

# Efficacy and Safety of Daclatasvir in Hepatitis C: An Overview

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## Abstract

Hepatitis C virus (HCV) infection is a growing public health concern, with 184 million people infected worldwide. During the past decade, interferon has been the backbone of HCV treatment, even though it remains far from ideal. The latest development of the new direct antivirals has drastically changed the treatment approach for chronic hepatitis C (CHC). Inhibitors of the HCV NS5A region have garnered remarkable interest among treating physicians, due to their high potency and favourable safety profile. In particular, treatment with daclatasvir (DCV) has yielded high rates of virologic response in patients infected with genotype (Gt) 1 and Gt 3, when used in combination with other antivirals of a different class, such as sofosbuvir. Although few data are available for DCV treatment of the other Gts, the results in patients with Gt 2 and Gt 4 infection appear promising, as do those for unique patient populations. NS5A-resistant viral variants can pre-exist or emerge after treatment failure for the HCV NS5A inhibitors. Nonetheless, DCV-resistant viral variants continue to be sensitive to interferon and other classes of antivirals such as NS3/4A and NS5B inhibitors. Herein, we aimed to provide an overview of the current knowledge about DCV in the treatment of CHC.

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## Introduction

Hepatitis C virus (HCV) infection is an increasing public health concern, with an estimated 184 million people infected across the globe.<sup>1</sup> Moreover, approximately 700,000 deaths annually are reported as resulting from HCV-related complications, most of which involving cirrhosis and hepatocellular carcinoma.<sup>2</sup>

For the past decade, interferon (IFN) has been the backbone of all anti-HCV treatment strategies. However, all of the regimens utilizing IFN yielded relatively unsatisfactory rates of virologic response, along with poor safety profiles. Indeed,

research to define the molecular mechanisms underlying HCV replication has facilitated the development of new antiviral molecules that halt the process by targeting/blocking various factors at a variety of steps.<sup>3</sup> The HCV viral genome encodes a single open reading frame of ~ 3000 amino acids. It is cleaved by host and viral proteases into three structural proteins (the nucleocapsid and envelope glycoproteins: core, E1, and E2) and seven non-structural ones (protein p7, derived from E2 cleavage and NS2, NS3, NS4A, NS4B, NS5A, and NS5B).<sup>3</sup>

A plethora of direct-acting antivirals (DAAs) is now available. The first generation DAAs consist of the NS3/4A serine protease inhibitors, such as telaprevir and boceprevir. The second-generation DAAs consist of the NS3/4A protease inhibitors, such as simeprevir, asunaprevir (ASN), paritaprevir and grazoprevir, the NS5B non-nucleoside polymerase inhibitors, such as dasabuvir, and nucleotide analogues, such as sofosbuvir (SOF). Recently, a new class of DAAs has been developed, and these include ledipasvir, daclatasvir (DCV), elbasvir, velpatasvir and ombitasvir. This new class is made up exclusively of NS5A replication complex inhibitors, and each has shown excellent antiviral efficacy and a low barrier to resistance.<sup>4</sup>

NS5A is a phosphorylated protein that has been studied extensively.<sup>5,6</sup> Its critical roles in viral replication, assembly and secretion<sup>6</sup> suggest it as a good target of antiviral molecules.

DCV/BMS-790052 (Bristol-Myers Squibb) is an NS5A inhibitor. This class of DAAs has two distinct mechanisms of action: a) blocking of HCV-RNA replication to prevent formation of the so-called 'membranous web' in which functional HCV-RNA replication takes place; and b) rapid inhibition of intracellular virion assembly.<sup>7</sup> The benefits of DCV include a picomolar potency, a pharmacokinetic profile that permits once-daily dosing, and broad genotypic coverage *in vitro*.<sup>8,9</sup> In addition, DCV is highly selective for HCV, as evidenced by its lack of clinically important antiviral activity against other viruses [e.g. human immunodeficiency virus (HIV)].<sup>10</sup> In June 2014, The European Medicines Agency's Committee for Medicinal Products for Human Use (CHMP) granted marketing approval for DCV in association with other medicines for the therapy of chronic hepatitis C (CHC). Moreover, the combination of DCV and ASN (an HCV NS3/4A protease inhibitor) was approved in Japan for the treatment of patients infected with Gt 1 HCV.<sup>11</sup>

