A randomized, placebo-controlled study of the NS5B inhibitor beclabuvir with peginterferon/ribavirin for HCV genotype 1

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INTRODUCTION

In the management of chronic hepatitis C virus (HCV) infection, treatment strategies have been evolving from peginterferon alfa plus ribavirin (pegIFN/RBV) towards regimens based on oral, direct-acting antiviral (DAA) agents. Treatments involving the NS3 inhibitor simeprevir, the polymerase inhibitor sofosbuvir, or the NS5A inhibitor daclatasvir, either in combination with pegIFN/RBV and/or other DAAs, have improved SVR rates and are associated

SUMMARY. Beclabuvir is a potent, non-nucleoside inhibitor of the HCV NS5B RNA polymerase, with nanomolar activity against HCV genotypes 1, 3, 4, 5 and 6 in vitro. This study evaluated the efficacy and safety of beclabuvir, in combination with peginterferon alfa-2a (pegIFN) and ribavirin (RBV), in HCV genotype 1. In this randomized (1:1:1), double-blinded, placebo-controlled, dose-ranging phase 2a study, 39 treatment-naive patients chronically infected with HCV genotype 1 were treated for 48 weeks with beclabuvir (75 mg or 150 mg) plus pegIFN (180 µg) and RBV (1000 mg/day [<75 kg] or 1200 mg/day [≥75 kg]) vs pegIFN/RBV alone. The primary efficacy endpoint of extended rapid virologic response (undetectable HCV RNA at treatment weeks 4 and 12) was achieved by 76.9% (10/13) of patients receiving beclabuvir 75 mg and 38.5% (5/13) receiving beclabuvir 150 mg vs 0% receiving pegIFN/RBV alone. Higher response rates were observed among patients receiving beclabuvir 75 mg for all secondary efficacy endpoints, including sustained virologic response at follow-up weeks 12 or 24. Three patients experienced virologic breakthrough on treatment, all in the beclabuvir 150-mg treatment group. Beclabuvir was well tolerated at both doses, with the most commonly observed adverse events (headache, fatigue, nausea, decreased appetite, irritability, depression and insomnia) consistent with those observed with pegIFN/RBV. In conclusion, beclabuvir was both effective and well tolerated when administered in combination with pegIFN/RBV for the treatment of chronic HCV GT 1, supporting the study of beclabuvir as part of an all-oral regimen for HCV GT1.

Keywords: all-oral regimen, direct-acting antiviral, IL28B non-CC, NS5B polymerase inhibitor, triple therapy.
with a favourable safety profile [1–6], with the first all-oral treatments recently approved in Japan, Europe, and the United States [7–10]. However, there is a need for additional treatment options involving other classes of DAAs that target alternative viral proteins to maximize efficacy and barrier to resistance, thereby optimizing therapeutic outcomes in all patients.

Beclabuvir (formerly BMS-791325) is a potent, non-nucleoside NS5B polymerase inhibitor that binds the NS5B thumb pocket 1 allosteric site and shows nanomolar activity against HCV GTs 1, 3, 4, 5 and 6, with 50% effective concentration (EC50) of 3 and 6 nM, respectively, for GT1a and GT1b [11]. In vitro, beclabuvir demonstrated additive or synergistic antiviral activity with pegIFN/RBV and in 2- or 3-drug combinations with a range of DAAs, such as HCV NS3 protease inhibitors, NS5A inhibitors and/or nucleoside NS5B inhibitors [11]. In a phase 1 study in patients with GT1, single doses of 100–600 mg of beclabuvir were well tolerated and resulted in maximum mean concentration (EC50) of 3 and 6 nM, respectively, for HCV GTs 1, 3, 4, 5 and 6, with 50% effective thumb pocket 1 allosteric site and shows nanomolar activity or safety of beclabuvir and host toxicokinetic (PK) profile supportive of once- or twice-daily dosing [12].

The preclinical and phase 1 data for beclabuvir supported its further development as a component of multiclass regimens for HCV GT1. Herein, we report the efficacy and safety results from a phase 2a study of beclabuvir administered over 48 weeks at 2 doses, each in combination with pegIFN and RBV, as compared with pegIFN/RBV alone.

