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BRIEF REPORT

In Vitro Assessment of Re-treatment Options for Patients with Hepatitis C Virus Genotype 1b Infection Resistant to Daclatasvir Plus Asunaprevir

Jacques Friborg · Nannan Zhou · Zhou Han · Xiaoyan Yang · Paul Falk · Patricia Mendez · Fiona McPhee

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ABSTRACT

Introduction: Daclatasvir is a non-structural protein 5A (NS5A) inhibitor with activity against hepatitis C virus (HCV) genotypes 1–6 in vitro, and asunaprevir is a non-structural protein 3 (NS3) protease inhibitor with activity against genotypes 1, 4, 5, and 6. This study evaluates potential options for the re-treatment of HCV genotype 1b-infected patients who have failed combination therapy with daclatasvir plus asunaprevir.

Methods: The antiviral activity of drug combination regimens in HCV subgenomic replicon cell lines representing genotype 1b (Con1 strain) wild-type or a variant with specific NS5A and NS3 amino acid substitutions conferring resistance to daclatasvir and asunaprevir were compared using replicon elimination assays. Drug concentrations representing multiple 50% effective concentrations (EC_{50}) derived in vitro and trough plasma concentrations observed in a clinical setting were utilized.

Results: At multiple EC_{50} values of each drug (3×, 10×, and 30× EC_{50}), combinations of daclatasvir plus sofosbuvir, sofosbuvir plus ledipasvir, sofosbuvir plus simeprevir, and sofosbuvir plus either a next-generation NS3 or NS5A inhibitor demonstrated comparable activity in wild-type and daclatasvir/asunaprevir-resistant cell lines. At clinically relevant drug trough concentrations, combination regimens of daclatasvir plus asunaprevir plus beclabuvir (±ribavirin), and daclatasvir plus asunaprevir plus beclabuvir plus sofosbuvir efficiently cleared daclatasvir + asunaprevir-resistant replicons from cells within 5 days of treatment.

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J. Friborg · N. Zhou · Z. Han · X. Yang · P. Falk · F. McPhee (✉)
Bristol-Myers Squibb Research and Development, 5 Research Parkway, Wallingford, CT 06492, USA
e-mail: fiona.mcphee@bms.com

P. Mendez
Bristol Myers-Squibb Research and Development, Lawrenceville, NJ, USA

Conclusion: Our in vitro results highlight a number of potential all-oral treatment options for patients who do not achieve a sustained virologic response following therapy with daclatasvir plus asunaprevir. These results require further evaluation in clinical studies.

Keywords: Asunaprevir; Beclabuvir; Daclatasvir; Hepatitis C virus; Ledipasvir; Replicon; Resistance; Re-Treatment; Simeprevir; Sofosbuvir

INTRODUCTION

Current options for the treatment of hepatitis C virus (HCV) infection are evolving rapidly with the recent approval of several direct-acting antiviral (DAA) agents. Daclatasvir (DCV) is a non-structural protein 5A (NS5A) inhibitor with activity against HCV genotypes 1–6 in vitro [1]. Asunaprevir (ASV) is a non-structural protein 3 (NS3) protease inhibitor with activity against genotypes 1, 4, 5, and 6 [2]. The all-oral, interferon-free combination of DCV + ASV provided high rates of sustained virologic response and was well tolerated in genotype 1b-infected patients in global and Japanese Phase III studies [3, 4]. Among genotype 1b-infected patients who experience virologic escape with DCV + ASV, the most common resistance-associated variants (RAVs) detected together after HCV RNA rebound occur at NS5A positions L31 and Y93, and NS3 position D168. Here, we aim to evaluate potential re-treatment options for genotype 1b-infected patients who have previously failed combination therapy with DCV + ASV using the in vitro HCV replicon system.