## Pharmacokinetics

The pharmacokinetic patterns of DCV have been studied in both healthy and chronically infected patients. In HCV-infected subjects, the peak plasma concentrations occurred within 2 hours after multiple oral doses of DCV tablet administered in doses ranging from 1 to 100 mg once daily.<sup>10</sup> In regards to

**Keywords:** Daclatasvir; NS5A inhibitor; Hepatitis C; Direct antivirals; New antiviral therapy.

**Abbreviations:** AE, adverse event; ASN, asunaprevir; DCV, daclatasvir; CTP, Child-Turcotte-Pugh; CHC, chronic hepatitis C; Gt, genotype; HCV, hepatitis C virus; Peg-IFN, pegylated-interferon; RBV, ribavirin; SAE, serious adverse event; SOF, sofosbuvir; SVR, sustained virologic response.

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effects of food intake, no reduction in DCV concentration was observed when the drug was administered after a light meal. Notably, DCV has been found to be primarily removed by hepatic metabolism and direct biliary excretion. As a consequence, renal impairment is not a contraindication for DCV administration.<sup>10</sup> Fundamentally, no dosage adjustment of DCV is necessary for subjects with hepatic impairment [all classes of Child-Turcotte-Pugh (CTP)].

### Clinical Trials with Daclatasvir

DCV has been evaluated in more than 13,000 treatment-naïve and treatment-experienced CHC patients, harbouring infections with all the various Gts. It has also been studied in combination with pegylated-interferon (Peg-IFN) or with other DAAs, with and without ribavirin (RBV). Based on Gt, we will subsequently describe all clinical trials that have been conducted to assess both efficacy and safety of DCV. To this end, we performed a standardized search of the MEDLINE, PubMed, Google Scholar and international conference abstract databases using the following search terms: 'BMS-790052', 'daclatasvir', 'directly acting antivirals' and 'hepatitis C.' A manual exploration of references was also implemented to recognize articles not found by the electronic searches.

#### Interferon-based therapeutic regimens (Table 1)

##### Genotype 1

In the phase IIb study known as COMMAND-1, Hezode *et al.*<sup>12</sup> assessed the effectiveness of Peg-IFN and RBV plus DCV in treatment-naïve Gt 1 and Gt 4 subjects. Three hundred and ninety-five Gt 1 patients were randomized to receive standard therapy plus DCV at 20 mg (arm A), DCV at 60 mg (arm B) or

placebo (arm C). The addition of DCV improved sustained virologic response (SVR) rates to 59%<sup>13</sup> and 60% (B) compared to 38% in arm C. Notably, the DCV arms (A and B) were similar to the placebo arm (C) in rates of discontinuation and serious adverse events (SAEs) without safety events related to the experimental drug.

Lok *et al.*<sup>14</sup> conducted a randomized, phase IIa, open-label, 24-week treatment trial. Subjects with Gt 1 infection who previously failed HCV antiviral treatment were randomized to the following 5 different regimens, all including DCV once daily (60 mg): 1) DCV plus ASN (NS3 protease inhibitor, 200 mg) twice-daily (n=18); 2) DCV plus ASN once-daily (n=20); 3) DCV plus ASN twice-daily plus Peg-IFN and RBV (n=20); 4) DCV plus ASN once-daily plus Peg-IFN and RBV (n=21); 5) DCV plus ASN twice-daily plus RBV (n=22). Indeed, rates of SVR24 were highest in group 3 and group 4 (90% and 95%, respectively) and without virologic breakthrough. However, the rate of such an event was rather high in group 5 (10/22, 46%, all Gt 1a). Headache, diarrhoea, and asthenia were the most common adverse events (AEs). Grade 3 or 4 hematologic laboratory alterations occurred only in the groups treated with IFN-based therapy.