PATIENTS AND METHODS
This was a randomized (1:1:1), double-blinded, placebo-controlled, dose-ranging phase 2a study (AI443-012; ClinicalTrials.gov: NCT 01193361) evaluating the safety and efficacy of beclabuvir combined with pegIFN/RBV in treatment-naive adults chronically infected with HCV GT1. Eligible patients received 48 weeks of twice-daily oral beclabuvir at 75 mg, beclabuvir at 150 mg, or placebo, each administered in combination with once-weekly subcutaneous pegIFN (180 µg) and twice-daily oral RBV (weight-based dosing of 1000 mg/day [<75 kg] or 1200 mg/day [≥75 kg]). The duration of post-treatment follow-up was 24 weeks (in patients with undetectable HCV RNA at end of treatment) or 48 weeks (in patients with detectable HCV RNA at end of treatment or relapse).

Patients were required to have HCV RNA ≥10–5 IU/mL (COBAS TaqMan HCV Test 2.0; Roche Molecular Diagnostics, Pleasanton, California; lower limit of quantitation [LLOQ] 25 IU/mL) at screening, with no evidence of cirrhosis by liver biopsy within 24 months of randomization. Key exclusion criteria included >4 weeks of prior treatment with interferon or RBV within 6 months prior to randomization; alanine aminotransferase (ALT) ≥5 × upper limit of normal (ULN); total bilirubin ≥34 µmol/L (≥2 mg/dL) or direct bilirubin >ULN; international normalisation ratio (INR) ≥1.7; confirmed creatinine clearance ≤50 mL/min; or concurrent diagnosis of chronic hepatitis B infection, HIV infection, hepatocellular carcinoma or other non-HCV liver disease.

The primary safety endpoints were the incidence of serious adverse events (SAEs) and discontinuations of study therapy for AEs. The primary efficacy endpoint was the proportion of patients with extended rapid virologic response (eRVR), defined as undetectable (<LLOQ) HCV RNA at both weeks 4 and 12 of treatment. Secondary efficacy endpoints included the proportion of patients with undetectable HCV RNA at week 4 (rapid virologic response; RVR), week 12 (complete early virologic response; cEVR), and post-treatment weeks 12 and 24 (sustained virologic response: SVR12 and SVR24, respectively). Exploratory analyses included PK of beclabuvir and its metabolite BMS-794712, and associations between antiviral activity or safety of beclabuvir and host IL28B genotype or beclabuvir exposure.

Population sequencing of NS5B was performed at baseline, and for all beclabuvir-treated patients experiencing futility (defined us [i] virologic breakthrough [increase in HCV RNA >1 log10 IU/mL above nadir, or HCV RNA ≥LLOQ following a confirmed undetectable measurement on treatment]; [ii] <1 log10 decrease in HCV RNA at week 4; [iii] failure to achieve EVR [defined as <2 log10 IU/mL decrease in HCV RNA at week 12]; or [iv] detectable HCV RNA at week 12 and ≥LLOQ at week 24) or relapse (undetectable HCV RNA at end of treatment followed by confirmed detectable HCV RNA at any post-treatment visit) and with HCV RNA ≥1000 IU/mL.

A target of 12 patients per arm provided 80% confidence intervals (CIs) of 29–71% and 52–90% for observed study eRVRs of 50% and 75%, respectively, expecting an eRVR rate for pegIFN/RBV of ≤15% (based on an 11% RVR rate in the phase 2 telaprevir PROVE study, which had an upper 80% CI bound of ~15% [13]). Efficacy analyses included all randomized patients and were performed using a modified intent-to-treat analysis and a sensitivity analysis of observed value approach. No prospective calculations of statistical power were made.

The study was conducted at 10 US sites between October 2010 and November 2012 in accordance with Good Clinical Practice (International Conference on Harmonisation) and with the ethical principles of the Declaration of Helsinki. Patients provided written informed consent prior to study procedures. The study protocol, amendments and informed consent forms were approved by the institutional review board of each site prior to study initiation.

RESULTS AND DISCUSSION
Overall, 69 patients were screened and 39 randomized; of these, 29 patients completed the study (Fig. S1). Baseline
characteristics were generally balanced across the three groups, except for a lower proportion of males, and higher proportions of white patients and patients with IL28B (rs12979860) CC genotypes in the beclabuvir 75-mg arm compared with the other arms (Table S1).

