1b infection. Recent evidence suggests that HCV genotype 3 may also be less responsive to treatment than other genotypes.[21,31] In our study, 16 of 18 patients with genotype 3 infection had a sustained virologic response at week 12 after treatment. With regard to host factors, lower response rates have been observed with non-CC IL28B genotypes than with the CC genotype in studies of peginterferon alfa–ribavirin with or without telaprevir or boceprevir[11,26,32]

Table 3. Adverse Events during the Treatment Period.

Adverse Event	Previously Untreated					Previously Treated	
	Treatment for 24 Wk			Treatment for 12 Wk		Treatment for 24 Wk	
	Groups A and B: Lead-in SOF and DCV (N=31)	*Groups C and D: DCV and SOF (N=28)*	*Groups E and F: DCV and SOF and RBV (N=29)*	*Group G: DCV and SOF (N=41)*	*Group H: DCV and SOF and RBV (N=41)*	*Group I: DCV and SOF (N=21)*	*Group J: DCV and SOF and RBV (N=20)*
	number of study participants (percent)						
Any adverse event	25 (81)	26 (93)	26 (90)	38 (93)	38 (93)	16 (76)	20 (100)
Adverse event occurring in ≥25% of patients in any group*							
Fatigue	9 (29)	14 (50)	9 (31)	16 (39)	15 (37)	6 (29)	9 (45)
Headache	5 (16)	8 (29)	11 (38)	14 (34)	9 (22)	7 (33)	7 (35)
Nausea	5 (16)	9 (32)	9 (31)	8 (20)	8 (20)	0	2 (10)
Grade 3 or 4 adverse event	0	2 (7)†	2 (7)	1 (2)	1 (2)	0	1 (5)
Discontinuation of treatment due to adverse event‡	0	1 (4)	1 (3)	0	0	0	0
Serious adverse event§	2 (6)	4 (14)	2 (7)	1 (2)	0	0	1 (5)
Grade 3 or 4 laboratory abnormality occurring in ≥3 patients across all groups							
Phosphorus <2.0 mg/dl	0	1 (4)	1 (3)	0	3 (7)	0	0
Glucose							
Fasting value >250 mg/dl	0	1 (4)	1 (3)	1 (2)	0	1 (5)	0
Random value >250 mg/dl	0	0	1 (5)¶	0	0	1 (5)	1 (5)

* All events listed were mild or moderate in intensity; further details are in Table S6 in the Supplementary Appendix. To convert values for glucose to millimoles per liter, multiply by 0.05551.
† Two patients had a total of four events.
‡ Further details are in Table S7 in the Supplementary Appendix.
§ Further details are in Table S8 in the Supplementary Appendix. Five events of overdose (extra study medication doses), classified as serious adverse events, are not included in the table; no clinically significant effects were reported from any of the overdoses.
¶ This percentage was calculated according to the number of available samples (21).

and in studies of some direct-acting antiviral combinations.[29,30] Most patients in our study had non-CC IL28B genotypes, and the rate of response was high among these patients.

Patients with HCV genotype 1 infection who have virologic failure during telaprevir-based or boceprevir-based therapy despite acceptable adherence are frequently infected with HCV genotype 1a, and they often have a poor response to interferon, as indicated by a non-CC IL28B genotype.[13,26] In our study, patients in whom prior treatment with protease inhibitors had failed were characterized according to prior virologic response: 71% had virologic breakthrough or nonresponse, indicating poor interferon responsiveness, HCV resistance, or both (Table S2 in the Supplementary Appendix). Furthermore, most of these patients were infected with subtype 1a (80%), had a non-CC IL28B genotype (98%), and had evidence of at least moderate hepatic fibrosis (score ≥2) at baseline (83%) (Table 1). Despite these characteristics, early HCV RNA suppression was similar in previously treated and untreated patients (Fig. S2 in the Supplementary Appendix), and all patients in whom prior treatment with protease inhibitors had failed had a sustained virologic response. This represents proof of concept that a sustained virologic response can be achieved in patients in whom previous treatment with telaprevir or boceprevir had failed, including patients who have persistent HCV variants with resistance to protease inhibitors (Fig. S4 in the Supplementary Appendix).