##### Genotype 2

Only one study reported data regarding DCV plus Peg-IFN and RBV in Gt 2 treatment-naïve subjects. Dore *et al.*<sup>15</sup> reported data of a randomized, double-blind, phase IIb study that involved subjects infected with Gt 2 and Gt 3. Patients were assigned to receive 12 or 16 weeks of DCV (60 mg once daily) or 24 weeks of placebo, each combined with Peg-IFN and RBV. Among the patients with Gt 2 infection, a similar SVR24 was achieved among those subjects treated with DCV for 12 or 16 weeks (83%) versus those who received placebo (63%).

**Table 1. Interferon-based trials utilizing daclatasvir**

First Author <sup>Ref</sup>	Design	Genotype (n of subjects)	Treatment Status	Cirrhosis	Treatment Arms	SVR (%)
Hezode <sup>12</sup>	Double-blind placebo controlled	1 (365)	Naïve	Yes	A- DCV (20 mg)+Peg-IFN+RBV B- DCV (60 mg)+Peg-IFN+RBV C- Placebo+Peg-IFN/RBV	A- 59% B- 60% C- 38%
		4 (30)	Naïve	Yes	A- DCV (20 mg)+Peg-IFN+RBV B- DCV (60 mg)+Peg-IFN+RBV C- Placebo+Peg-IFN/RBV	A- 67% B- 100% C- 50%
Lok <sup>14</sup>	Open-label	1 (101)	Null-responders	No	1- DCV+ASV twice daily 2- DCV+ASV once daily 3- DCV+ASV twice daily+Peg-IFN+RBV 4- DCV+ASV once daily+Peg-IFN+RBV 5- DCV+ASV twice daily+RBV	1- 83% 2- 60% 3- 90% 4- 95% 5- 23%
Dore <sup>15</sup>	Double-blind placebo control	2 (71)	Naïve	Yes	A- DCV+Peg-IFN+RBV (12w) B- DCV+Peg-IFN+RBV (16w) C- Placebo+Peg-IFN/RBV	A- 83% B- 83% C- 63%
		3 (80)	Naïve	Yes	A- DCV+Peg-IFN+RBV (12w) B- DCV+Peg-IFN+RBV (16w) C- Placebo	A- 69% B- 67% C- 59%

Abbreviations: Peg-IFN, pegylated-Interferon; DCV, daclatasvir; SOF, sofosbuvir; RBV, ribavirin; ASV, asunaprevir; BEC, beclabuvir.

## Genotype 3

Only the above-reported study by Dore *et al.*<sup>15</sup> assessed DCV plus standard therapy in Gt 3 treatment-naïve patients. The rates of SVR24 attained were 69%, 67%, and 59% of patients in the 3 groups, respectively.

## Genotype 4

In the above-cited COMMAND-1 study,<sup>12</sup> 30 Gt 4 treatment-naïve patients were randomized to receive DCV (20 mg or 60 mg) or placebo plus Peg-IFN for 24 weeks. Notably, SVR rates for both subgroups (DCV 20 mg, 66.7% and 60 mg, 100%) were high when compared with placebo (50%) and patients infected with Gt 1.

**Interferon-free therapeutic regimens (Table 2)**

## Genotype 1

Everson *et al.*<sup>16</sup> reported data from 66 Gt 1 treatment-naïve, non-cirrhotic subjects that had been randomly allocated to receive DCV (60 mg, once daily), ASN (200 mg, twice daily), and the NS5B non-nucleoside polymerase inhibitor BMS-791325 (75 mg or 150 mg, twice daily) for 12 or 24 weeks. Sixty-one of the patients (92%) achieved SVR12. Notably, the rates of SVR were similar among the 12- and 24-week groups. During the study, two patients experienced virologic breakthrough and one patient relapsed (3/66, 4.5%). There were no deaths or discontinuations resulting from SAEs, or even AEs, related to the study drugs.