The primary efficacy endpoint of eRVR was achieved by a higher proportion of patients in the beclabuvir 75-mg (76.9%) and 150-mg (38.5%) groups than in the pegIFN/RBV group (0%) (Fig. 1). Patients in the beclabuvir 75-mg group demonstrated the highest response rates for the secondary efficacy endpoints RVR, cEVR, SVR12 and SVR24 (Fig. 1). Similar results were obtained when patients with missing measurements were excluded from the analysis (on-treatment analysis; Fig. S2). Response rates were also generally higher in the beclabuvir treatment arms compared with placebo for both IL28B CC and non-CC genotypes (Fig. 1), although numbers were small. The higher response rates with beclabuvir 75 mg vs 150 mg may be explained by 2 factors: (i) a lower proportion of patients with IL28B (rs1297860) CC genotypes – known to be associated with higher response rates to pegIFN/RBV-based regimes than non-CC genotypes [14–16] – in the 150-mg group (15%) than in the 75-mg group (46%), and (ii) a higher proportion of patients in the 150-mg arm with missing HCV RNA data at post-treatment week 24. When missing data were accounted for, SVR24 rates in the 150-mg treatment arm improved, with observed values of SVR24 rates in both the beclabuvir 150-mg and the placebo arms of 71% (5/7) and 90% (9/10) in the beclabuvir 75-mg arm.

In the beclabuvir 75-mg group, no patient experienced virologic breakthrough or relapse post-treatment; among the four patients without SVR24, one had detectable HCV RNA at the end of treatment, whereas the remaining 3 (all with undetectable HCV RNA prior to post-treatment follow-up week 24) had missing week 24 post-treatment data, were lost to follow-up, or discontinued due to other reasons (incarceration) prior to this time point. In the beclabuvir 150-mg group, among the eight patients without SVR24, three had virologic breakthrough and one had detectable HCV RNA at end of treatment; no post-treatment relapse was observed. The remaining four patients (all with undetectable HCV RNA prior to post-treatment week 24) either had missing RNA data at post-treatment week 24 (n = 1) or were lost to follow-up (n = 3). In the placebo group, among the eight patients without SVR24, two had detectable HCV RNA at end of treatment, three experienced post-treatment relapse, whereas the remaining

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**Fig. 1** Virologic outcomes overall and by IL28B rs1297860 genotype (intent-to-treat analysis). *Primary endpoint. cEVR, complete early virologic response; CI, confidence interval; EOTR, end-of-treatment response; eRVR, extended rapid virologic response; ND, not determined; RVR, rapid virologic response; SVR, sustained virologic response.

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three patients (all with undetectable HCV RNA prior to post-treatment week 24) had missing RNA data or withdrew consent.

Among the three patients with virologic breakthrough (all beclabuvir 150 mg), all had GT1a infection, were IL28B non-CC genotype and had evidence of the NS5B substitutions A421V and P495S at breakthrough; one patient also had the NS5B substitution M426L at baseline. The observation that all patients with virologic breakthrough had GT1a and NS5B variants at A421 and P495 is consistent with prior data for beclabuvir and other thumb pocket 1 NS5B inhibitors. Variants at P495 are signature resistance-associated substitutions for thumb pocket-1 inhibitors [17], and A421V has previously been observed following in vitro passage of GT1a replicon cells in the presence of beclabuvir alone and when combined with the NS3 inhibitor asunaprevir [18].

Beclabuvir was well tolerated at both doses, with no unexpected safety events (Table 1). No deaths were reported. The nature and incidence of on-treatment AEs were similar across the three groups (≥92%), with the most common AEs typically associated with pegIFN/RBV treatment. The most frequent on-treatment grade 3/4 laboratory abnormalities were haematologic and were also events expected with pegIFN/RBV. While on treatment and during post-treatment follow-up, three patients experienced SAEs; of these, two patients had SAEs considered unrelated to study drug. The third patient (beclabuvir 75 mg) had grade 3 anaemia with grade 2 leukopenia at post-treatment follow-up week 4, which were considered related to study drug. Six patients discontinued from study therapy due to AEs (one receiving 75 mg beclabuvir, two receiving 150 mg beclabuvir, and three receiving placebo). Four discontinuations were protocol-mandated for confirmed conjugated hyperbilirubinemia (bilirubin ≥3 × baseline and >ULN) within the first 2 weeks (days 5–10; one in each beclabuvir arm, two in placebo arm). All four events were mild (grade 1) or moderate (grade 2) in intensity, with direct bilirubin levels ranging from 0.4 to 1.0 mg/dL (normal range, 0–0.2 mg/dL). Bilirubin abnormalities resolved completely or returned to just above baseline in three patients following discontinuation of all study drugs, but persisted in 1 placebo patient who initiated commercial pegIFN/RBV treatment.