Virologic breakthrough and relapse were rare

in our population and were not observed in any of the 193 patients infected with HCV genotype 1 or 2, despite preexisting daclatasvir-resistant variants in 27 patients. Of the 5 patients infected with HCV genotype 1 or 2 who were classified as not having a sustained virologic response at week 12 after treatment, 3 had missing data at week 12 but had a sustained virologic response at week 24 after treatment (including 1 who returned after the database lock) and 2 were lost to follow-up. Among the 18 patients with HCV genotype 3 infection, virologic relapse occurred in 1 of 5 patients with a preexisting daclatasvir-resistant variant, and in a second patient, who did not have preexisting daclatasvir-resistant variants, an HCV RNA level below 25 IU per milliliter was detected at weeks 8 and 10. Although HCV RNA was undetectable at week 12, this response pattern was predefined in the original protocol as virologic breakthrough, and rescue therapy was initiated. Because of low virus levels during the treatment period and a sustained virologic response at week 12 after treatment, we could not assess the role of viral variants in this patient. Sofosbuvir-resistant variants were not detected in any of the patients. Our observations suggest that the development of resistance is uncommon with daclatasvir plus sofosbuvir.

Many HCV treatment regimens have been assessed with and without ribavirin, and the response rates have been lower in the absence of ribavirin. This has been observed with peginterferon alfa–ribavirin plus a protease inhibitor[33] and with interferon-free regimens, including sofosbuvir with or without ribavirin in patients infected with genotype 2 or 3.[23,29,34] In our study, response rates were similar among patients treated with ribavirin and those treated without it; however, ribavirin recipients had a greater decrease in the hemoglobin level. Our findings may reflect the antiviral potency and high resistance barrier of the daclatasvir–sofosbuvir combination and suggest that ribavirin is not required with every oral direct-acting antiviral regimen. Ribavirin requires twice-daily dosing, is associated with hemolytic anemia, and is highly teratogenic. Ribavirin-sparing regimens are therefore desirable and warrant further investigation.

In conclusion, once-daily, oral treatment with the NS5A inhibitor daclatasvir plus the NS5B polymerase inhibitor sofosbuvir was associated with high rates of sustained virologic response in untreated patients infected with genotype 1, 2, or 3 and in patients with genotype 1 infection in whom previous treatment with protease inhibitors had failed and who had no current treatment options. The response rate was high across subgroups of patients defined according to IL28B genotype, HCV genotype 1 subtype, receipt of ribavirin, and the presence of HCV protease inhibitor–resistant variants.

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REFERENCES

1. World Health Organization. Global alert and response (GAR). Hepatitis C. 2012 (http://www.who.int/csr/disease/hepatitis/whocdscsrlyo2003/en/index4.html).
2. Perz JF, Armstrong GL, Farrington LA, Hutin YJ, Bell BP. The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. J Hepatol 2006;45:529-38.
3. Kanwal F, Hoang T, Kramer JR, et al. Increasing prevalence of HCC and cirrhosis in patients with chronic hepatitis C virus infection. Gastroenterology 2011;140(4):1182.e1-1188.e1.
4. Deuffic-Burban S, Poynard T, Sulkowski MS, Wong JB. Estimating the future health burden of chronic hepatitis C and human immunodeficiency virus infections in the United States. J Viral Hepat 2007;14:107-15.
5. Negro F, Alberti A. The global health burden of hepatitis C virus infection. Liver Int 2011;31:Suppl 2:1-3.
6. Ghany MG, Strader DB, Thomas DL, Seeff LB, American Association for the Study of Liver Diseases. Diagnosis, management, and treatment of hepatitis C: an update. Hepatology 2009;49:1335-74.
7. Cornberg M, Razavi HA, Alberti A, et al. A systematic review of hepatitis C virus epidemiology in Europe, Canada and Israel. Liver Int 2011;31:Suppl 2:30-60.
8. Sievert W, Altraif I, Razavi HA, et al. A systematic review of hepatitis C virus epidemiology in Asia, Australia and Egypt. Liver Int 2011;31:Suppl 2:61-80.
9. McHutchison JG, Lawitz EJ, Shiffman ML, et al. Peginterferon alfa-2b or alfa-2a with ribavirin for treatment of hepatitis C infection. N Engl J Med 2009;361:580-93. [Erratum, N Engl J Med 2009;361:1027.]
10. Hadziyannis SJ, Sette H Jr, Morgan TR, et al. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. Ann Intern Med 2004;140:346-55.
11. Jacobson IM, McHutchison JG, Dusheiko G, et al. Telaprevir for previously untreated chronic hepatitis C virus infection. N Engl J Med 2011;364:2405-16.
12. Poordad F, McCone J Jr, Bacon BR, et al. Boceprevir for untreated chronic HCV genotype 1 infection. N Engl J Med 2011;364:1195-206.
13. Zeuzem S, Andreone P, Pol S, et al.