**Table 2. Interferon-free trials utilizing daclatasvir**

First Author <sup>Ref</sup>	Design	Genotype (n of subjects)	Status	Cirrhosis	Treatment Arms	Svr (%)
Lok <sup>14*</sup>	Open-label	1 (101)	Null-responders	No	A- DCV+ASV twice daily B- DCV+ASV once daily C- DCV+ASV twice daily +Peg-IFN+RBV D- DCV+ASV once daily +Peg-IFN+RBV E- DCV+ASV twice daily +RBV	A- 83% B- 60% C- 90% D- 95% E- 23%
Everson <sup>16</sup>	Open-label	1 (66)	Naïve	No	DCV+ASV+BMS-791325	92%
Sulkowski <sup>17</sup>	Open-label	1 (167)	Naïve and Experienced	No	DCV+SOF±RBV	Naïve 98% Experienced 98%
		2 (26)	Naïve and Experienced	No	DCV+SOF±RBV	Naïve 92% Experienced 92%
		3 (18)	Naïve and Experienced	No	DCV+SOF±RBV	Naïve 89% Experienced 89%
Manns <sup>18</sup>	Open-label	1b (747)	Naïve, Experienced and IFN-ineligible	Yes	DCV+ASV	Naïve 90% Experienced 82% Ineligible 82%
Poordad <sup>19</sup>	Open-label	1 (415)	Naïve and Experienced	No	DCV+ASV+BEC	Naïve 92% Experienced 89%
Muir <sup>21</sup>	Open-label	1 (202)	Naïve and Experienced	Yes	DCV+ASV+BEC±RBV	Naïve 93% Experienced 87%
Nelson <sup>24</sup>	Open-label	3 (152)	Naïve and Experienced	Yes	DCV+SOF	Naïve 90% Experienced 86%
Leroy <sup>25</sup>	Open-label	3 (50)	Naïve and Experienced	Yes	DCV/SOF+RBV	Naïve 92% Experienced 89%
Welzel <sup>22</sup>	Open-label	3 (102)	Naïve and experienced	Yes	DCV+SOF±RBV	Naïve 94% Experienced 82%
Hezode <sup>26</sup>	Open-label	3 (282)	Naïve and experienced	Yes	DCV+SOF±RBV	With RBV: 87% Without RBV: 83%
Hassanein <sup>28</sup>	Open-label	4 (21)	Naïve	No	A- DCV+BEC (75 mg) +ASV B- DCV+BEC (150 mg) +ASV	A- 91% B- 90%

Abbreviations: DCV, daclatasvir; SOF, sofosbuvir; RBV, ribavirin; ASV, asunaprevir; BEC, beclabuvir.  
\* cited also in Table 1.

The antiviral combination of DCV and SOF, with or without RBV, was evaluated in an open-label study by Sulkowski *et al.*<sup>17</sup> in which 167 Gt 1 subjects (126 naïve, 41 experienced) and 44 Gt 2 or Gt 3 treatment-naïve patients were randomly assigned to receive DCV (60 mg) plus SOF (400 mg), with or without RBV, for 12 or 24 weeks. One hundred and sixty-four of the 167 Gt 1 patients (98%) attained SVR12. Notably, rates of SVR12 were similar for patients infected with the various viral subtypes (Gt 1a, 98%; Gt 1b, 100%), IL28B genotype (CC, 93%; non-CC, 98%), and RBV treatment (with, 94%; without, 98%). As expected, RBV recipients had a major reduction in haemoglobin level. Fatigue, headache, and nausea were the most common AEs reported. Serious AEs occurred in 10 patients, and in 2 of those cases (<1%) led to treatment discontinuation.

