Dual Therapy With the Nonstructural Protein 5A Inhibitor, Daclatasvir, and the Nonstructural Protein 3 Protease Inhibitor, Asunaprevir, in Hepatitis C Virus Genotype 1b–Infected Null Responders

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Patients with chronic hepatitis C virus (HCV) infection and previous null response to pegylated interferon (Peg-IFN) and ribavirin (RBV) have limited therapeutic options. HCV genotype 1 is the most common worldwide and the most difficult to treat; genotype 1b is the most common subtype of genotype 1 outside North America. The enhanced antiviral activity achieved by combining two direct-acting antiviral (DAA) agents may improve clinical outcomes. This open-label, phase IIa study included 10 patients with chronic HCV genotype 1b infection and previous null response (<2 $\log_{10}$ reduction in HCV RNA after 12 weeks) to Peg-IFN and RBV. Patients received dual DAA treatment for 24 weeks with the nonstructural protein 5A replication complex inhibitor, daclatasvir (60 mg once-daily), and the nonstructural protein 3 protease inhibitor, asunaprevir (initially 600 mg twice-daily, then subsequently reduced to 200 mg twice-daily). The primary efficacy endpoint was the proportion of patients with sustained virologic response (SVR) at 12 weeks post-treatment (SVR_{12}). Nine patients completed 24 weeks of treatment; 1 patient discontinued treatment after 2 weeks. In the 9 patients who completed the full course of treatment, HCV RNA was undetectable at week 8 and remained undetectable through the end of treatment; all 9 patients achieved SVR_{12} and SVR_{24}. HCV RNA also remained undetectable post-treatment in the patient who discontinued after 2 weeks. There was no viral breakthrough. Diarrhea and headache, generally mild, were the most common adverse events; transaminase elevations were reported in 3 patients, but did not result in discontinuation. *Conclusions*: Dual therapy with daclatasvir and asunaprevir, without Peg-IFN and RBV, can achieve high SVR rates in difficult-to-treat patients with HCV genotype 1b infection and previous null response to Peg-IFN and RBV. (HEPATOLOGY 2012;55:742-748)

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Chronic hepatitis C virus (HCV) infection affects approximately 180 million individuals worldwide and is a common cause of chronic liver disease and hepatocellular carcinoma (HCC) in Japan, the United States, and many European countries.[1,2] Among the six major HCV genotypes, genotype 1 is the most common and the most difficult to treat, and its two main subtypes may differentially influence therapeutic outcomes.[3,4] Genotype 1b is the most prevalent worldwide and predominates in Japan and China, whereas genotype 1a is most common in the United States; subtype prevalence in Europe is similar.[5-7]

Abbreviations: ALT, alanine aminotransferase; cEVR, complete early virology response: undetectable HCV RNA at week 12; DAA, direct-acting antiviral; EOTR, end-of-treatment response: undetectable HCV RNA at week 24; eRVR, extended rapid virologic response: undetectable HCV RNA at weeks 4 and 12; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IL28B*, interleukin-28B; INR, international normalized ratio; LLQ, lower limit of quantitation; NS3, nonstructural protein 3; NS5A, nonstructural protein 5A; Peg-IFN-α, pegylated interferon alpha; PCR, polymerase chain reaction; RBV, ribavirin; RVR, rapid virologic response: undetectable HCV RNA at week 4; SNP, single-nucleotide polymorphism; SVR, sustained virologic response: undetectable HCV RNA post-treatment; SVR_{12}, sustained virologic response 12 weeks post-treatment; SVR_{24}, sustained virologic response 24 weeks post-treatment; ULN, upper limit of normal.*

