

ORIGINAL ARTICLE

Preliminary Study of Two Antiviral Agents for Hepatitis C Genotype 1

Anna S. Lok, M.D., David F. Gardiner, M.D., Eric Lawitz, M.D., Claudia Martorell, M.D., Gregory T. Everson, M.D., Reem Ghalib, M.D., Robert Reindollar, M.D., Vinod Rustgi, M.D., Fiona McPhee, Ph.D., Megan Wind-Rotolo, Ph.D., Anna Persson, Ph.D., Kurt Zhu, Ph.D., Dessislava I. Dimitrova, M.D., Timothy Eley, Ph.D., Tong Guo, Ph.D., Dennis M. Grasela, Pharm.D., Ph.D., and Claudio Pasquinelli, M.D., Ph.D.

ABSTRACT

BACKGROUND

From the University of Michigan, Ann Arbor (A.S.L.); Bristol-Myers Squibb Research and Development, Hopewell, NJ (D.F.G., K.Z., D.I.D., T.E., D.M.G., C.P.), Wallingford, CT (F.M., T.G.), and Princeton, NJ (M.W.-R., A.P.); Alamo Medical Research, San Antonio, TX (E.L.); Research Institute, Springfield, MA (C.M.); University of Colorado—Denver, Aurora (G.T.E.); Texas Clinical Research Institute, Arlington (R.G.); Carolinas Center for Liver Disease, Statesville, NC (R.R.); and Metropolitan Research, Fairfax, VA (V.R.). Address reprint requests to Dr. Lok at the Department of Internal Medicine, Division of Gastroenterology, 3912 Taubman Center, SPC 5362, University of Michigan Medical Center, Ann Arbor, MI 48109, or at aslok@med.umich.edu.

Patients with chronic hepatitis C virus (HCV) infection who have not had a response to therapy with peginterferon and ribavirin may benefit from the addition of multiple direct-acting antiviral agents to their treatment regimen.

METHODS

This open-label, phase 2a study included an exploratory cohort of 21 patients with chronic HCV genotype 1 infection who had not had a response to previous therapy (i.e., had not had $\geq 2 \log_{10}$ decline in HCV RNA after ≥ 12 weeks of treatment with peginterferon and ribavirin). We randomly assigned patients to receive the NS5A replication complex inhibitor daclatasvir (60 mg once daily) and the NS3 protease inhibitor asunaprevir (600 mg twice daily) alone (group A, 11 patients) or in combination with peginterferon alfa-2a and ribavirin (group B, 10 patients) for 24 weeks. The primary end point was the percentage of patients with a sustained virologic response 12 weeks after the end of the treatment period.

RESULTS

A total of 4 patients in group A (36%; 2 of 9 with HCV genotype 1a and 2 of 2 with genotype 1b) had a sustained virologic response at 12 weeks after treatment and also at 24 weeks after treatment. Six patients (all with HCV genotype 1a) had viral breakthrough while receiving therapy, and resistance mutations to both antiviral agents were found in all cases; 1 patient had a viral response at the end of treatment but had a relapse after the treatment period. All 10 patients in group B had a sustained virologic response at 12 weeks after treatment, and 9 had a sustained virologic response at 24 weeks after treatment. Diarrhea was the most common adverse event in both groups. Six patients had transient elevations of alanine aminotransferase levels to more than 3 times the upper limit of the normal range.

CONCLUSIONS

This preliminary study involving patients with HCV genotype 1 infection who had not had a response to prior therapy showed that a sustained virologic response can be achieved with two direct-acting antiviral agents only. In addition, a high rate of sustained virologic response was achieved when the two direct-acting antiviral agents were combined with peginterferon alfa-2a and ribavirin. (Funded by Bristol-Myers Squibb; ClinicalTrials.gov number, NCT01012895.)

N Engl J Med 2012;366:216-24.
Copyright © 2012 Massachusetts Medical Society.

APPROXIMATELY 180 MILLION PEOPLE worldwide are infected with hepatitis C virus (HCV), including 4.1 million in the United States.^{1,2} HCV infection is the most common cause of chronic liver disease in the United States and a leading cause of cirrhosis and hepatocellular carcinoma globally.^{3,4} HCV is classified into six major genotypes; genotype 1 is predominant in the United States and is the most difficult to treat.^{5,6}

Treatment of HCV genotype 1 infection with peginterferon alfa and ribavirin for 48 weeks results in 24-week sustained virologic response (undetectable HCV RNA 24 weeks after the end of therapy) in 40 to 50% of patients who have not received previous treatment.⁷⁻¹⁰ Patients are considered as not having had a response if they do not have a reduction in HCV RNA levels by at least 2 log₁₀ IU per milliliter after a minimum of 12 weeks of treatment with peginterferon and ribavirin.⁶ Retreatment with triple therapy — a protease inhibitor, peginterferon, and ribavirin — yields rates of sustained virologic response of only 14 to 33%.^{11,12} Gane and colleagues found that treatment for 13 days with a combination of two direct-acting antiviral agents without peginterferon or ribavirin can suppress HCV RNA levels in patients who have not had a response to previous treatment with peginterferon and ribavirin.¹³ These results suggest that additional studies of combinations of direct-acting antiviral agents are warranted.