HALLMARK-DUAL<sup>18</sup> was a phase III multicohort study concerning Gt 1b patients who were treatment-naïve and previous non-responders/ineligible for Peg-IFN/RBV, including patients with cirrhosis. The treatment-naïve patients (n=307) received DCV (60 mg, once daily) plus ASN (100 mg, twice daily) or placebo for 12 weeks. The non-responders (n=205) and ineligible, intolerant patients (n=235) received the same therapy for 24 weeks. This IFN-free regimen led to SVR12 in 182 (90%) of the patients in the treatment-naïve cohort, 168 (82%) in the non-responder cohort, and 192 (82%) in the ineligible, intolerant cohort. Among the treatment-naïve patients, no differences were found in terms of SVR12 between patients with or without cirrhosis (91% and 89%, respectively). Likewise, no meaningful differences were observed in terms of SVR among the previously-treated or IFN-ineligible patients with cirrhosis. Overall, the rates of SAEs were low (5%–7%) across all treatment groups. Similarly, grade 3 or 4 laboratory anomalies and AE-related discontinuations were uncommon. No deaths were recorded.

UNITY-1 was an open-label international study<sup>19</sup> that involved 312 treatment-naïve and 103 treatment-experienced patients with Gt 1 infection who had been enrolled and administered DCV (30 mg), ASN (200 mg), and beclabuvir<sup>20</sup> (an NS5B non-nucleoside inhibitor; 75 mg) for 12 weeks. SVR12 was obtained by 379 of the 415 patients (91.3%; 95% CI: 88.6%–94.0%). In particular, this finding involved 287 of the 312 treatment-naïve patients (92.0%) and 92 of the 103 treatment-experienced patients (89.3%). Rates of virologic failure were low (n=34/415, 8%). AEs leading to treatment discontinuation occurred in less than 1%. Notably, one patient died at post-treatment week 3 for a reason not related to the study.

The all-oral regimen of DCV plus ASN and BEC, with the addition of RBV, was also evaluated for patients with Gt 1 infection and compensated cirrhosis in the UNITY-2 study.<sup>21</sup> One hundred and twelve treatment-naïve and 90 treatment-experienced patients were treated for 12 weeks. In the treatment-naïve patients, SVR12 was achieved by 98% and 93% with and without RBV, respectively. Comparable rates of SVR12 were achieved among the treatment-experienced patients (93% and 87%, with and without RBV, respectively). In total, SVR12 was achieved by 88% of those receiving the fixed-dose combination alone and by 95% of those with RBV added to the regimen. Based on further analysis of the subgroups, the investigators suggested that inclusion of RBV with the regimen may be considered for patients with Gt 1a infection. The treatment was considered safe and well tolerated with only three serious AEs (2.5%) reported that had been considered as treatment-related, and in 4 cases (3.3%) an AE led to treatment discontinuation.

An important real-world study by Welzel *et al.*<sup>22</sup> evaluated a large cohort of 458 patients infected with Gt 1, 2, 3, 4 and 5, among which 42% had decompensated cirrhosis. The recommended therapeutic regimen was DCV plus SOF with or without RBV for 24 weeks. Patients were infected primarily with HCV GT 1b (36%) and 1a (33%). In the modified intention-to-treat analysis, SVR12 was achieved by 149/155 (96%) of GT 1a patients and 150/169 (89%) of GT 1b patients. Notably, rates of virologic response were high irrespective of the severity of hepatic disease.<sup>22</sup>

#### Genotype 2

In the above-mentioned trial by Sulkowski *et al.*,<sup>17</sup> the DCV plus SOF combination (with or without RBV) was evaluated in 26 treatment-naïve Gt 2 patients, including those with cirrhosis. After randomization, all subjects received the DCV (60 mg) plus SOF (400 mg) treatment for 24 weeks. The overall rate of SVR12 was 92%. Notably, the addition of RBV did not provide any additional benefit in terms of SVR but did increase side effects.<sup>17</sup>