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Treatment of chronic HCV infection with pegylated interferon alpha (Peg-IFN-α) and ribavirin (RBV) elicits a sustained virologic response (SVR) in 40%-50% of treatment-naïve patients with genotype 1 infections; SVR rates in this population increase to 66% or 75% when boceprevir or telaprevir, respectively, is added to the regimen.[8-12] Response rates are influenced by viral load and genotype and by patient demographics, disease history, and genetics.[10] Peg-IFN/RBV retreatment of patients with previous nonresponse to Peg-IFN/RBV is frequently unsuccessful, with SVR rates of only 6%-9%.[13,14] Null responders are the subset of nonresponders who have responded most poorly to Peg-IFN/RBV, and their urgent need for more potent therapies has prompted the evaluation of regimens containing direct-acting antivirals (DAAs). SVR rates of 27% (genotype 1a) and 37% (genotype 1b) were achieved in null responders with a regimen combining telaprevir with Peg-IFN/RBV in a study of nonresponders.[15] These results suggest that DAA-containing regimens can benefit this population, but greater antiviral potency is needed to increase response rates further.

Combinations of two DAAs may overcome IFN nonresponsiveness in null responders by increasing antiviral activity and reducing the risk of developing resistance-associated variants.[16] In HCV-infected human hepatocyte chimeric mice, dual DAA treatment eradicated HCV without resistance, whereas resistance emerged rapidly with single DAA treatment.[17] In a clinical study that included null responders, marked antiviral effects were observed after 13 days of dual DAA treatment, supporting the evaluation of longer term dual DAA therapy reported in this study.[18] Daclatasvir (BMS-790052) is a first-in-class, highly selective nonstructural protein 5A (NS5A) replication complex inhibitor with picomolar potency and broad genotypic coverage; asunaprevir (BMS-650032) is a nonstructural protein 3 (NS3) protease inhibitor active against HCV genotypes 1a and 1b.[19,20] Daclatasvir and asunaprevir are associated with different resistance-associated variants, consistent with their different molecular targets, and showed no meaningful pharmacokinetic interactions in healthy volunteers.[20-22]

In a 24-week study of null responders in the United States, daclatasvir and asunaprevir demonstrated potent antiviral effects, both as a dual DAA regimen and in a quadruple regimen that included Peg-IFN/RBV.[23] Overall, 36% of dual-therapy recipients achieved SVR, including both of the 2 patients with genotype 1b infection. However, patients with genotype 1a experienced frequent viral breakthrough with the dual regimen and only 2 of 9 achieved SVR, suggesting subtype-associated differences in resistance barrier and response. We present the results of an open-label trial evaluating dual therapy with daclatasvir and asunaprevir in Japanese patients with chronic HCV genotype 1b infection and previous null response to Peg-IFN/RBV.

Patients and Methods

Study Design. This open-label, phase IIa study (clinicaltrials.gov identifier NCT01051414) evaluated the antiviral activity and safety of daclatasvir combined with asunaprevir in patients with HCV genotype 1 infection and previous null response to treatment with Peg-IFN/RBV, defined as <2 $\log_{10}$ reduction of HCV RNA after 12 weeks of therapy. This sentinel cohort provided safety data for review by an independent study safety committee before the enrollment of additional cohorts that will be described in a subsequent report. Written informed consent was obtained from all patients. The study was approved by institutional review boards at each site and was conducted in compliance with the Declaration of Helsinki, Good Clinical Practice Guidelines, and local regulatory requirements.

Patients. Patients eligible for enrollment in the sentinel cohort included men and women 20-75 years in age (women of childbearing potential were required to use adequate contraception) with chronic HCV genotype 1 infection for at least 6 months (all enrolled patients were genotype 1b because of the high prevalence of this subtype in Japan) and HCV RNA $\geq 10^5$ IU/mL. Eligible patients met criteria defining null responders and had no evidence of cirrhosis documented by laparoscopy, imaging, or liver biopsy within 2 years.