Daclatasvir is a first-in-class, highly selective HCV NS5A replication complex inhibitor with picomolar potency *in vitro*.¹⁴ Asunaprevir is a highly active HCV NS3 protease inhibitor.¹⁵ Both daclatasvir and asunaprevir produce robust declines in HCV RNA levels in patients with HCV genotype 1 infection,^{16,17} and administration of the two drugs in combination did not produce a clinically meaningful pharmacokinetic interaction.¹⁸ In this study, daclatasvir and asunaprevir were administered in combination, with or without peginterferon alfa-2a and ribavirin, for 24 weeks in patients with HCV genotype 1 infection who had not had a response to previous treatment with peginterferon and ribavirin. We report preliminary data from the exploratory cohort, including the percentages of patients with sustained virologic response at 12, 24, and 48 weeks after the end of the treatment period.

METHODS

PATIENTS

We screened 56 patients and enrolled 21 of them in an exploratory cohort to assess safety and antiviral activity. Patients were enrolled at seven centers in the United States during the period from December 2009 through February 2010. Eligible patients were men and women, 18 to 70 years of age, who had chronic HCV genotype 1 infection, an HCV RNA level of 10⁵ IU per milliliter or higher, and no evidence of cirrhosis, as documented by means of either liver biopsy or assessment of serum markers of cirrhosis (a FibroTest score of ≤0.72, on a scale of 0 to 1, with higher scores indicating more severe fibrosis, and a score on the aspartate aminotransferase:platelet ratio index [APRI] of ≤2, with higher scores indicating a greater likelihood of extensive fibrosis).¹⁹⁻²¹ Patients were required to have had no response to previous HCV therapy, as defined previously. Written informed consent was obtained from all patients.

STUDY OVERSIGHT

The study was approved by the institutional review board at each participating site and was conducted in compliance with the Declaration of Helsinki, Good Clinical Practice guidelines, and local regulatory requirements. The study was designed and conducted by the sponsor (Bristol-Myers Squibb) in collaboration with the principal investigators. The sponsor collected the data, monitored the study conduct, and performed the statistical analyses. The first draft of the manuscript was prepared by the principal academic and sponsor authors, with assistance from a medical writer paid by Bristol-Myers Squibb and with input from all the other authors. The principal academic and sponsor authors reviewed and edited every version of the manuscript. All the authors reviewed major changes to the drafts and approved the final manuscript. All the authors had access to the data and assume responsibility for the veracity and completeness of the reported data and for the fidelity of the study to the protocol. The study protocol, including the statistical analysis plan, is available with the full text of this article at NEJM.org.

STUDY DESIGN

This was an open-label, proof-of-concept, phase 2a study. Eligible patients were randomly assigned,

in a 1:1 ratio, to receive daclatasvir and asunaprevir for 24 weeks (group A) or to receive daclatasvir, asunaprevir, peginterferon alfa-2a (Pegasys, Roche), and ribavirin (Copegus, Roche) for 24 weeks (group B) (see Fig. 1 in the Supplementary Appendix, available at NEJM.org). Daclatasvir was administered orally at a dose of 60 mg once daily, and asunaprevir was administered orally at a dose of 600 mg twice daily. No dose reductions of daclatasvir or asunaprevir were permitted. Patients in group B also received 180 μ g per week of peginterferon alfa-2a, administered subcutaneously, and ribavirin, administered orally twice daily, with doses determined according to body weight (1000 mg daily in patients with body weight of <75 kg, and 1200 mg daily in patients with body weight of \geq 75 kg). Randomization was stratified according to HCV subtype (1a or 1b), with no more than two patients with genotype 1b enrolled in each treatment group. Patients in group A were eligible to have peginterferon alfa-2a and ribavirin added to their regimens for up to 48 weeks in the event of viral breakthrough.

The IL28B genotype was assayed with the use of a TaqMan genotyping assay (Applied Biosystems) for the rs12979860 single-nucleotide polymorphism (SNP).

EFFICACY ASSESSMENTS

Plasma HCV RNA levels were measured with the use of the COBAS TaqMan HCV test, version 2.0 (Roche), with a lower limit of quantification of 25 IU per milliliter and a lower limit of detection of 10 IU per milliliter. HCV RNA assays were performed at baseline and on days 1 through 7, 9, 11, and 14; at week 3; and every 2 weeks from week 4 through week 12. Between weeks 12 and 24, HCV RNA levels were measured every 4 weeks in the patients in group B and every 2 weeks in the patients in group A who did not have viral breakthrough. In the patients in group A who had viral breakthrough, HCV RNA levels were measured every 4 weeks after the addition of peginterferon and ribavirin. In patients from both groups who completed 24 weeks of treatment, HCV RNA was measured at weeks 4 and 12 after the end of the treatment period and every 12 weeks thereafter until week 48. All patients who did not have viral breakthrough have completed 48 weeks of post-treatment follow-up.

SAFETY ASSESSMENTS

Adverse events and results of clinical laboratory tests were recorded throughout the treatment and follow-up periods. Physical examinations and electrocardiographic studies were performed at the time of screening, at baseline, at weeks 1, 2, and 4, and every 4 weeks thereafter until the end of the treatment period.