METHODS

HCV subgenomic replicon cell lines representing genotype 1b (Con1 strain) wild-type or a variant with specific NS5A and NS3 amino acid substitutions conferring resistance to DCV and ASV (NS5A-L31M-Y93H and NS3-D168V, respectively) were established as previously described [5]. Peginterferon alfa-2a (PEGASYS®) was purchased from Hoffman–La Roche, Inc. (Nutley, NJ, USA) and ribavirin (RBV) was purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). Simeprevir (SMV; NS3 inhibitor), sofosbuvir (SOF; NS5B inhibitor), and ledipasvir (LDV; NS5A inhibitor) were synthesized at Bristol-Myers Squibb, and have been described previously [6–8]. DCV, ASV, beclabuvir (BCV; BMS-791325; NS5B thumb 1 inhibitor), BMS-1 (next-generation NS5A inhibitor), and BMS-2 (next-generation NS3 inhibitor) were also synthesized at Bristol-Myers Squibb. The antiviral activities of the individual compounds and combination regimens were assessed using phenotypic analyses (to determine 50% effective concentrations [EC_{50}]) and replicon elimination assays, as described previously [9, 10]. The ability of drug combinations to clear replicons was evaluated using two different approaches. First, wild-type and DCV + ASV-resistant replicon cell lines with a neo-selectable marker were incubated without G418 for 1, 3, 7, 11 or 14 days with multiples of EC_{50} values for each drug (3×, 10×, and 30 × EC_{50}) determined against wild-type replicon. Combination regimens of DCV + ASV, DCV + SOF, SOF + LDV, SOF + SMV, DCV + ASV + BCV and DCV + ASV + BCV + SOF were assessed initially using multiple EC_{50} values of each agent estimated against wild-type replicon. RBV was examined at 1 × EC_{50} concentration, tenfold below any

Table 1 Cell potency of compounds against genotype 1b wild-type (Con1) and DCV + ASV-resistant replicons and C_{trough} concentration observed in clinical settings

Agent	EC_{50} (±SD), nM*			C_{trough}, nM*
	GT 1b (Con1) replicon	GT 1b NS3-D168V, NS5A-L31M-Y93H	Fold change	
Asunaprevir	2.0 ± 0.4	401 ± 102	201	40†
Daclatasvir	0.002 ± 0.001	49 ± 9	24,500	250†
Beclabuvir	3.4 ± 0.2	4.0 ± 0.7	1	500†
Ledipasvir	0.002 ± 0.0004	131 ± 40	65,500	120 [20]
Sofosbuvir	147 ± 27	102 ± 12	1	1,100†
Simeprevir	1.9 ± 0.1	6,296 ± 203	3,313	2,200†
Next-gen NS5A (BMS-1)	0.010 ± 0.002	0.354 ± 0.05	39	–
Next-gen NS3 PI (BMS-2)	0.7 ± 0.1	4.1 ± 0.6	6	100‡
Ribavirin*	8.1 ± 1.2	7.8 ± 5.7	1	2.5 [21]
Peginterferon alfa*	1.2 ± 0.2	2.6 ± 0.6	2	15 [22]

C_{trough} trough plasma concentrations, *EC_{50}* 50% effective concentrations, *GT* genotype, *NS3* non-structural protein 3, *NS5A* non-structural protein 5A, *PI* protease inhibitor, *SD* standard deviation

* All EC_{50} and C_{trough} concentrations are nM, except for ribavirin (μg/mL) and peginterferon alfa (ng/mL)

† BMS data on file

‡ Estimated value

observed cell toxicity. In another approach, replicon cell lines were incubated without G418 for 1, 2, 3, 5 and 7 days with drug concentrations representing trough plasma concentrations (C_{trough}) observed in a clinical setting. In both approaches, the drug regimen was removed at the end of the incubation period and the cell cultures were further maintained for 2 weeks in growth medium supplemented with G418 (0.5 mg/mL) to monitor replicon elimination. Surviving replicon colonies were fixed and stained with crystal violet as described previously [10].

RESULTS

Phenotypic analyses indicated that DCV + ASV-resistant replicon cell lines conferred high levels of resistance to DCV, LDV, ASV and SMV, relative to the wild-type reference replicon (Table 1). In contrast, EC_{50} values for BCV, peginterferon alfa, RBV and SOF were similar in both cell lines. The next-generation NS5A (BMS-1) and NS3 (BMS-2) protease inhibitors demonstrated improved potency (39-fold and 6-fold reduction in anti-HCV activity, respectively, relative to wild type) in the DCV + ASV-resistant replicon when compared to the activities of DCV and ASV (24,500-fold and 201-fold reduction in anti-HCV activity, respectively). HCV replicon elimination results for days 3, 7 and 14 are shown in Fig. 1 (complete results for Days 1, 3, 7, 11 and 14 are provided in Supplementary Fig. 1). With 14 days of treatment, two-DAA regimens of DCV + ASV, DCV + SOF, SOF + LDV and SOF + SMV demonstrated comparable activity