Median time to maximum plasma concentrations (Tmax) for beclabuvir and its metabolite BMS-794712 were 2 h at each dose (Fig. S3). Exposure to both beclabuvir and BMS-794712 at treatment week 12 was greater than dose proportional, with a metabolite-parent AUC ratio of ~0.23–0.25, which is consistent with previous single-dose data (100–900 mg) from a beclabuvir phase 1 study [12]. No association was observed between composite trough concentrations of beclabuvir and achievement of a virologic response (eRVR, SVR24, RVR, cEVR, SVR12; Fig. S4a and data not shown), incidence of SAEs, or discontinuations for AEs (Fig. S4a). There was also no continuous association between drug exposure and changes from baseline in select clinical laboratory endpoints, including total bilirubin (Fig. S4b), ALT, haemoglobin or absolute neutrophils (data not shown); however, the caveat exists that trough levels are an indirect indication of peak exposure.

Together, these data show that beclabuvir is effective and well tolerated in combination with pegIFN/RBV for the treatment of chronic HCV GT1, and support the use of beclabuvir in all-oral regimens. Based on these results and similar data involving the DAAs daclatasvir (DCV; NS5A inhibitor) [5] and asunaprevir (ASV) [19], a phase 2b study in GT1 was initiated with the triple DAA combination beclabuvir (75 mg or 150 mg), DCV (60 mg) and ASV (200 mg). In a pilot cohort (N = 66), this all-oral combination achieved SVR12 in 92% of patients after 12 or 24 weeks of treatment, with response rates apparently independent of either IL28B genotype and treatment duration [20]. Similar efficacy and safety findings with this three-DAA regimen, using either the 75-mg or 150-mg beclabuvir dose, were subsequently reported for a larger (N = 166) cohort of patients treated for 12 weeks [21]. Further phase 3 studies of the triple regimen of beclabuvir (75-mg dose) plus DCV and ASV as a fixed-dose combination pill are being conducted, targeting treatment-naïve as well as -experienced patient populations.

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STATEMENTS OF INTEREST

H Tatum has served as a speaker and advisory board member for Gilead. PJ Thuluvath has received research funding from Bristol-Myers Squibb. E Lawitz has served as a speaker and advisory board member for Abbvie, Achillion, BioCryst, Biotica, Enanta, Gilead, GlaxoSmithKline, Idenix, Kadmon, Janssen, Merck & Co, Novalartis, Santaris, Theravance and Vertex, and has received research grants from AbbVie, Achillion, Boehringer Ingelheim, Bristol-Myers Squibb, Gilead, GlaxoSmithKline, Idenix, Intercept, Janssen, Medtronic, Merck & Co, Novalartis, Presidio, Roche, Santaris and Vertex. C Martorell has served as a speaker and advisory board member for AbbVie, Bristol-Myers Squibb, Gilead, Merck & Co and Viiv, and has received research funding from AbbVie, Boehringer Ingelheim, Bristol-Myers Squibb, Gilead, Idenix and Janssen. S Cohen has served as a speaker and consultant for, and has received research funding from, Bristol-Myers Squibb and Gilead. V Rustgi
Table 1 On-treatment adverse event summary

|                          | Beclabuvir 75 mg BID (n = 13) | Beclabuvir 150 mg BID (n = 13) | Placebo (n = 13) |
|--------------------------|-------------------------------|-------------------------------|-----------------|
| SAEs                     | 1*† (7.7)                    | 0                             | 1‡ (7.7)        |
| AE-related discontinuations | 1 (7.7)                     | 2 (15.4)                     | 3 (23.1)        |
| Protocol-mandated§       | 1 (7.7)                      | 1 (7.7)                      | 2 (15.4)        |
| Grade 3/4 AEs¶           | 2** (15.4)                   | 0                             | 1 (7.7)         |
| Deaths                   | 0                             | 0                             | 0               |
| Grade 3/4 laboratory abnormalities |                       |                               |                 |
| Haemoglobin              | 3 (23.1)                     | 0                             | 1 (7.7)         |
| White blood count        | 3 (23.1)                     | 0                             | 2 (15.4)        |
| Lymphocytes              | 6 (46.2)                     | 0                             | 5 (38.5)        |
| Neutrophils + bands      | 5 (38.5)                     | 3 (23.1)                     | 3 (23.1)        |
| ALT                      | 1 (7.7)                      | 1 (7.7)                      | 0               |
| AST                      | 1 (7.7)                      | 2 (15.4)                     | 1 (7.7)         |
| Lipase                   | 0                             | 1 (7.7)                      | 0               |