Patients were excluded if they had a history of HCC, coinfection with hepatitis B virus or human immunodeficiency virus, other chronic liver disease, or

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evidence of hepatic decompensation. Patients were also excluded if they had other severe or unstable conditions or evidence of organ dysfunction in excess of that consistent with the age of the patient, were unable to tolerate oral medication or had conditions that could affect the absorption of study drug, or were exposed to any investigational drug within 4 weeks of study participation or had any previous exposure to inhibitors of NS5A or NS3 protease. Laboratory findings that excluded participation were the following: alanine aminotransferase (ALT) >5× the upper limit of normal (ULN); total bilirubin ≥2 mg/dL; direct bilirubin >1.5× ULN; international normalized ratio (INR) ≥1.7; albumin ≤3.5 g/dL; hemoglobin <9.0 g/dL; white blood cells <1,500/mm^3; absolute neutrophil count <750/mm^3; platelets <50,000/mm^3; or creatinine >1.8× ULN.

Prohibited concomitant medications included inducers or inhibitors of cytochrome P450/3A4, non-study medications with anti-HCV activity, any prescription medication or herbal product not prescribed for a specific condition, liver-protection drugs, proton pump inhibitors, and erythropoiesis-stimulating agents. H_2 receptor antagonists were permitted, but administered ≥10 hours before or ≥2 hours after daclatasvir; other acid-modifying agents had to be taken ≥2 hours before or after daclatasvir.

Study Drug Dosing. All patients received oral combination therapy with daclatasvir and asunaprevir from the beginning of the study. Daclatasvir was dosed as two 30-mg tablets once-daily. Asunaprevir was initially dosed as three 200-mg tablets twice-daily; subsequently, the dose of asunaprevir was reduced to 200 mg twice-daily after reports of hepatic enzyme elevations in a clinical study of asunaprevir and Peg-IFN/RBV.[24]

Treatment was continued to week 24 for patients with HCV RNA below the assay lower limit of quantitation (LLQ; 15 IU/mL) on or after week 2; treatment was discontinued for patients with <2 $\log_{10}$ IU/mL decrease of HCV RNA from baseline or on or after week 2. For patients with viral rebound on or after week 2, or HCV RNA above LLQ on or after week 4, treatment was discontinued or weight-based Peg-IFN-RBV therapy was added for up to 48 additional weeks at the investigator's discretion, based on expected tolerance of Peg-IFN-RBV. Viral rebound was defined as an increase ≥1 $\log_{10}$ IU/mL from nadir at more than one time point or HCV RNA ≥15 IU/mL after declining to below that level.

Safety and Efficacy Assessments. Assessments, including HCV RNA, physical examination, vital signs, adverse events, laboratory tests, and review of concomitant medications, were conducted at screening, on study days 1 (baseline) through 7 and days 9, 11, and 14, at weeks 3, 4, 6, 8, 10, 12, 16, 20, and 24, and at post-treatment weeks 4, 8, 12, and 24. Twelve-lead electrocardiograms were recorded at all visits, except those at weeks 3 and 6. Additional pretreatment assessments included HCV genotype and host interleukin-28B (*IL28B*) genotype.

Serum HCV RNA levels were determined at a central laboratory using the Roche COBAS TaqMan HCV Auto assay (LLQ = 15 IU/mL; Roche Diagnostics KK, Tokyo, Japan). HCV genotype and subtype were determined at the central laboratory by polymerase chain reaction (PCR) amplification and sequencing. *IL28B* genotype was determined by PCR amplification and sequencing of the rs12979860 single-nucleotide polymorphism (SNP).

Outcome Measures. The primary efficacy endpoint was the proportion of patients with undetectable HCV RNA at 12 weeks post-treatment (SVR_{12}). Secondary endpoints included the proportions of patients with rapid virologic response (RVR; defined as undetectable HCV RNA at week 4), extended RVR (eRVR; undetectable HCV RNA at weeks 4 and 12), complete early virologic response (cEVR; undetectable HCV RNA at week 12), end-of-treatment response (EOTR; undetectable HCV RNA at week 24), and SVR at 24 weeks post-treatment (SVR_{24}).