A recently published small trial conducted by Mangia *et al.*<sup>23</sup> explored the IFN-free and RBV-free combination treatment with DCV plus SOF among patients with Gt 2 infection and including treatment-experienced patients and those with CPT class A&B cirrhosis. A small cohort of 20 patients were included in this study, and 19 received the DCV plus SOF combination for 12 or 24 weeks. Surprisingly, all patients attained SVR, supporting the use of this regimen for 12 weeks in non-cirrhotics or 24 weeks in cirrhotic Gt 2 patients who are RBV-intolerant, including those with decompensated disease.<sup>23</sup>

#### Genotype 3

The combination of DCV plus SOF, with or without RBV, was assessed in the same trial by Sulkowski *et al.*<sup>17</sup> in 18 Gt 3 infected, treatment-naïve patients, including patients with cirrhosis. In this subgroup, the SVR12 was 89%. As for patients infected with Gt 1 and Gt 2, the inclusion of RBV produced a negative impact on the safety profile, without adding any additional benefit in terms of SVR.

In the phase III ALLY-3 study, Nelson *et al.*<sup>24</sup> evaluated a 12-week therapeutic protocol with DCV (60 mg) plus SOF (400 mg) in patients infected with Gt 3 and who were either treatment-naïve (n=101) or treatment-experienced (n=51). Patients with cirrhosis (up to 50% in each cohort) were eligible for the study as well. During the course of treatment, no virologic breakthrough was registered. SVR12 rates were 90% (91/101) and 86% (44/51) in the treatment-naïve and treatment-experienced subjects, respectively. Among the non-responders, 5 of 7 patients (71.4%) previously treated with a SOF-based regimen and 2 of 2 (100%) who previously failed treatment with alisporivir achieved SVR12. As expected, SVR rates were lower in patients with severe fibrosis (63% vs. 96%). The administration of this DCV plus SOF treatment regimen led to no AEs that necessitated treatment withdrawal. Only one SAE (<1%), not related to study medications, was registered.

ALLY-3+ is a recently published phase III study that evaluated DCV plus SOF and RBV in treatment-naïve and treatment-experienced Gt 3 patients with advanced fibrosis or cirrhosis. The subjects had been randomized in a 1:1 manner to receive either a 12- or 16-week treatment regimen. Overall, 50 patients were treated and 45 of those achieved SVR12 (90% overall; 92% among the treatment-naïve and

89% among the treatment-experienced). All patients with advanced fibrosis attained SVR12 in both the 12- and 16-week arms. Among the patients with cirrhosis, SVR12 was achieved in 83% and 89% of cases in the 12- and 16-week arms, respectively. This treatment regimen was considered safe and well tolerated since no treatment interruptions related to AEs were observed.<sup>25</sup>

A similar study was the real-life experience of the European DCV compassionate use program (CUP; AI444-237), in which the combination of DCV plus SOF, with or without RBV, for 24 weeks in Gt 3 patients with advanced liver disease was evaluated. Approximately 70% of the participants were treatment-experienced. In total, 485 patients of different genotypes were included in this program and 460 were included in the primary efficacy analysis. SVR12 was achieved by 82/93 (88%) of the patients infected with Gt 3, including 88% among those treated with DCV plus SOF and 89% treated with DCV plus SOF and with RBV.<sup>22</sup>

Of note, improvements in liver function were observed, with few cases of discontinuation due to AEs, of treatment-correlated serious AEs, or of grade 3/4 laboratory anomalies.

The French ATU (Temporary Authorisation for Use) program for DCV provided early, pre-market authorization access to DCV for HCV patients with advanced liver disease and without other antiviral treatment options. The primary efficacy population (n=284) was composed of patients within the safety population (n=468) who had available HCV-RNA data at 12 weeks after treatment discontinuation. Approximately 73% of the enrolled subjects were treatment-experienced, including patients with advanced fibrosis (15%) and cirrhosis (79%). All patients were randomized to receive either a 12- or 24-week course of DCV plus SOF, with or without RBV at the discretion of the investigator. The interim analysis confirmed high rates of overall SVR (82%–100%). However, the secondary analysis of the subgroups demonstrated a rather unsatisfactory SVR12 rate among the cirrhotic patients who had undergone the 12-week treatment course (72%); thus, 24 weeks was suggested as the optimal duration to treat such patients.<sup>26</sup>

Such data about the duration of treatment in cirrhotic patients were confirmed by Eley *et al.*<sup>27</sup> who concluded that cirrhotic patients infected with Gt 3 required a longer duration of treatment (>12 weeks) with the DCV plus SOF combination therapy or with the addition of RBV, in case they should be treated for only 12 weeks.