Telaprevir for retreatment of HCV infection. N Engl J Med 2011;364:2417-28.
14. Bronowicki JP, Davis M, Flamm S, et al. Sustained virologic response (SVR) in prior peginterferon/ribavirin (PR) treatment failures after retreatment with boceprevir (BOC) + PR: the PROVIDE study interim results. J Hepatol 2012;56:Suppl 2:S6. abstract.
15. Gao M, Nettles RE, Belema M, et al. Chemical genetics strategy identifies an HCV NS5A inhibitor with a potent clinical effect. Nature 2010;465:96-100.
16. Sofia MJ, Bao D, Chang W, et al. Discovery of a β-d-2′-deoxy-2′-α-fluoro-2′-β-C-methyluridine nucleotide prodrug (PSI-7977) for the treatment of hepatitis C virus. J Med Chem 2010;53:7202-18.
17. Lam AM, Espiritu C, Bansal S, et al. Genotype and subtype profiling of PSI-7977 as a nucleotide inhibitor of hepatitis C virus. Antimicrob Agents Chemother 2012;56:3359-68.
18. Nettles RE, Gao M, Bifano M, et al. Multiple ascending dose study of BMS-790052, a nonstructural protein 5A replication complex inhibitor, in patients infected with hepatitis C virus genotype 1. Hepatology 2011;54:1956-65.
19. Pol S, Ghalib RH, Rustgi VK, et al. Daclatasvir for previously untreated chronic hepatitis C genotype-1 infection: a randomised, parallel-group, double-blind, placebo-controlled, dose-finding, phase 2a trial. Lancet Infect Dis 2012;12:671-7.
20. Dore GJ, Lawitz E, Hezode C, et al. Twelve- or 16-week treatment with daclatasvir combined with peginterferon alfa and ribavirin for hepatitis C virus genotype 2 or 3 infection: command GT2/3 study. Hepatology 2012;56:Suppl 4:558A-559A. abstract.
21. Lawitz E, Mangia A, Wyles D, et al. Sofosbuvir for previously untreated chronic hepatitis C infection. N Engl J Med 2013;368:1878-87.
22. Lawitz E, Lalezari JP, Hassanein T, et al. Sofosbuvir in combination with peginterferon alfa-2a and ribavirin for non-cirrhotic, treatment-naive patients with genotypes 1, 2, and 3 hepatitis C infection: a randomised, double-blind, phase 2 trial. Lancet Infect Dis 2013;13:401-8.
23. Gane EJ, Stedman CA, Hyland RH, et al. Nucleotide polymerase inhibitor sofosbuvir plus ribavirin for hepatitis C. N Engl J Med 2013;368:34-44.
24. Wai CT, Greenson JK, Fontana RJ, et al. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. Hepatology 2003;38:518-26.
25. Imbert-Bismut F, Ratziu V, Pieroni L, et al. Biochemical markers of liver fibrosis in patients with hepatitis C virus infection: a prospective study. Lancet 2001;357:1069-75.
26. Poordad F, Bronowicki JP, Gordon SC, et al. Factors that predict response of patients with hepatitis C virus infection to boceprevir. Gastroenterology 2012;143(3):608.e5-618.e5.
27. Hezode C, Hirschfield GM, Ghesquiere W, et al. Daclatasvir, an NS5A replication complex inhibitor, combined with peginterferon alfa-2a and ribavirin in treatment-naive HCV-genotype 1 or 4 subjects: phase 2b COMMAND-1 SVR12 results. Hepatology 2012;56:Suppl:553A-554A. abstract.
28. Lok AS, Gardiner DF, Lawitz E, et al. Preliminary study of two antiviral agents for hepatitis C genotype 1. N Engl J Med 2012;366:216-24.
29. Zeuzem S, Soriano V, Asselah T, et al. Interferon (IFN)-free combination treatment with the HCV NS3/4A protease inhibitor BI 201335 and the non-nucleoside NS5B inhibitor BI 207127 ± ribavirin (R): final results of SOUND-C2 and predictors of response. Hepatology 2012;56:Suppl 4:308A-309A. abstract.
30. Sulkowski M, Rodriguez-Torres M, Lawitz E, et al. High sustained virologic response rate in treatment-naive HCV genotype 1a and 1b patients treated for 12 weeks with an interferon-free all-oral QUAD regimen: interim results. J Hepatol 2012;56:Suppl 2:S560. abstract.
31. Jacobson IM, Gordon SC, Kowdley KV, et al. Sofosbuvir for hepatitis C genotype 2 or 3 in patients without treatment options. N Engl J Med 2013;368:1867-77.
32. Ge D, Fellay J, Thompson AJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature 2009;461:399-401.
33. Hézode C, Forestier N, Dusheiko G, et al. Telaprevir and peginterferon with or without ribavirin for chronic HCV infection. N Engl J Med 2009;360:1839-50.
34. Drenth JPH. HCV treatment — no more room for interferonologists? N Engl J Med 2013;368:1931-2.