The possible presence of HCV-resistance polymorphisms was analyzed using stored specimens. Resistance testing was performed on all samples at baseline and on samples indicative of virologic failure, defined as either (1) <2 $\log_{10}$ HCV RNA decrease from baseline at week 2, (2) virologic rebound (HCV RNA detectable after previously undetectable or ≥1 $\log_{10}$ increase from nadir), or (3) detectable HCV RNA at weeks 4 or 12 or at the end of therapy. Resistance analysis methodology included isolation of HCV RNA, PCR amplification, and population sequencing of HCV NS3 protease and NS5A domains.

Statistical Analysis. Categorical variables were summarized using counts and percents; continuous variables were summarized with univariate statistics.

Results

Patient Characteristics and Disposition. Twelve patients were screened; 2 patients failed to meet entry criteria (for HCC and elevated direct bilirubin, respectively), and 10 were enrolled and treated. Enrolled patients were generally older (median, 62 years); 6

Table 1. Baseline Demographic and Disease Characteristics

Parameter	Value
N	10
Age, median years (range)	62 (52-70)
Male sex, n (%)	4 (40)
Japanese race, n (%)	10 (100)
Host *IL28B* genotype,* n (%)	
CC	2 (20)
CT	8 (80)
HCV genotype 1b, n (%)	10 (100)
HCV RNA, mean $\log_{10}$ IU/mL (SD)	6.8 (0.61)
ALT, mean U/L (SD)	60.6 (32.9)
Platelets $\times$ 10^9 cells/mL, median (min, max)	150.5 (84.0, 166.0)
Total bilirubin, median mg/dL (min, max)	0.8 (0.6, 1.2)
Albumin, median g/dL (min, max)	3.9 (3.1, 4.2)
INR, median (min, max)	1.0 (1.0, 1.1)

*SNP rs12979860.

Abbreviation: *IL28B*, interleukin-28B; HCV, hepatitis C virus; SD, standard deviation; ALT, alanine aminotransferase; min, minimum; max, maximum; INR, international normalized ratio; SNP, single-nucleotide polymorphism.

were female and all were Japanese (Table 1). All enrolled patients were infected with genotype 1b, reflecting the predominance of this subtype in Japan, although the study protocol did not exclude patients with HCV genotype 1a.[6] Two patients were *IL28B* genotype CC (SNP rs12979860) and 8 were CT. Nine patients completed 24 weeks of therapy; 1 patient discontinued at week 2 because of a grade 4 total bilirubin elevation (see below). Among the 9 patients treated for 24 weeks, asunaprevir was dosed at 600 mg twice-daily for 12-21 weeks before the dose was reduced to 200 mg twice-daily (Fig. 1).

Virologic Response. Serum HCV RNA levels decreased rapidly in all patients (Fig. 2); mean reductions from baseline were 4.4 $\log_{10}$ IU/mL at week 1,

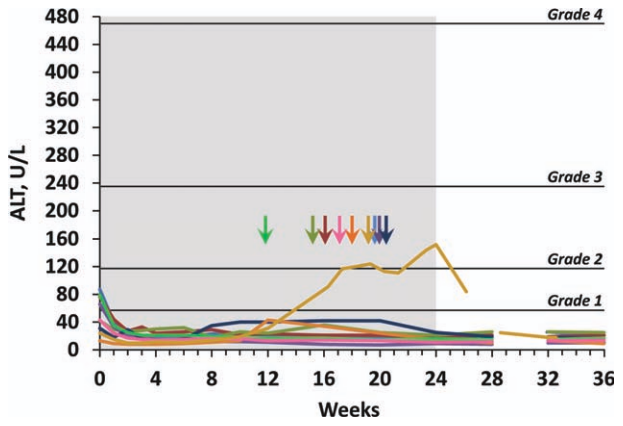

Fig. 1. ALT levels: individual patients. Serum ALT levels for the 9 patients who completed 24 weeks of treatment; the patient who discontinued at week 2 is not presented. Shaded area indicates the treatment period; arrows indicate the points at which the dose of asunaprevir was reduced from 600 to 200 mg twice-daily. Arrow and line colors are the same for each patient.