All-grade, all-cause AEs occurring in at least 3 (>20%) patients in any arm

|                          | Beclabuvir 75 mg BID (n = 13) | Beclabuvir 150 mg BID (n = 13) | Placebo (n = 13) |
|--------------------------|-------------------------------|-------------------------------|-----------------|
| Abdominal pain           | 4 (30.8)                     | 1 (7.7)                      | 0               |
| Anaemia                  | 4 (30.8)                     | 1 (7.7)                      | 5 (38.5)        |
| Arthralgia               | 3 (23.1)                     | 1 (7.7)                      | 0               |
| Chills                   | 3 (23.1)                     | 0                             | 0               |
| Cough                    | 3 (23.1)                     | 3 (23.1)                     | 3 (23.1)        |
| Decreased appetite       | 3 (23.1)                     | 4 (30.8)                     | 2 (15.4)        |
| Depression               | 4 (30.8)                     | 2 (15.4)                     | 3 (23.1)        |
| Diarrhoea                | 4 (30.8)                     | 1 (7.7)                      | 1 (7.7)         |
| Fatigue                  | 7 (53.8)                     | 5 (38.5)                     | 5 (38.5)        |
| Headache                 | 8 (61.5)                     | 4 (30.8)                     | 3 (23.1)        |
| Influenza-like illness   | 4 (30.8)                     | 2 (15.4)                     | 7 (53.8)        |
| Insomnia                 | 5 (38.5)                     | 4 (30.8)                     | 3 (23.1)        |
| Irritability             | 4 (30.8)                     | 3 (23.1)                     | 3 (23.1)        |
| Myalgia                  | 3 (23.1)                     | 2 (15.4)                     | 0               |
| Nausea                   | 4 (30.8)                     | 5 (38.5)                     | 2 (15.4)        |
| Neutropenia              | 4 (30.8)                     | 1 (7.7)                      | 1 (7.7)         |
| Pruritus                 | 2 (15.4)                     | 3 (23.1)                     | 4 (30.8)        |
| Pyrexia                  | 3 (23.1)                     | 0                             | 1 (7.7)         |
| Rash                     | 1 (7.7)                      | 1 (7.7)                      | 4 (30.8)        |
| Weight decreased         | 2 (15.4)                     | 0                             | 3 (23.1)        |
| Drug-induced liver injury†† | 0                             | 0                             | 0               |
| Dose reductions‡‡        |                               |                               |                 |
| Peginterferon dose        | 3 (23.1)                     | 1 (7.7)                      | 1 (7.7)         |
| Ribavirin dose           | 7 (53.8)                     | 2 (15.4)                     | 10 (76.9)       |

AE, adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase; SAE, serious adverse event. *Grade 2 constipation and grade 3 febrile neutropenia (on-treatment week 24). †One additional patient experienced an SAE (grade 3 anaemia and grade 2 leukopenia) during post-treatment follow-up week 4. ‡Grade 3 serotonin syndrome considered unrelated to treatment (grade 3 SAE with hospitalization: on-treatment week 32). §All protocol-mandated discontinuations were for increases in direct bilirubin ≥3× baseline and >upper limit of normal. ¶No grade 4 AEs were reported. **Both neutropenia. ††ALT ≥5× baseline and ≥10× ULN, and total bilirubin >2× ULN, with no other apparent possible causes of ALT elevation and hyperbilirubinemia. ‡‡In all 3 treatment groups, the most frequently reported AEs leading to a dose reduction were anaemia and neutropenia.
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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

**Figure S1.** Patient disposition.
**Figure S2.** Virologic outcomes, on-treatment observed values.
**Figure S3.** Steady-state (week 12) pharmacokinetics of beclabuvir and its equipotent metabolite BMS-794712.
**Figure S4.** Scatterplots of beclabuvir 24-week composite troughs.
**Table S1.** Demographics and baseline characteristics.