VIRAL BREAKTHROUGH, RELAPSE, AND RESISTANCE MONITORING

Viral breakthrough during the treatment period was defined as an increase from the nadir in HCV RNA of at least 1 log₁₀ IU per milliliter or an HCV RNA level of 25 IU per milliliter or more in patients who had had undetectable HCV RNA levels at or after week 4. Viral relapse was defined as confirmed detectable HCV RNA levels during the post-treatment follow-up period in patients who had had undetectable HCV RNA levels at the end of treatment. All samples that were obtained during or after the treatment period from patients with viral breakthrough or viral relapse were analyzed for resistance genotypes. The HCV NS3 protease domain and the NS5A domain were analyzed by means of population sequencing and clonal analysis.

END POINTS

The primary efficacy end point was the proportion of patients with undetectable HCV RNA (HCV RNA levels of <10 IU per milliliter) 12 weeks after the completion of the study treatment. Secondary efficacy end points included the proportions of patients with undetectable HCV RNA at weeks 4, 12, and 24 during the treatment period and at weeks 24 and 48 after the end of the treatment period. Safety end points included the frequencies of adverse events, serious adverse events, discontinuations due to adverse events, and changes in clinical laboratory-test values.

STATISTICAL ANALYSIS

For binary end points related to antiviral activity, including the primary efficacy end point, the analyses included all patients who received at least one dose of study medication (modified intention-to-treat population). The numerator comprised patients who received treatment and who met the criteria for response, with patients in group A who

had peginterferon and ribavirin added to their regimens as rescue therapy classified as having had treatment failure. Selected antiviral-activity end points were also summarized according to HCV subtype (1a or 1b). No adjustments have been made for multiple testing.

RESULTS

PATIENTS

A total of 21 patients were enrolled (11 in group A and 10 in group B) (Fig. 1 in the Supplementary Appendix). The baseline characteristics of the treatment groups are shown in Table 1. As expected in the case of a cohort of patients who did not have a response to prior treatment with peginterferon and ribavirin, genotyping of the IL28B rs12979860 SNP showed that 19 patients had a CT or TT genotype.

VIROLOGIC RESPONSE

Group A

HCV RNA levels in the patients in group A declined rapidly after the initiation of treatment (Fig. 1A). At week 2, the median decrease in HCV RNA from baseline was 5.1 log₁₀ IU per milliliter, and seven patients (64%) had undetectable HCV RNA at week 4 (rapid virologic response). Four patients (36%) had a sustained virologic response at 12 weeks after the end of the treatment period, and all four also had a sustained virologic response at week 24 after treatment (Table 2). Three of these patients also had a sustained virologic response at week 48 after treatment; the fourth had HCV RNA of less than 25 IU per milliliter at week 48 after treatment and undetectable HCV RNA 43 days later.

Viral relapse occurred in one patient (with HCV genotype 1a) at week 4 after treatment. This patient had had undetectable HCV RNA at week 4 during the treatment period, and the levels had remained undetectable through week 24 (end of treatment). An analysis of baseline samples revealed a preexisting variant conferring resistance to asunaprevir (see below). Viral breakthrough occurred in six patients (55%), all with HCV genotype 1a, as early as week 3 and as late as week 12. All six patients had peginterferon and ribavirin added to their regimen (rescue therapy), resulting in initial reductions in HCV RNA in all

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

Characteristic	Group A (N = 11)	Group B (N = 10)
Age — yr		
Median	54.0	56.5
Range	36–61	38–63
Male sex — no. (%)	9 (82)	4 (40)
Race — no. (%)†		
White	9 (82)	7 (70)
Black	2 (18)	3 (30)
HCV genotype — no. (%)		
1a	9 (82)	9 (90)
1b	2 (18)	1 (10)
IL28B rs12979860 genotype — no. (%)		
CT or TT	10 (91)	9 (90)
CC	1 (9)	1 (10)
HCV RNA log ₁₀ — IU/ml	6.8±0.6	6.6±0.8
HCV RNA distribution — no. (%)		
<800,000	0	2 (20)
≥800,000	11 (100)	8 (80)
Baseline ALT — U/liter	70.5±57.65	57.9±29.94

* Plus-minus values are means ±SD. Group A included patients who received the NS5A replication complex inhibitor daclatasvir and the NS3 protease inhibitor asunaprevir alone for 24 weeks. Group B included patients who received daclatasvir and asunaprevir in combination with peginterferon alfa-2a and ribavirin for 24 weeks. ALT denotes alanine aminotransferase, and HCV hepatitis C virus.

† Race was self-reported.

cases (Fig. 1B). Peak HCV RNA levels at the initiation of rescue therapy ranged from 6.5×10² to 3.2×10⁶ IU per milliliter. In four of these six patients, HCV RNA became undetectable while they were receiving rescue therapy; two of them had a relapse after the treatment period, whereas HCV RNA remained undetectable in the other two at the most recent testing, one at 14 weeks and one at 42 weeks after treatment. The remaining two patients never achieved undetectable HCV RNA, and treatment was discontinued.

