

A randomized trial of daclatasvir in combination with asunaprevir and beclabuvir in patients with chronic hepatitis C virus genotype 4 infection

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To the Editor:

While most hepatitis C virus (HCV) treatment studies have focused on genotypes 1–3, genotype 4 studies are lacking, even though it is common in the Middle East and Africa and has a growing prevalence elsewhere [1,2]. The most effective genotype 4 treatment currently approved is peginterferon (PegIFN)/ribavirin with sofosbuvir. However, both PegIFN and ribavirin carry a significant tolerability burden, and there remains a need for effective and better tolerated all-oral combinations for genotype 4 treatment.

The combination of daclatasvir (NS5A inhibitor), asunaprevir (NS3 protease inhibitor), and beclabuvir (BMS-791325) – a non-nucleoside NS5B polymerase inhibitor with comparable activity against genotypes 1a, 1b, 3a, 4a, and 5a (50% effective concentration 3–18 nM) and more variable activity against genotype 6a (9–125 nM) [3] – has demonstrated sustained virologic response (SVR) rates >90% in treatment-naïve genotype 1 infection [4]. Each of these agents is active *in vitro* against genotype 4. We therefore undertook a randomized, open-label, phase 2a exploratory evaluation of this regimen in treatment-naïve patients with genotype 4 infection.

The patients (n = 21) were enrolled at nine sites in the USA as an expansion of a larger study (ClinicalTrials.gov number NCT01455090) where the design and methods have been fully described elsewhere [4]. Briefly, treatment-naïve adults with chronic genotype 4 HCV infection were randomized 1:1 to receive a twice-daily oral regimen comprising of 75 mg or 150 mg of beclabuvir, each with daclatasvir (30 mg) and asunaprevir (200 mg), for 12 weeks with 48 weeks of post-treatment follow-up. The primary endpoint was SVR at post-treatment Week 12 (SVR₁₂), defined as HCV RNA below 25 IU/ml (COBAS TaqMan V2 assay; Roche Molecular Diagnostics, Pleasanton, CA, USA), with or without target detected. In the primary modified intention-to-treat analysis, missing HCV RNA data at post-treatment Week 12 was considered failure. An imputed analysis with missing Week 12 SVR data backwards-imputed from the next available RNA measurement was also performed. Sample size was not derived on the basis of formal statistical power. At an assumed population SVR₁₂ of 90%, 12 patients per arm was predicted to yield an 11% probability of underestimating – and a 28% probability of overestimating – SVR₁₂ by $\geq 10\%$, with a 72% probability of observing a safety event with an incidence of 10%. Patients with compensated cirrhosis were permitted although none were enrolled.

Viral subtype was assessed by phylogenetic alignment. Baseline NS5A amino acid sequences (positions 9–213) were aligned with 39 genotype 4 sequences from the European HCV Database (<http://euhcvdb.ibcp.fr/euHCVdb/>) using the ClustalW algorithm in the AlignX program of Vector NTI (Invitrogen, Carlsbad, CA, USA). Neighbor-joining trees (1000 bootstrap replicates) were generated from these alignments using Mega version 5.05 and were rooted on the ED43 genotype 4 reference sequence (Genbank accession number Y11604).

Resistance-associated polymorphisms (RAPs) associated with reduced susceptibility to daclatasvir (e.g. NS5A-L28, -L30, -Y93 variants), beclabuvir (e.g. NS5B-P495 variants) or asunaprevir (e.g. NS3-Q80, -D168, -R155 variants) were determined at baseline by population-based sequencing.