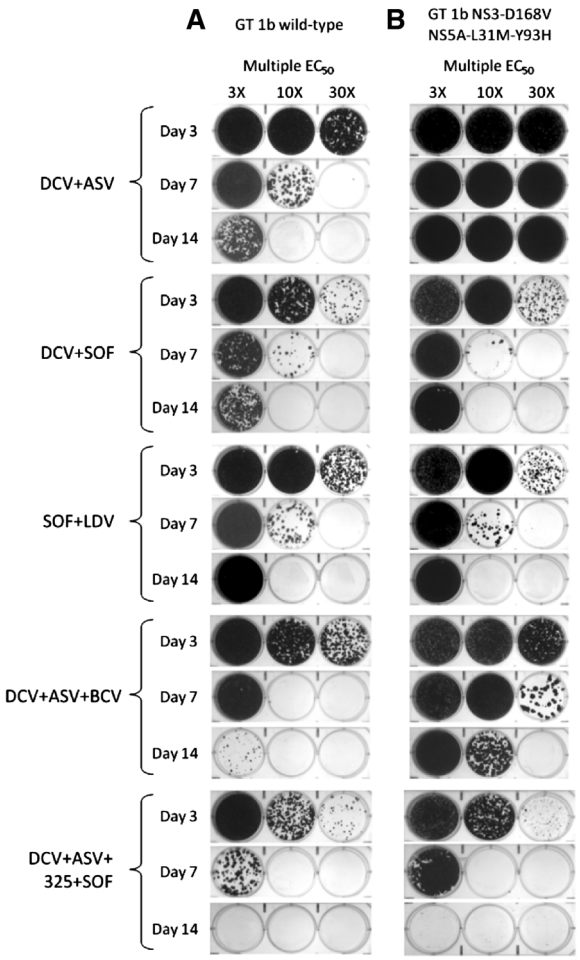

Fig. 1 HCV replicon elimination assays using **a** wild-type GT1b and **b** DCV + ASV-resistant (NS3-D168V, NS5A-L31M-Y93H) replicon cell lines treated with indicated combination regimens at multiple EC_{50} values for each agent (determined in wild-type replicon). Data for Days 3, 7, and 14 are shown; complete data are shown in Supplementary Fig. 1. *ASV* asunaprevir, *BCV* beclabuvir, *DCV* daclatasvir, *GT* genotype, *HCV* hepatitis C virus, *LDV* ledipasvir, *NS3* non-structural protein 3, *NS5A* non-structural protein 5A, *SMV* simeprevir, *SOF* sofosbuvir

in eliminating wild-type replicons at 10 × EC_{50} values (Fig. 1a). The three-DAA regimen of DCV + ASV + BCV and the four-DAA regimen of DCV + ASV + BCV + SOF demonstrated increased efficacy with complete clearance of wild-type replicons observed with the four-DAA regimen by day 11 at 3 × EC_{50} values. Similar results were observed with SOF in combination with either the next-generation NS3 protease inhibitor or a next-generation NS5A inhibitor (Supplementary Fig. 1). As expected, DCV + ASV did not eliminate replicons harboring NS5A-L31M-Y93H and NS3-D168V, which confer reduced susceptibilities to both compounds (Fig. 1b). The elimination of replicons by DCV + SOF, SOF + LDV, SOF + SMV, and SOF + next-generation NS3 protease or NS5A inhibitor was comparable in wild-type and DCV + ASV-resistant cell lines. The combination of DCV + ASV + BCV showed reduced activity against DCV + ASV-resistant replicons compared with wild type. To further evaluate the use of these combination regimens, replicon elimination assays were performed at drug concentrations based on clinically relevant C_{trough} concentrations (Table 1). Monotherapy at C_{trough} concentrations demonstrated the high potency of the NS5A inhibitors, DCV and LDV, compared with the other agents tested (Fig. 2a). With EC_{50} values in the picomolar range that are well below the high plasma C_{trough} concentrations obtained in clinical settings, treatment with these DAAs was sufficient in eliminating wild-type replicons within 7 days. Conversely, none of the NS5A inhibitors and NS3 protease inhibitors tested at C_{trough} concentrations were able to eliminate DCV + ASV-resistant replicons (Fig. 2b). Moreover, SOF as a single agent exhibited low clearance activity in this assay. Although the C_{trough} of SOF (1,100 nM) is higher than the EC_{50} (147 nM), it is below the estimated SOF EC_{90} (1,230 nM). Furthermore, the metabolism and efficiency of phosphorylation of SOF appear to be lower in hepatoma cell lines compared with primary hepatocytes. An analysis of the mechanism of activation of SOF and its analogs has demonstrated that some enzymes in these metabolic pathways, such as CES1, are expressed at significantly lower levels in Huh7