Group B

HCV RNA levels declined rapidly and remained suppressed throughout the study in all the patients in group B (Fig. 1C). At week 2, the median decrease from baseline in HCV RNA was 5.3

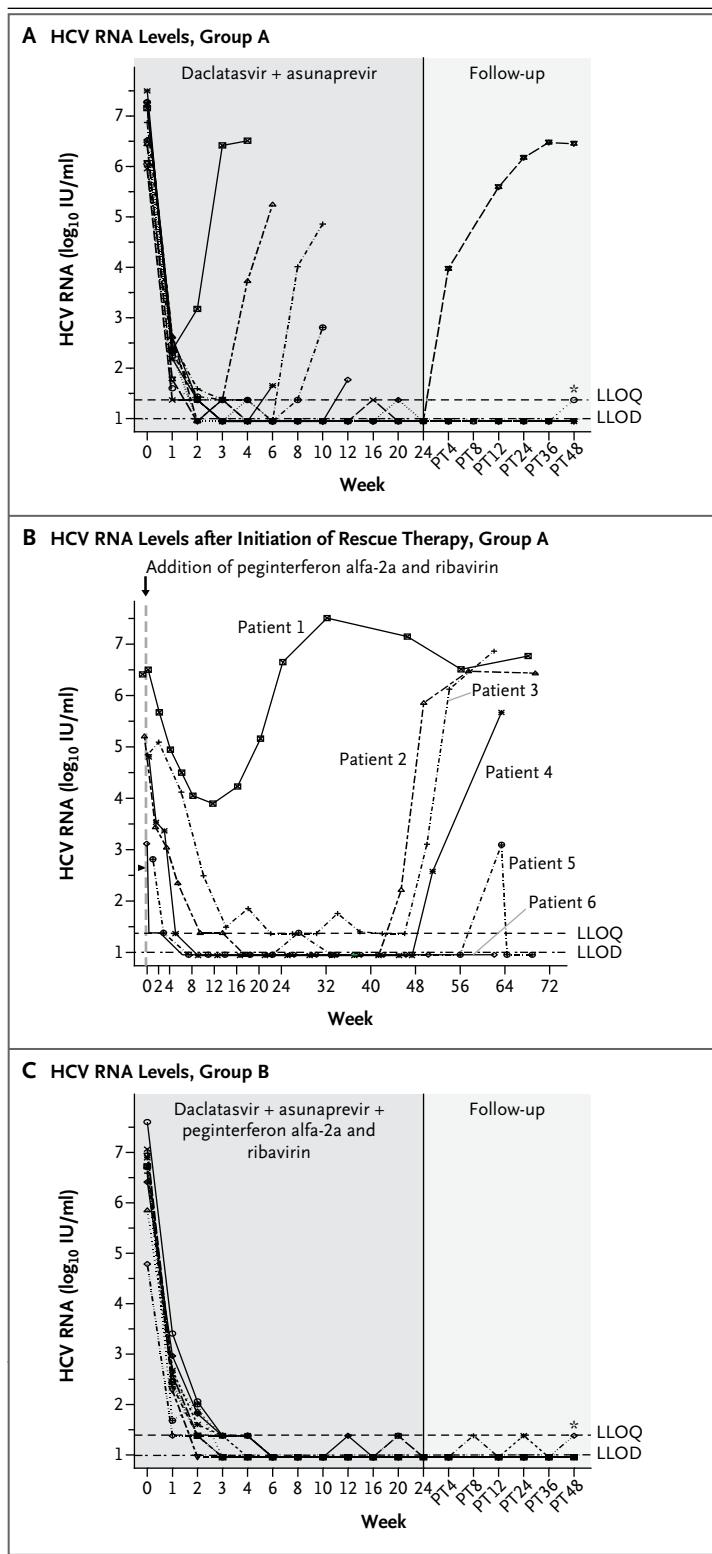


Figure 1. HCV RNA Levels in Groups A and B.

Panel A shows HCV RNA levels over time in the patients in group A, who received the NS5A replication complex inhibitor daclatasvir and the NS3 protease inhibitor asunaprevir alone for 24 weeks. For patients who had viral breakthrough, HCV RNA levels are shown up to the time rescue therapy was initiated. In one patient, HCV RNA levels were less than the lower limit of quantification (LLOQ; 25 IU per milliliter) at post-treatment (PT) week 48 (asterisk) and were undetectable on retesting 43 days later. Panel B shows HCV RNA levels after the initiation of rescue therapy (peginterferon alfa-2a and ribavirin added to direct-acting antiviral therapy) in the six patients in group A who had viral breakthrough (the same symbols are used in Panels A and B). HCV RNA levels are shown from the initiation of rescue therapy (day 0) until the time the database was locked. Patients received rescue therapy for up to 48 weeks, after which all treatment was discontinued. Panel C shows HCV RNA levels in the patients in group B, who received daclatasvir and asunaprevir in combination with peginterferon alfa-2a and ribavirin for 24 weeks. In one patient, HCV RNA levels were less than the LLOQ at week 48 after treatment (asterisk) and were undetectable on retesting 13 days later. The lower limit of detection (LLOD) of HCV RNA was less than 10 IU per milliliter.

