Research Article

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Characterization of virologic escape in hepatitis C virus genotype 1b patients treated with the direct-acting antivirals daclatasvir and asunaprevir

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See Editorial, pages 643–645

Background & Aims: Daclatasvir and asunaprevir are NS5A and NS3 protease-targeted antivirals currently under development for treatment of chronic hepatitis C virus infection. Clinical data on baseline and on-treatment correlates of drug resistance and response to these agents are currently limited.

Methods: Hepatitis C virus genotype 1b Japanese patients (prior null responders to PegIFN-α/RBV [n = 21] or PegIFN-α/RBV ineligible or intolerant [n = 22]) were administered daclatasvir/asunaprevir for 24 weeks during a phase 2a open-label study. Genotypic and phenotypic analyses of NS3 and NS5A substitutions were performed at baseline, after virologic failure, and post-treatment through follow-up week 36.

Results: There were three viral breakthroughs and four relapsers. Baseline NS3 polymorphisms (T54S, Q80L, V170M) at amino acid positions previously associated with low-level resistance (<9-fold) to select NS3 protease inhibitors were detected in four null responders and three ineligibles, but were not associated with virologic failure. Baseline NS5A polymorphisms (L28M, L31M, Y93H) associated with daclatasvir resistance (<25-fold) were detected in five null responders and six ineligibles. All three viral breakthroughs and 2/4 relapsers carried a baseline NS5A-Y93H polymorphism. NS3 and NS5A resistance-associated variants were detected together (NS3-D168A/V, NS5A-L31M/V-Y93H) after virologic failure. Generally, daclatasvir-resistant substitutions persisted through 48 weeks post-treatment, whereas asunaprevir-resistant substitutions were no longer detectable. Overall, 5/10 patients with baseline NS5A-Y93H experienced virologic failure, while 5/10 achieved a sustained virologic response.

Conclusions: The potential association of a pre-existing NS5A-Y93H polymorphism with virologic failure on daclatasvir/asunaprevir combination treatment will be examined in larger studies. The persistence of treatment-emergent daclatasvir- and asunaprevir-resistant substitutions will require assessment in longer-term follow-up studies.

Keywords: Asunaprevir; Daclatasvir; Drug resistance; Direct-acting antivirals; Hepatitis C; Peginterferon-sparing.

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Abbreviations: DAA, direct-acting antiviral; HCV, hepatitis C virus; SVR, sustained virologic response; GT, genotype; PegIFN-α/RBV, peginterferon alfa and ribavirin; DCV, daclatasvir; ASV, asunaprevir; LLOQ, lower limit of quantitation; PCR, polymerase chain reaction; FU, follow-up; RAV, resistance-associated variant; BL, baseline; VBT, viral breakthrough; SD, standard deviation.

Introduction

The introduction of direct-acting antivirals (DAAs) targeting hepatitis C virus (HCV) NS3 protease activity has substantially increased sustained virologic response (SVR) in chronic HCV genotype 1 (GT1) infection. In combination with peginterferon-alfa and ribavirin (PegIFN-α/RBV), treatment with the recently approved protease inhibitors boceprevir or telaprevir results in SVR rates of around 70–75% in treatment-naive patients [1,2]. Despite these improvements, SVR rates vary by genotype and remain suboptimal in some patients, such as null responders to PegIFN-α/RBV [3], and patients for whom PegIFN-α/RBV is poorly tolerated or medically contraindicated. Furthermore, PegIFN-α/RBV is associated with frequent side effects [3], and the addition of these DAAs results in elevated rates of anemia and additional events such as dysgeusia (boceprevir), or rash, pruritus, and nausea (telaprevir) [4,5].

Daclatasvir (DCV) and asunaprevir (ASV) are currently undergoing clinical development for HCV infection. DCV (BMS-790052) is a first-in-class, highly selective NS5A replication complex inhibitor with picomolar potency and broad HCV genotypic coverage [6] that has demonstrated antiviral efficacy and good tolerability in combination with PegIFN-α/RBV [7]. ASV (BMS-650032) is a selective inhibitor of NS3 protease with antiviral activity *in vitro* against GT1 and GT4 [8]; it has also been shown to be

efficacious and generally well tolerated in combination with PegIFN-α/RBV [9]. Clinical interest is increasingly focusing on exploring DAA-only regimens without PegIFN-α/RBV, whose potential benefits might include better tolerability and compliance, and a reduced duration of therapy. One recent PegIFN-α/RBV-sparing study of DCV plus ASV (AI447017) has examined the efficacy and safety of this combination for 24 weeks in a small cohort of ten GT1b null responders, in whom an SVR rate of 90% was observed [10]. The study was then expanded to include an additional cohort of null responders and a group of patients ineligible to receive, or intolerant of, PegIFN-α/RBV [11].

As with other antiviral agents, the efficacy of DCV and ASV can be compromised by the development of drug resistance. *In vitro* data suggest that DCV and ASV should provide additive or synergistic activity that enhances the genetic barrier to resistance [8]. Here we characterize virologic escape observed on DCV plus ASV treatment in the expanded AI447017 study [11]; its associations with baseline characteristics, including *IL28B* genotype and HCV polymorphisms; and an assessment of on- and off-treatment genotypic changes in NS5A and NS3 protease and their phenotypic consequences.