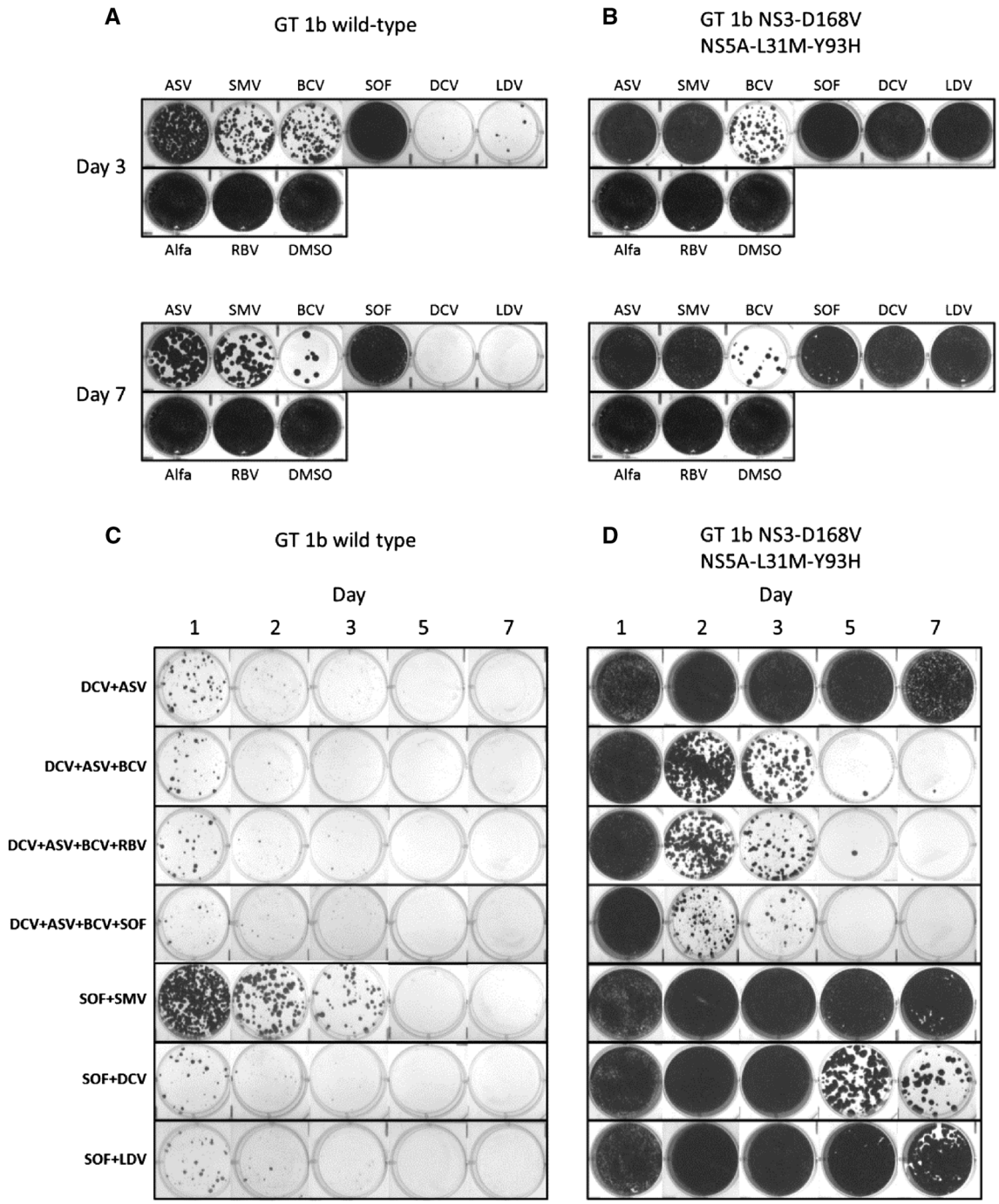

Fig. 2 HCV replicon elimination assays with single agents and combination regimens using concentrations representing C_{trough} values observed in a clinical setting in wild-type GT-1b (**a** and **c**) and DCV + ASV-resistant (NS3-D168V, NS5A-L31M-Y93H) replicon cell lines (**b** and **d**). *Alfa* peginterferon alfa, *ASV* asunaprevir, *BCV* beclabuvir, C_{trough} trough plasma concentrations, *DCV* daclatasvir, *DMSO*, dimethyl sulfoxide, *GT* genotype, *HCV* hepatitis C virus, *LDV* ledipasvir, *NS3* non-structural protein 3, *NS5A* non-structural protein 5A, *RBV* ribavirin, *SMV* simeprevir, *SOF* sofosbuvir

cells compared with primary hepatocytes [11]; thus, the anti-HCV activity of SOF in replicon-based assays may not correlate with its activity in vivo. Similar instances of low activity with nucleosides in hepatoma-derived Huh7 cells harboring replicons have been reported [12, 13]. However, SOF has a high barrier to resistance and has demonstrated efficacy in combination regimens. For wild-type replicons, all DAA combinations at C_{trough} concentrations that included an NS5A inhibitor eliminated replicons with high efficiency (Fig. 2c). In comparison, elimination of wild-type replicons was less efficient with SOF + SMV and peginterferon alfa-based combinations (Fig. 2c and Supplementary Fig. 2). Complete elimination of DCV + ASV-resistant replicons occurred by day 7 with the three-DAA regimen (DCV + ASV + BCV) ± RBV or with the four-DAA regimen (DCV + ASV + BCV + SOF) (Fig. 2d). Replicon elimination profiles were comparable in wild-type and DCV + ASV-resistant cell lines following treatment with peginterferon alfa/RBV-based regimens combined with SOF or BCV, or the DAA-only combination of SOF with a next-generation NS3 protease inhibitor (Supplementary Fig. 2).

DISCUSSION