had viral breakthrough. Several patients had transiently detectable HCV RNA at levels below the limit of quantification (<25 IU per milliliter) during or after therapy (Fig. 1C). All 10 patients had a sustained virologic response at week 12 after treatment and all but 1 had a sustained virologic response at week 24 after treatment (Table 2, and Fig. 1 in the Supplementary Appendix). One patient had transiently detectable HCV RNA (<25 IU per milliliter) at week 8 after treatment; HCV RNA was undetectable at week 12 after treatment (Fig. 1C). Another patient had transiently detectable HCV RNA (<25 IU per milliliter) at week 24 after treatment, and undetectable HCV RNA when levels were measured again 35 days later. Nine of the 10 patients had a sustained virologic response at week 48 after treatment; 1 patient had HCV RNA of less than 25 IU per milliliter at week 48 after treatment and undetectable HCV RNA 13 days later.

RESISTANCE

An analysis of samples from the six patients in group A with viral breakthrough showed that there were no resistance variants at baseline and that resistance variants to both daclatasvir and asunaprevir had emerged in all cases by the time of viral breakthrough. Viral variants in the NS5A domain included Q30R, L31 M/V, and Y93C/N; variants in the NS3 protease domain included

\log_{10} IU per milliliter, and 6 patients (60%) had a rapid virologic response. All 10 patients had undetectable HCV RNA by week 6, and no patient

R155K and D168A/E/T/V/Y. The patient with viral relapse had a preexisting NS3 protease resistance variant, R155K, at baseline and at the time of viral relapse, whereas the NS5A resistance variant Q30E was detected only at relapse.

SAFETY

All the patients completed at least 24 weeks of therapy. The most common adverse events were diarrhea, fatigue, headache, and nausea (Table 3). Most adverse events were mild to moderate. There were no deaths, serious adverse events, or discontinuations of study treatment due to adverse events during the analysis period. One serious adverse event of hospitalization for alcohol abuse was reported 1 month after the completion of rescue therapy. Grade 3 or 4 adverse events and laboratory-test abnormalities included fatigue (one patient in group A), neutropenia (six patients, all of whom were receiving peginterferon alfa-2a and ribavirin), and elevations of alanine aminotransferase levels (two patients in group A and one in group B). There were no instances of grade 3 or 4 anemia or thrombocytopenia. There were no clinically relevant changes in electrocardiographic findings. There were seven adverse events leading to dose reductions of peginterferon or ribavirin in four patients in group B, including neutropenia (three events), anemia (two), leukopenia (one), and depression (one).

Six patients (four from group A, including two who were receiving peginterferon and ribavirin after viral breakthrough, and two from group B) had transient elevations of alanine aminotransferase levels to greater than 3 times the upper limit of the normal range (Fig. 2A and 2B in the Supplementary Appendix). Elevated aminotransferase levels generally occurred between weeks 8 and 20. The peak alanine aminotransferase level was 370 U per liter, and the maximum direct bilirubin level was 0.6 mg per deciliter. There was no evidence of an association between elevations of alanine aminotransferase levels and response to therapy or viral breakthrough. No discontinuations or interruptions of study treatment were required, and alanine aminotransferase levels stabilized or improved in all patients.

DISCUSSION

In this study, we assessed the effect of coadministration for 24 weeks of two direct-acting antiviral agents with different mechanisms of action,

Table 2. Virologic Response during and after Treatment.*

Timing of Virologic Response	Group A (N=11)	Group B (N=10)
	number (percent)	
During 24-wk treatment period		
Wk 4: rapid virologic response	7 (64)	6 (60)
Wk 4 and wk 12: extended rapid virologic response	4 (36)	6 (60)
Wk 12: Complete early virologic response	5 (45)	9 (90)†
Wk 24	5 (45)	10 (100)
During post-treatment period		
Wk 4	4 (36)	10 (100)
Wk 12	4 (36)	10 (100)
Wk 24	4 (36)	9 (90)‡
Wk 48	3 (27)§	9 (90)¶

* Patients were considered to have had virologic response if they had undetectable HCV RNA, which was defined as HCV RNA levels of less than 10 IU per milliliter. The analyses were performed in the modified intention-to-treat population (all patients who received at least one dose of study medication). Patients who had viral breakthrough or relapse were classified as having had treatment failure.

† One patient in group B had detectable but not quantifiable HCV RNA levels (<25 IU per milliliter) at week 12 during treatment and undetectable HCV RNA levels (<10 IU per milliliter) on retesting.

‡ One patient in group B had detectable but not quantifiable HCV RNA levels at week 24 after treatment and undetectable HCV RNA levels on retesting 35 days later.

§ One patient in group A who had virologic response at weeks 4, 12, and 24 after treatment had detectable but not quantifiable HCV RNA levels at 48 weeks after treatment and undetectable HCV RNA levels on retesting 43 days later.