Patients and methods

Study design and patients

This was an open-label, phase 2a study (AI447017; clinicaltrials.gov identifier NCT01051414) evaluating the antiviral activity and safety of DCV plus ASV in 43 patients with HCV GT1 infection. Patients comprised (a) 21 PegIFN-α/RBV null responders (<2 $\log_{10}$ decline in plasma HCV RNA after 12 weeks) and (b) 22 patients who discontinued previous PegIFN-α/RBV within 12 weeks for intolerance or were considered medically poor candidates for PegIFN-α/RBV for reasons such as advanced age, complications of depression, anemia, myelosuppression, diabetes, or cardiovascular or renal dysfunction. Patients enrolled in four cohorts; two each of null responders and ineligible/intolerant patients. The initial sentinel cohort of null responders has been described previously [10]. All enrolled patients were infected with GT1b.

Patients received DCV 60 mg once daily with ASV 200 mg twice daily for 24 weeks, with a further 48 weeks of post-treatment follow-up. ASV dosing in the expanded study was reduced from the 600 mg twice-daily administration used in the sentinel cohort, following reports of hepatic enzyme elevations at this dose, in another clinical study [12].

The full study design, including inclusion/exclusion criteria, and safety/efficacy endpoints, is described elsewhere [11]. Briefly, eligible patients were men and women aged 20–75 years with HCV GT1 infection ⩾6 months and HCV RNA ⩾10^5 IU/ml. Patients were excluded if they had evidence of liver cirrhosis within 24 months of screening; a history of hepatocellular carcinoma, other chronic liver disease, variceal bleeding, hepatic encephalopathy, or ascites requiring diuretics or paracentesis; co-infection with hepatitis B virus or HIV; or other clinically significant medical conditions.

Laboratory assessments

Plasma samples for resistance testing were collected at baseline and study weeks 1, 2, 4, 6, 8, 10, 12, 16, 20, and 24 and post-treatment weeks 4, 8, 12, 24, 36, and 48. HCV RNA was determined at a central laboratory using the Roche COBAS® TaqMan® HCV Auto assay (Roche Diagnostics KK, Tokyo, Japan) with a lower limit of quantitation (LLOQ) of 15 IU/ml. HCV genotype and subtype and *IL28B* genotype (rs12979860 single-nucleotide polymorphism) were determined by polymerase chain reaction (PCR) amplification and sequencing.

Genotypic and phenotypic analysis of clinical samples

Testing was performed on all baseline samples and on samples indicative of slow virologic response at week 1 or virologic failure with HCV RNA levels ⩾1000 IU/ml. Virologic failure, for the purpose of the study, was defined as an HCV RNA level (a) ⩾LLOQ at week 4 (futility rule), (b) >1 $\log_{10}$ IU/ml above nadir or ⩾LLOQ after confirmed undetectable (virologic breakthrough), or (c) ⩾LLOQ at any follow-up visit after being undetectable at the end of treatment (relapse).

Population sequencing of PCR amplicons was performed using methods described elsewhere [13–15]. For clonal analysis, amplicons were cloned into the TOPO vector and transformed into TOP10 *Escherichia coli* using a commercially available kit (TOPO® TA-cloning® kit, Invitrogen, Carlsbad, CA) according to manufacturer's instructions, with ⩾20 individual colonies expanded and sequenced for each analysis.

Phenotypic analyses of resistance-associated substitutions were performed by employing *in vitro* HCV replicon systems according to previously published methodologies [15–17].

Results

Viral response to DCV and ASV

Overall, plasma HCV RNA was undetectable in 77% (33/43) of patients at 24 weeks post-treatment. SVR was higher among the null responders than in the PegIFN-α/RBV ineligible population; all viral breakthroughs (n = 3) and relapses (n = 4) occurred in the ineligible/intolerant subpopulation. Three patients discontinued the study without subsequent SVR or virologic failure (Tables 1 and 2) [11].

Null responders

Virologic response.

Rapid and similar decreases in plasma HCV RNA levels were observed among patients who initiated treatment with ASV 600 mg (Fig. 1A) or ASV 200 mg (Fig. 1B). Mean reduction in HCV RNA at week 1 was comparable for both groups (−4.4 *vs.* −4.3 $\log_{10}$ IU/ml, respectively). Of the patients still receiving treatment (P-6 discontinued at day 16 due to an AE), all but one patient (P-13) had HCV RNA <15 IU/ml at week 4 and 52% had undetectable HCV RNA at this time.

Baseline analysis. Baseline *IL28B* genotype and naturally occurring polymorphisms associated with ASV or DCV resistance (resistance-associated variants [RAVs]) are shown in Table 1. As anticipated for this prior null responder population, the majority (18/21) were non-CC *IL28B*. The NS5A polymorphism Y93H (24-fold DCV resistance [13]) was observed in three patients. Other polymorphisms conferring minimal (two- to three-fold) DCV resistance were detected in two patients (NS5A-L28M-R30Q and NS5A-L31M). Polymorphisms associated with minimal to low-level resistance to select NS3 protease inhibitors (one patient, NS3-T54S-Q80L; one patient, NS3-Q80L-V170I/M; two patients, NS3-Q80L) [4,5,18] were also observed.

Baseline polymorphisms and *IL28B* genotype did not appear to influence either the week 1 response or SVR rate (Fig. 2A). Five patients had RNA levels ⩾1000 IU/ml after 1 week, of whom one (P-21) had significantly slower initial HCV RNA declines when compared with mean reductions (standard deviation [SD]) in HCV RNA for null responders on the study (−3.4 *vs.* −4.35 ± 0.49 $\log_{10}$ IU/ml). This patient had a CC *IL28B* genotype and an NS5A polymorphism (Q54L; no fold-change in DCV resistance). The other four patients had polymorphisms that have been associated with DCV and NS3 protease inhibitor low-level resistance [13,19]—specifically NS5A-Q54H/Q-Q62Q/E-Y93H/Y with NS3-T54S-Q80L (P-1, no fold-change to DCV/ASV), NS3-Q80L-V170I/M (P-2, no fold-change to ASV), NS5A-R30Q