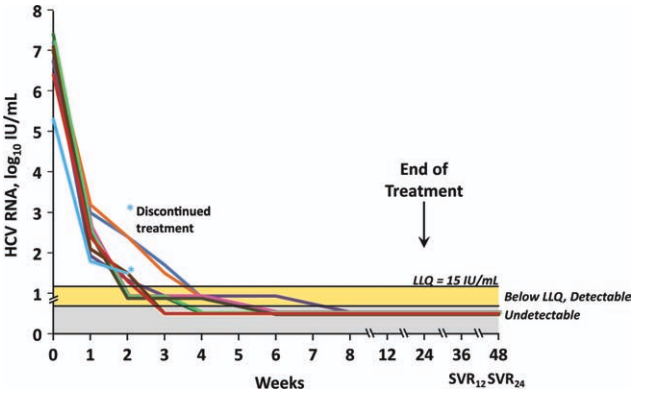

Fig. 2. HCV RNA levels: individual patients. Individual patient plasma HCV RNA levels during 24 weeks of treatment and through 24 weeks post-treatment (week 48) are shown. LLQ = 15 IU/mL.

5.3 $\log_{10}$ IU/mL at week 2, and 5.8 $\log_{10}$ IU/mL from week 4 through the end of treatment. At week 4, HCV RNA was undetectable (RVR) in 4 of 10 (40%) patients and below the assay LLQ in 9 of 10 (90%; Fig. 3). No patients qualified for discontinuation or addition of pegIFN/RBV. At week 8, HCV RNA was undetectable in 9 of 10 patients (all who remained on treatment) and remained undetectable through the end of treatment and follow-up. SVR_{12}, the primary endpoint, and SVR_{24} were achieved by 90% of patients, including all 9 who completed 24 weeks of therapy. The patient who discontinued treatment at week 2 had low-level HCV RNA at discontinuation (1.8 $\log_{10}$ IU/mL), but HCV RNA was undetectable at follow-up visits 2, 3, 4, 13, and 24 weeks after discontinuation.

Viral Breakthrough and Relapse. There was no viral breakthrough during treatment or relapse of HCV RNA post-treatment. Analysis of baseline samples revealed variants reported to confer minimal to low

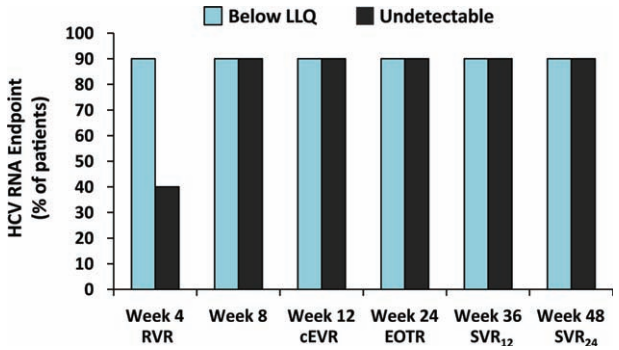

Fig. 3. HCV RNA endpoints. Categorical HCV RNA endpoints are indicated for the 10 study patients. One patient discontinued at week 2 and was counted as a treatment failure at the time points shown. However, HCV RNA was undetectable in this patient at 2, 3, 13, and 24 weeks post-treatment.