¶ One patient in group B had detectable but not quantifiable HCV RNA levels at week 48 after treatment and undetectable HCV RNA levels on retesting 13 days later.

daclatasvir and asunaprevir, alone or with peginterferon alfa-2a and ribavirin, in an exploratory cohort of patients with HCV infection, who typically do not have a response to retreatment with peginterferon and ribavirin and are expected to have a poor response to triple therapy with peginterferon and ribavirin plus a protease inhibitor (boceprevir or telaprevir).^{11,12} A total of 90% of the patients carried IL28B genotypes (the rs12979860 SNP CT or TT), which are associated with poor response to peginterferon and ribavirin,²² and most of the patients had HCV genotype 1a infection.

The low rate of sustained virologic response achieved with peginterferon and ribavirin among patients with HCV genotype 1 infection, coupled with high rates of adverse effects, have stimulated the development of direct-acting antiviral agents and the study of regimens that exclude peginterferon, ribavirin, or both.^{13,23,24} Coadministration

Table 3. Adverse Events during the Treatment Period.*

Adverse Event	Group A† (N=11)	Group B (N=10)
	<i>number of patients (percent)</i>	
Diarrhea	8 (73)	7 (70)
Fatigue	6 (55)	7 (70)
Headache	5 (45)	5 (50)
Nausea	2 (18)	5 (50)
Cough	3 (27)	2 (20)
Insomnia	3 (27)	3 (30)
Pyrexia	3 (27)	1 (10)
Chills	3 (27)	1 (10)
Dizziness	2 (18)	2 (20)
Dyspnea	2 (18)	2 (20)
Urinary tract infection	2 (18)	2 (20)

* All adverse events that occurred in at least four patients in the two groups combined are included.

† The patients in group A were assigned to receive direct-acting antiviral therapy alone; however, six patients in the group received rescue therapy with peginterferon alfa-2a and ribavirin (in addition to the direct-acting antiviral therapy) after viral breakthrough occurred. Adverse events that occurred in those patients are included here.

of daclatasvir and asunaprevir alone in group A initially led to a rapid reduction in HCV RNA, with all 11 patients achieving greater than a 4- \log_{10} decline. Ultimately, 5 patients had undetectable HCV RNA at the end of the treatment period, and 4 had sustained virologic response at weeks 12 and 24 after treatment, establishing proof-of-concept that sustained virologic response can be achieved without peginterferon or ribavirin.

Both of the patients in group A who were infected with HCV genotype 1b had sustained virologic response at weeks 12 and 24 after treatment. Six patients in group A (all with HCV genotype 1a infection) had viral breakthrough. Previous studies have also shown that some patients receiving combinations of direct-acting antiviral agents without interferon have had viral breakthrough.^{23,24} In our study, viral breakthrough correlated with baseline HCV RNA levels (Table 1 in the Supplementary Appendix) and did not appear to be associated with pharmacokinetic findings at day 14 (data not shown). Although there were only 2 patients with HCV genotype 1b infection in group A, the observation that both of the patients had a sustained virologic response after treatment

with two direct-acting antiviral agents alone may reflect a higher resistance barrier for this combination of drugs in patients with HCV genotype 1b infection than in patients with HCV genotype 1a infection. A similar finding of a high rate of sustained virologic response among patients with HCV genotype 1b infection was shown in a pilot study of combination therapy with asunaprevir and daclatasvir in 10 Japanese patients who had HCV genotype 1b infection and who had not had a response to previous therapy with peginterferon and ribavirin.²⁵

Although all six patients with viral breakthrough initially had a response to the addition of peginterferon and ribavirin as rescue therapy, most ultimately had therapeutic failure during or after the treatment period, which was consistent with their previous lack of response to those agents. The high frequency of resistance to two classes of drugs that was seen in patients with HCV genotype 1a infection who were treated with direct-acting antiviral agents alone suggests that studies of combinations of direct-acting antiviral agents without peginterferon and ribavirin in patients with HCV genotype 1a infection should proceed carefully.

In contrast to group A, no viral breakthrough occurred in patients in group B, who were receiving quadruple therapy. All 10 patients in group B achieved a sustained virologic response at week 12 after treatment and all but 1 had a sustained virologic response at week 24 after treatment. The inclusion of peginterferon alfa-2a and ribavirin with daclatasvir and asunaprevir provided sufficient antiviral activity to suppress the emergence of resistance, an observation that is consistent with *in vitro* data showing a synergism of interferon and ribavirin with both direct-acting antiviral agents.^{14,15} Retreatment with peginterferon and ribavirin in patients who have not had a previous response to those drugs is generally unsuccessful, and even triple therapy with peginterferon, ribavirin, and telaprevir in these patients produced rates of sustained virologic response of approximately 30% at week 24 after treatment.¹² Gane and colleagues found that a combination of two direct-acting antiviral agents can suppress HCV RNA in patients who have not had a response to peginterferon and ribavirin, but the treatment with the combination of direct-acting

antiviral agents was limited to 13 days and was followed immediately by treatment with peginterferon and ribavirin, thus preventing the assessment of sustained response to the direct-acting antiviral agents only.¹³

Among the 14 patients who had a sustained virologic response at week 12 after treatment, an HCV RNA level of less than 25 IU per milliliter was detected in 1 patient at week 24 after treatment and in 2 other patients at week 48 after treatment. All 3 patients had undetectable HCV RNA on retesting. These data indicate that late relapse is unlikely to occur in patients who have a sustained virologic response with combination direct-acting antiviral agents at week 12 after treatment.