Baseline characteristics were comparable between treatment arms and are shown in [Supplementary Table 1](#). Overall patients were white and male (91% and 62% respectively), with a mean age of 51 years. Ethnic ancestry was not collected for study entry, but post hoc enquiries established 8/21 patients (38%) to be of Near or Middle Eastern origin (5 Egyptian, 1 Saudi, 1 Afghan, 1 not specified). All 21 patients completed randomized therapy and adherence to study medication was high, with 90% (19/21) assessed as $\geq 95\%$ adherent to all three study drugs, assessed by pill counts and dosing diaries. There were no virologic failures or post-treatment rebounds in either treatment arm. HCV RNA decline was rapid: median (range) log₁₀ change from baseline at day 7 was -4.39 (-4.91, -2.95) IU/ml in the 75 mg beclabuvir group and -4.01 (-5.03, -3.47) IU/ml in the 150 mg group. All patients had <25 IU/ml by Week 2 on-treatment. Two patients, one in each treatment arm, were missing data at post-treatment Week 12 but were confirmed to have <25 IU/ml of HCV RNA at their next attended visits at post-treatment Weeks 24 and/or 36. SVR₁₂ was therefore achieved by 90.9% (10/11) of patients receiving beclabuvir 75 mg and by 90.0% (9/10) patients receiving 150 mg in the primary mITT analysis, and by 100% of patients in both arms by the imputed analysis ([Table 1](#)). Concordance between SVR₁₂ and SVR₂₄ was 100% in the 9 patients in each arm with available HCV RNA data at both these post-treatment time points.

There were no serious adverse events, discontinuations for adverse events, or grade 3–4 adverse events in the study. Fifteen patients (71%) experienced adverse mild-moderate events, seven patients in the 75 mg arm and eight in the 150 mg arm; all events were of mild (11/15 patients) or moderate (4/15) intensity. Only



Table 1. Virologic responses.

HCV RNA <25 IU/ml, n/N (%)	DCV + ASV + BCV 75 mg	DCV + ASV + BCV 150 mg
Week 2	11/11 (100)	10/10 (100)
Week 4	11/11 (100)	10/10 (100)
Week 12 (end of treatment)	11/11 (100)	10/10 (100)
Post-treatment Week 4 (SVR₄)		
mITT ^a	8/11 (72.7)	9/10 (90.0)
Post-treatment Week 12 (SVR₁₂) (Primary endpoint)		
mITT ^b	10/11 (90.9)	9/10 (90.0)
Imputed (documented on or after posttreatment Week 12)	11/11 (100)	10/10 (100)
Post-treatment Week 24 (SVR₂₄) (Observed data)		
	10/10 (100)	9/9 (100)

^aThree patients in the beclabuvir 75 mg and one patient in the 150 mg group had missing data at post-treatment Week 4; all four patients achieved SVR₁₂, SVR₂₄, or SVR₃₆.
^bOne patient in each group had missing data at post-treatment Week 12, but was established to have an SVR result at their next visits (SVR₂₄ and SVR₃₆).

one grade 3 laboratory abnormality was observed: an isolated incident of hypophosphatemia (1.9 mg/dl; lower limit of normal = 2.4 mg/dl) was noted at the end of treatment/Week 12 visit and normalized by post-treatment Week 4. No grade 3 or 4 transaminase or bilirubin elevations were observed, and there was no evidence of beclabuvir dose-related differences in the regimen safety profile. Headache, insomnia, nausea and pain were reported in more than 10% of patients (Supplementary Table 2).

In the phylogenetic analysis, virus from most patients (n = 16) grouped with genotype 4a reference sequences, with the remainder grouping with 4l (n = 2), 4d, 4n or 4o (n = 1 each; Supplementary Fig. 1). No baseline RAPs in NS3 or NS5B were observed. Twelve NS5A RAPs at positions 28, 30 and/or 93 were identified in nine patients: L28M (n = 2), L30R (n = 4), L30H (n = 1), L28M + L30A (n = 1) and L28L/M + L30L/H + Y93Y/C (n = 1). In a previous study, L28 and L30 substitutions emerged together in genotype 4 infected patients with virologic failure who had received daclatasvir combined with PegIFN α and ribavirin [5]. A further 22 NS5A polymorphisms were observed at positions 54, 58, and 62; polymorphisms at these positions are associated with minimal changes in daclatasvir susceptibility in replicon assays (<2-fold elevation in EC₅₀). Most patient samples (n = 19) had a D62E substitution relative to the ED43 reference strain. Other minor polymorphisms were D62N (n = 1), P58T (n = 1) and P58S (n = 1). These RAPs did not affect virologic response in this study and all patients achieved SVR.