Table 2. On-Treatment Adverse Events Occurring in ≥2 Patients

Event	Patients, n (%)
Diarrhea	7 (70)
Headache	4 (40)
ALT increased	3 (30)
AST increased	3 (30)
Lymphopenia	2 (20)
Abdominal discomfort	2 (20)
Malaise	2 (20)
Pyrexia	2 (20)
Nasopharyngitis	2 (20)
Lipase increased	2 (20)
Back pain	2 (20)

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase.

levels of resistance to daclatasvir.[22] NS5A substitutions L28M and L31M were detected in 1 patient each, and Y93H was detected in 2 other patients. NS3 protease substitutions reported to confer resistance to telaprevir, boceprevir, and TMC-435 were detected[25]; T54S was identified in 1 patient, and Q80L was identified in 3. In 1 patient, both NS3 protease substitutions (T54S and Q80L) and an NS5A substitution (Y93H) were detected. There was no consistent association between detection of these variants and virologic outcomes.

Safety. The most frequently reported adverse events were diarrhea and headache, all of which were mild (grade 1) (Table 2). The patient who discontinued (see below) experienced multiple grade 3 or 4 adverse events and laboratory abnormalities on treatment. In the other 9 patients, there were no grade 3 or 4 transaminase elevations or other grade 3 or 4 events, no clinically relevant changes in electrocardiogram parameters, and no lymphopenia of any severity. Two transient grade 1 ALT elevations were reported, and 1 grade 2 elevation that began at week 16 and persisted until the end of treatment, after which it normalized within 2 weeks (Fig. 1). There were no notable differences in ALT before and after asunaprevir dose reduction.

There were two serious adverse events. A 54-year-old male was hospitalized with grade 3 pyrexia and persistent diarrhea 11 days after initiating study treatment. Loxoprofen was initiated, and body temperature normalized and diarrhea improved after 4 days. The patient remained on study treatment. The second event concerned a 60-year-old woman with a history of ulcerative colitis who discontinued study treatment after 2 weeks because of a grade 4 bilirubin elevation with multiple complicating features. Five days before discontinuation, she presented with infectious gastroenteritis and was treated with cefotiam and was subsequently hospitalized with fever, vomiting, and diarrhea. Meropenem, human serum albumin, and furosemide were initiated. At discontinuation of study drugs, laboratory findings included total bilirubin of 7.7 mg/dL and grade 3 lymphopenia and serum phosphorus reduction; transaminases and alkaline phosphatase were within normal ranges. In the week after discontinuation, white cell and eosinophil counts became elevated; total bilirubin improved and transaminases remained normal. Two weeks after discontinuation, grade 4 ALT and aspartate-aminotransferase elevations and a grade 3 lipase elevation were reported. Six weeks after discontinuation, bilirubin and transaminase elevations were resolved and lipase improved to within 2× ULN.

Discussion

This study assessed combination oral DAA therapy in a difficult-to-treat population with multiple adverse prognostic features, including HCV genotype 1b infection, primarily *IL28B* CT genotype, generally older age, and null response to previous Peg-IFN/RBV therapy.[10,13,14] These patients represent a group with a significant need for new therapeutic options.

A DAA-only therapeutic strategy may be particularly appropriate for null responders, who have previously shown only marginal response to Peg-IFN/RBV.[13,14] The combination of two highly potent DAAs cleared detectable virus rapidly in this study; HCV RNA was undetectable by week 8 in all 9 patients treated for 24 weeks. This outcome compares favorably with those observed when null responders received a combination of Peg-IFN/RBV and a single NS3 protease inhibitor, telaprevir or TMC435.[15,26] In these studies, HCV RNA remained detectable in 36% to approximately 50% of patients after 12 weeks.

HCV RNA remained undetectable 12 (SVR_{12}) and 24 weeks (SVR_{24}) post-treatment in all patients who completed treatment. This contrasts with the poor results obtained with Peg-IFN/RBV retreatment and the reported 37% SVR rate of genotype 1b null responders who received Peg-IFN/RBV and telaprevir.[10,13-15] Additional follow-up of patients from this study will assess whether SVR_{24} is predictive of long-lasting viral clearance with this dual DAA therapy, as it is with Peg-IFN/RBV. It is interesting that HCV RNA was persistently undetectable post-treatment in the patient who discontinued after only 2 weeks of treatment. With early discontinuation data from only this single case, at present, the result must be considered an anomaly. The factors that contributed to viral

clearance are uncertain, although the patient's *IL28B* CC genotype suggests increased sensitivity to endogenous interferon[27]; the possible influence of concurrent acute gastroenteritis or other complicating factors is unknown. However, coupled with the attainment of SVR_{12} in all other patients, this outcome suggests that required duration of therapy, which is currently predicated on data from Peg-IFN-based regimens, may need reassessment for DAA-only regimens, and, possibly, that certain patient populations can be treated for very short durations.