The most common adverse event was diarrhea, which was mild or moderate in all cases. Grade 3 or 4 neutropenia occurred in six patients, all of whom were receiving peginterferon and ribavirin in addition to the two direct-acting antiviral agents. No grade 3 or 4 events related to hemoglobin levels or platelet counts were observed. Transient elevations of alanine aminotransferase levels were observed in six patients; these elevations were not associated with clinically significant increases in bilirubin level. In addition, we did not find any association between these elevations and a reduction in HCV RNA levels, viral breakthrough, or pharmacokinetic findings at day 14. Similar elevations of alanine aminotransferase levels were observed in a separate dose-ranging study of asunaprevir in combination with peginterferon and ribavirin,²⁶ and a lower dose of asunaprevir was selected for ongoing and future studies.

In conclusion, therapy with daclatasvir, asunaprevir, peginterferon alfa-2a, and ribavirin for 24 weeks provided a high rate of sustained virologic response in patients with HCV genotype 1 infection who had not had a response to previous therapy with peginterferon and ribavirin. The response in some patients to the combination of daclatasvir and asunaprevir alone showed proof-of-concept that a sustained virologic response can be achieved without peginterferon and ribavirin therapy. Both patients with HCV genotype 1b infection who were treated with the combination of direct-acting antiviral agents alone had a sustained virologic response. In con-

trast, the response rate was low in patients with genotype 1a infection. Overall, these results suggest that further research with combinations of direct-acting antiviral agents, with or without peginterferon and ribavirin, is warranted. Future studies should evaluate other combinations of direct-acting antiviral agents with high potency and nonoverlapping resistance profiles and investigate treatments tailored to specific characteristics of the patient and the virus.

Presented in part at the annual meeting of the American Association for the Study of Liver Diseases, Boston, October 29–November 2, 2010; and at the European Association for the Study of the Liver International Liver Congress, Berlin, March 30–April 3, 2011.

Dr. Lok reports receiving consulting fees from Abbott, Bristol-Myers Squibb, and Gilead Sciences and grant support from Bristol-Myers Squibb, Gilead Sciences, GlaxoSmithKline, Merck-Schering-Plough, and Roche; Dr. Gardiner, being an employee of and owning stock in Bristol-Myers Squibb; Dr. Lawitz, receiving consulting fees from Abbott, Achillion Pharmaceuticals, Anadys Pharmaceuticals, and Pharmasset, lecture fees from Merck and Vertex Pharmaceuticals, and grant support from Abbott, Achillion Pharmaceuticals, Anadys Pharmaceuticals, Bioplex, Bristol-Myers Squibb, Gilead Sciences, GlaxoSmithKline, GlobeImmune, Idera Pharmaceuticals, Inhibitex, Medtronic, Merck, Novartis, Pharmasset, Roche, Santaris Pharma, Scynexis, Tibotec, Vertex Pharmaceuticals, and ZymoGenetics (now part of Bristol-Myers Squibb); Dr. Martorell, receiving lecture fees from Bristol-Myers Squibb, Genentech (now Roche-Genentech), Gilead Sciences, Merck, Tibotec, and ViiV Healthcare and grant support from Abbott, Bristol-Myers Squibb, Gilead Sciences, Kowa, Novartis, and ViiV Healthcare; Dr. Everson, receiving consulting fees from Abbott, Roche-Genentech, and Vertex Pharmaceuticals, serving on a data and safety monitoring board for Merck, receiving grant support from Abbott, Bristol-Myers Squibb, Conatus Pharmaceuticals, Gilead Sciences, GlobeImmune, Human Genome Sciences-Novartis, Merck-Schering-Plough, Pfizer, Roche-Genentech, Tibotec, and Vertex Pharmaceuticals, owning a patent in conjunction with the University of Colorado for tests of liver function, and owning HepQuant, which develops these tests (but for which he has received no income or payment to date); Dr. Ghalib, receiving grant support from Abbott, Achillion Pharmaceuticals, Anadys Pharmaceuticals, Bristol-Myers Squibb, Duke Clinical Research, Roche-Genentech, Gilead Sciences, Johnson & Johnson, Medtronic, Merck, Novartis, Roche, Merck-Schering-Plough, Tibotec, Vertex Pharmaceuticals, ViroChem, and ZymoGenetics (Bristol-Myers Squibb); Dr. Rustgi, receiving consulting fees from Merck, Novartis, and Tibotec and lecture fees from Roche-Genentech, Gilead, and Merck; Drs. McPhee, Zhu, and Dimitrova, being employees of Bristol-Myers Squibb; and Drs. Wind-Rotolo, Persson, Eley, Guo, Grasela, and Pasquinelli, being employees of Bristol-Myers Squibb and owning stock in Bristol-Myers Squibb. No other potential conflict of interest relevant to this article was reported.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

We thank the patients and their families, the research staff at all participating sites, and colleagues at Bristol-Myers Squibb Research and Development. Editorial assistance was provided by Jennifer Tobin at Articulate Science and was funded by Bristol-Myers Squibb.