#### Genotype 4

In the study by Hassanein *et al.*,<sup>28</sup> 21 Gt 4 treatment-naïve adults were randomized in a 1:1 manner to receive twice-daily oral regimen of BEC at 75 mg or at 150 mg, each with DCV (30 mg) and ASN (200 mg), for 12 weeks. In this exploratory study, SVR12 was achieved by 90.9% (10/11) of the patients who received BEC at 75 mg and by 90% (9/10) of the patients who received 150 mg. No serious AEs or discontinuations due to AEs were reported.

Hezode *et al.*<sup>26</sup> explored the efficacy of DCV plus SOF, with or without RBV, in the French ATU programme; patients were infected with HCV Gt 4 (n=2015), 5 (n=26) and 6 (n=5). This study protocol included adult patients with the following conditions: 1) METAVIR fibrosis score of F3 or above; and 2) with any fibrosis score: extrahepatic manifestations, post-liver transplant HCV recurrence or indication for liver or kidney transplant. Patients were treated for 12 or 24 weeks. In this

real-life study, the rates of SVR12 were generally high, 91%, 100% and 100% among the Gt 4, Gt 5 and Gt 6 patients, respectively. Treatment terminations due to AEs were uncommon (1%) and none were considered drug-related.

## Unique patient populations

### Liver transplant recipients

HCV recurrence after liver transplantation is associated with lower rates of patient survival. Indeed, SVR is associated with increased life expectancy in such cases.<sup>30,31</sup>

Herzer *et al.*<sup>32</sup> were the first to describe the first use of DCV, SMV and RBV as an all-oral triple regimen administered to 6 liver transplant recipients with recurrent CHC; this cohort included one patient that was infected with Gt 1a and 5 patients infected with Gt 1b. All subjects were treated for 24 weeks. Notably, although none of the 6 patients responded to previous treatment using the standard IFN-based therapy, 4 exhibited SVR24. Furthermore, clinical measures of liver function improved substantially for all patients and AEs were few and limited to RBV-induced moderate anaemia. Importantly, adjustments to the immunosuppressant dosage were not required.

In the phase 3 ALLY-1 trial, Poordad and colleagues<sup>33</sup> used a 12-week course of SOF plus DCV with RBV to treat 53 patients with HCV recurrence after liver transplant and 60 patients with advanced cirrhosis; infections with Gt 1 (76%), Gt 2, Gt 3, Gt 4, and Gt 6 were represented. Patients were both treatment-naïve and treatment-experienced. Enrolled patients had CPT class A, B, or C cirrhosis and Model for End-Stage Liver Disease (MELD) scores ranging from 8 to 27. Interestingly, the overall SVR12 rate was 82% for the Gt 1 patients with advanced cirrhosis and 95% and 91% for the patients with Gt 1 and Gt 3, respectively, in the post-transplant arm. No deaths occurred during the study. SAEs were observed in 17% of the advanced cirrhosis patients and 9% of the post-transplant subjects. However, no drug-related events were considered to have been related to the study drugs.

A larger scale study conducted by Fontana *et al.*<sup>34</sup> explored the DCV-based regimens in liver transplant recipients who also had severe recurrent CHC. The study included 97 liver transplant recipients, of which 93% had Gt 1 HCV infection and 31% had biopsy-proven cirrhosis. The mean MELD score was 13±6, and 12% of the patients were CTP class C. Antiviral regimens comprised DCV plus SOF (n=77), DCV plus SMV (n=18), and DCV plus SMV and SOF (n=2); RBV was administered to 35% of the patients. Overall, 84/97 (87%) patients attained SVR12. Eight deaths occurred during the study and none were attributed to therapy. Notably, hepatic function, as expressed by both CTP and MELD scores, considerably improved with the beginning of antiviral therapy.