The high SVR rate is consistent with limited data from a related U.S.-based study, in which 2 of 2 null responders with HCV genotype 1b and who were treated with daclatasvir and asunaprevir achieved SVR_{24}.[23] However, only 2 of 9 patients with genotype 1a achieved SVR_{24} with the dual DAA regimen, compared with 9 of 10 patients who received both DAAs and Peg-IFN/RBV. These differences suggest that viral genotype can influence responses to DAA regimens that do not include Peg-IFN/RBV, and outcomes can be optimized with individualized therapy that considers viral genotype, among other factors. Because of the high SVR rate, the potential influence of other baseline and on-treatment parameters could not be assessed, other than to observe that unfavorable predictors of Peg-IFN/RBV response, such as older age and *IL28B* CT genotype,[27,28] had no measureable impact on outcomes.

There was no viral breakthrough on treatment. In view of the rapid emergence of resistance in some studies of short-term DAA monotherapy,[29,30] these findings support the concept that dual DAA therapy reduces the risk of viral breakthrough, in addition to increasing antiviral activity. Resistance analyses revealed that before treatment, some patients carried NS5A and NS3 polymorphisms predicted to reduce sensitivity to daclatasvir and some HCV protease inhibitors, respectively.[22,25] There was no clear relationship between the presence of these polymorphisms and minor interpatient differences in the rate of early virologic response; however, further study in larger patient cohorts will help determine whether baseline polymorphisms can influence virologic response with this regimen.

The adverse event profile of the dual DAA regimen compares favorably with the more frequent and severe events reported with Peg-IFN/RBV, although patient numbers in this study were limited. The mild diarrhea experienced by several patients has been reported previously with asunaprevir and is common with other drugs of this class.[15,18,24] Though a role for daclatasvir and/or asunaprevir in the two serious adverse events could not be ruled out and the investigator considered these events drug related, multiple confounding factors existed. The case of pyrexia was consistent with a viral infection and resolved with treatment. In the case of hyperbilirubinemia that led to discontinuation, the time course of laboratory abnormalities and related events suggests a link to the use of cefotiam and meropenem for treatment of infectious gastroenteritis. Both of these agents have been associated with vomiting, diarrhea, and hyperbilirubinemia.[31,32]

The asunaprevir dose was reduced during treatment because of transaminase elevations observed with 600 mg twice-daily in a concurrent study.[24] In this sentinel cohort, viral suppression was maintained in all patients after dose reduction, and no grade 3 or 4 transaminase elevations occurred during treatment at either dose of asunaprevir. One patient experienced grade 2 transaminase elevations that began at week 16 and persisted during treatment, despite asunaprevir dose reduction at week 19. Although these elevations were not severe, their rapid normalization post-treatment suggests a possible relationship to study treatment. None of the 9 patients treated for 24 weeks experienced transaminase elevations post-treatment. Although grade 4 transaminase elevations occurred 2 weeks post-treatment in the patient who discontinued, the timing of these events and multiple other complications suggest that they were not related directly to study treatment.

In conclusion, the combination of daclatasvir and asunaprevir achieved a high rate of SVR_{24} in patients with HCV genotype 1b infections and previous null response to Peg-IFN/RBV. These results support the concept that HCV infection can be cured with two DAAs without Peg-IFN/RBV, even in difficult-to-treat populations that lack robust IFN responsiveness. Further research will assess the benefits of DAA combinations in larger, more diverse patient populations.

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