Thus, in this exploratory study, 12 weeks of twice-daily therapy with daclatasvir, asunaprevir, and beclabuvir (75 mg or 150 mg) resulted in a rapid and sustained decline in HCV viraemia in treatment-naïve patients with genotype 4 infection, with SVR₁₂ rates similar to the 96% SVR₁₂ seen in genotype 4 after 12 weeks of sofosbuvir plus PegIFN/ribavirin [6]. There were no notable safety or tolerability issues, consistent with previous data for this regimen with genotype 1 [7]. Thus, this combination of oral agents for treatment of genotype 4 may potentially provide an improved safety and tolerability profile compared with current PegIFN/ribavirin-containing regimens. It may also offer a tolerability advantage compared with several ribavirin-containing all-oral regimens for genotype 4 which are currently under investigation [8–10]. Adherence to the three components of the regimen (supplied in separate bottles) was high, and may be further improved by the use of a fixed-dose combination tablet currently under phase 3 evaluation.

Although baseline RAPs in NS5A were observed in just under half the patients assessed, there were no on-treatment virologic

failures, and no post-treatment relapses through the primary endpoint or in any patient for whom post-SVR₁₂ data were available at time of analysis. A combination of three different mechanistic classes of direct-acting agent may conceivably reduce the virological impact of reduced susceptibility to any one of them, but the overall impact of baseline RAPs on response to this regimen will require evaluation in a larger dataset.

The primary limitations of this study are its small sample size and the relatively mild stage of liver disease presented, both of which limit the extent to which study outcomes can be extrapolated to the broader virus and patient populations. Coupled with the present results, ongoing phase 3 studies in genotype 1 infection will help inform future studies in patients with genotype 4. Most infections were with genotype 4a, and although the consistent virologic results in the 24% of patients with non-4a infections suggest that this regimen will be effective across subtypes, a larger study in a more diverse range of genotype 4 infections will be needed to confirm this. In addition, there were no cirrhotic patients and only four patients with a FibroTest-derived fibrosis staging above F2 (FibroTest score >0.58). The absence of patients with advanced liver disease precludes conclusions about safety or efficacy in this population, although it is relevant that SVR rates above 90% have subsequently been observed for this regimen \pm ribavirin in genotype 1 infected, treatment-naïve patients with compensated cirrhosis in the phase 3 UNITY-2 study [11]. The sample size is also insufficient for detection of low-frequency safety-related events; however, this limitation is mitigated to some extent by the similarly favorable safety profile observed with this regimen in larger populations of patients with genotype 1 infection [7,11,12].

In summary, in this exploratory evaluation, 12 weeks of treatment with the IFN- and ribavirin-free combination of daclatasvir, asunaprevir and beclabuvir was well tolerated and resulted in a high rate of SVR in previously untreated patients with chronic HCV genotype 4 infection, consistent with previous results in genotype 1.

Conflict of interest

T. Hassanein has received consulting fees from Bristol-Myers Squibb. E. Lawitz has received research grants from AbbVie, Achillion, Boehringer Ingelheim, Bristol-Myers Squibb, Gilead Sciences, GlaxoSmithKline, Idenix, Janssen, Merck, Novartis, Presidio, Roche, Santaris, and Vertex, and consulting fees and/or

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Authors' contributions

K.D. Sims, D.M. Grasela, E. Chung, A. Griffies, and D.F. Gardiner designed the study. T. Hassanein, M. Bennett, N. Gitlin, E. Lawitz, T. Nguyen, L. Webster, Z. Younossi, H. Schwartz, and P.J. Thuluvath recruited patients and obtained data. H. Zhou, B. Rege, F. McPhee, N. Zhou, and M. Wind-Rotolo analyzed the data. All authors interpreted the data, participated in writing the manuscript, and approved the final version of the manuscript.

Supplementary data

Supplementary data associated with this article can be found in the online version, at <http://dx.doi.org/10.1016/j.jhep.2014.12.025>.

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