REFERENCES

1. Armstrong GL, Wasley A, Simard EP, McQuillan GM, Kuhnert WL, Alter MJ. The prevalence of hepatitis C virus infection in the United States, 1999 through 2002. *Ann Intern Med* 2006;144:705-14.
2. Global alert and response (GAR): hepatitis C. Geneva: World Health Organization (<http://www.who.int/csr/disease/hepatitis/whocdscsrlyo2003/en/index4.html>).
3. The ABCs of hepatitis. Atlanta: Centers for Disease Control and Prevention (<http://www.cdc.gov/hepatitis/Resources/Professionals/PDFs/ABCTable.pdf>).
4. 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.
5. 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.
6. Ghany M, Nelson DR, Strader DB, Thomas DL, Seeff LB. An update on treatment of genotype 1 chronic hepatitis C virus infection: 2011 practice guideline by the American Association for the Study of Liver Diseases. *Hepatology* 2011;54:1433-44.
7. Fried MW, Shiffman ML, Reddy KR, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002;347:975-82.
8. 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.
9. Rumi MG, Aghemo A, Prati GM, et al. Randomized study of peginterferon-alpha2a plus ribavirin vs peginterferon-alpha2b plus ribavirin in chronic hepatitis C. *Gastroenterology* 2010;138:108-15.
10. 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.]
11. Schiff E, Poordad F, Jacobson I, et al. Boceprevir (B) combination therapy in null responders (NR): response dependent on interferon responsiveness. *J Hepatol* 2008;48:Suppl 2:S46. abstract.
12. Zeuzem S, Andreone P, Pol S, et al. Telaprevir for retreatment of HCV infection. *N Engl J Med* 2011;364:2417-28.
13. Gane EJ, Roberts SK, Stedman CA, et al. Oral combination therapy with a nucleoside polymerase inhibitor (RG7128) and danoprevir for chronic hepatitis C genotype 1 infection (INFORM-1): a randomised, double-blind, placebo-controlled, dose-escalation trial. *Lancet* 2010;376:1467-75.
14. 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.
15. McPhee F. Identification and preclinical profile of the novel HCV NS3 protease inhibitor BMS-650032. *J Hepatol* 2010;52:Suppl 1:S296. abstract.
16. 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.
17. Pasquinelli C, Eley T, Villegas C, et al. Safety, tolerability, pharmacokinetics and antiviral activity following single- and multiple-dose administration of BMS-650032, a novel HCV NS3 inhibitor, in subjects with chronic genotype 1 HCV infection. *Hepatology* 2009;50:Suppl:411A. abstract.
18. Bifano M, Sevinsky H, Bedford BR, et al. Coadministration of BMS-790052 and BMS-650032 does not result in a clinically meaningful pharmacokinetic interaction in healthy subjects. *Hepatology* 2010;52:Suppl:719A. abstract.
19. Wai CT, Greenon 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.
20. Poynard T, Imbert-Bismut F, Ratziu V, et al. Biochemical markers of liver fibrosis in patients infected with hepatitis C virus: longitudinal validation in a randomized trial. *J Viral Hepat* 2002;9:128-33.
21. Poynard T, McHutchison J, Manns M, Myers RP, Albrecht J. Biochemical surrogate markers of liver fibrosis and activity in a randomized trial of peginterferon alfa-2b and ribavirin. *Hepatology* 2003;38:481-92.
22. Ge D, Fellay J, Thompson AJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature* 2009;461:399-401.
23. Zeuzem S, Buggisch P, Agarwal K, et al. Dual, triple, and quadruple combination treatment with a protease inhibitor (GS-9256) and a polymerase inhibitor (GS-9190) alone and in combination with ribavirin (RBV) or PegIFN/RBV for up to 28 days in treatment naive, genotype 1 HCV subjects. *Hepatology* 2010;52:Suppl:400A-401A. abstract.
24. Zeuzem S, Asselah T, Angus PW, et al. Strong antiviral activity and safety of IFN-sparing treatment with the protease inhibitor BI 201335, the HCV polymerase inhibitor BI 207127 and ribavirin in patients with chronic hepatitis C. *Hepatology* 2010;52:Suppl:876A. abstract.
25. Chayama K, Takahashi S, Toyota J, et al. Dual therapy with the NS5A inhibitor BMS-790052 and the NS3 protease inhibitor BMS-650032 in HCV genotype 1b-infected null responders. *Hepatology* 2011 October 10 (Epub ahead of print).
26. Bronowicki J-P, Pol S, Thuluvath PJ, et al. BMS-650032, an NS3 inhibitor, in combination with peginterferon alpha-2a and ribavirin in treatment-naive subjects with genotype 1 chronic hepatitis C infection. *J Hepatol* 2011;54:Suppl 1:S472. abstract.

Copyright © 2012 Massachusetts Medical Society.

AN NEJM APP FOR IPHONE

The NEJM Image Challenge app brings a popular online feature to the smartphone. Optimized for viewing on the iPhone and iPod Touch, the Image Challenge app lets you test your diagnostic skills anytime, anywhere. The Image Challenge app randomly selects from 300 challenging clinical photos published in NEJM, with a new image added each week. View an image, choose your answer, get immediate feedback, and see how others answered. The Image Challenge app is available at the iTunes App Store.