In summary, these in vitro experiments demonstrate that there are a number of potential alternate all-oral DAA treatment options available for genotype 1b-infected patients who experience virologic escape during DCV + ASV therapy. These include the three-DAA regimen of DCV + ASV + BCV combined with RBV or SOF, or other SOF-based combinations. Furthermore, the results suggest that a peginterferon alfa/RBV-based regimen with agents targeting NS5B could also provide effective therapy for these patients. Results from this in vitro study will require further evaluation in clinical studies. The efficacy of re-treating patients who have failed prior boceprevir or telaprevir therapy with multiple DAAs (DCV + SOF, SOF + LDV) has already been demonstrated, with sustained virologic response rates of up to 99% achieved with 24 weeks of treatment [14, 15]. However, studies on the treatment of patients with resistance to multiple DAAs are more limited; existing data suggest that effective options are available, and that it may also be possible to re-treat patients with DAAs from the same drug class when combining with additional agents targeting complementary mechanisms of action [16–19].

CONCLUSION

Our in vitro results indicate that re-treatment with DAAs of the same class plus additional DAAs targeting different mechanisms of action resulted in clearance of replicons similar to wild-type replicons. This was observed when DCV + ASV-resistant replicons were treated with DCV + SOF, SOF + LDV and DCV + ASV + BCV + SOF. Re-treatment data in the clinic are currently minimal; however, patients have been successfully retreated with the same DAAs plus the addition of another agent.

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Conflict of interest. Jacques Friborg is an employee of Bristol-Myers Squibb. Nannan Zhou is an employee of Bristol-Myers Squibb. Zhou Han is an employee of Bristol-Myers Squibb. Xiaoyan Yang is an employee of Bristol-Myers Squibb. Paul Falk is an employee of Bristol-Myers Squibb. Patricia Mendez is an employee of Bristol-Myers Squibb. Fiona McPhee is an employee of Bristol-Myers Squibb.

Compliance with ethics guidelines. This article does not contain any new studies with human or animal subjects performed by any of the authors.

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