### HCV/HIV co-infected patients

The DCV plus SOF combination has shown efficacy in patients with HCV and HIV type 1 (HIV-1) co-infection. ALLY-2<sup>35</sup> was an open-label clinical trial that included 151 treatment-naïve and 52 treatment-experienced CHC patients, all infected with HIV-1. The treatment-naïve subjects were randomly assigned in a 2:1 manner to receive either 12 weeks or 8 weeks of DCV (60 mg, once daily, with dose modification for concomitant antiretroviral therapy) plus SOF (400 mg, once daily). The treatment-experienced patients were assigned to undergo

12 weeks of therapy at the same doses. The patients were infected with HCV Gt 1 (83%), Gt 2, Gt 3 and Gt 4, and 14% had compensated cirrhosis. Rates of SVR12 across all genotypes were 97% after 12 weeks of treatment, irrespective of their anti-HCV treatment history or a concomitant antiretroviral regimen, without disruption in their HIV-1 virologic control. Nonetheless, HCV relapse was more common (24%) after 8 weeks of treatment than after 12 weeks of treatment, suggesting that the 12-week course might be the preferred duration of treatment for most patients with HIV/HCV co-infection. Notably, there were no study drug discontinuations because of AEs, and SAEs were rarely reported.

An interesting Austrian real-life study<sup>36</sup> investigated both safety and efficacy of DCV and SOF combination in 31 HIV/HCV co-infected patients with advanced liver disease. Approximately half of the patients in the study were treatment-experienced, and the various treatment durations were: 12 weeks in the Gt 1 and Gt 4 patients without cirrhosis; 24 weeks in the Gt 1 and Gt 4 patients with cirrhosis; and 24 weeks in the Gt 3 patients. If HCV-RNA was detectable at 4 weeks before the end of therapy, the duration was extended by 4 weeks at a time. Surprisingly, 100% of the enrolled patients attained SVR12. Notably, HCV eradication determined a remarkable improvement in liver stiffness (median change, -3.6 kPa) and in liver enzymes.

### **Chronic haemodialysis patients with CHC**

The DCV plus ASN combination therapy was assessed in Japanese haemodialysis subjects with Gt 1 infection, including patients with compensated cirrhosis.<sup>37</sup> Overall, 21 patients completed a 24-week treatment course of DCV (60 mg, once daily) and ASN (100 mg, twice daily). Rates of SVR12 were high (20/21, 95%). Furthermore, this combination had an excellent safety profile and even significantly improved levels of serum alanine aminotransferase and albumin. Recently, Toyoda *et al.*<sup>38</sup> investigated the safety and viral responses of the same combination (DCV plus ASN for 24 weeks) in 28 patients with Gt 1 infection who were receiving hemodialysis, and 56 who had no renal dysfunction. The two groups included cirrhotic subjects (39%). The rates of SVR were comparable between the two groups; the rate of SVR12 was 100% in patients receiving haemodialysis and 94.6% in subjects without renal dysfunction. In addition, no important AEs were reported.

### **Warnings and precautions**

In clinical trials, approximately 2400 subjects with CHC have been treated with the recommended dose of DCV in combination with another anti-HCV drug in clinical studies. Overall, this drug was found to be safe and well tolerated in all tested combinations. In particular, fatigue (14%), headache (14%), nausea (8%), and diarrhoea (5%) were among the most commonly reported AEs among patients treated with DCV/SOF combination with and without RBV.<sup>10</sup> However, in 2015, the FDA advised against the concomitant use of amiodarone with DCV in combination with SOF, as this can cause serious symptomatic bradycardia.<sup>39</sup>

### **Drug-drug interactions**

DCV is a CYP3A4 substrate, as well as a substrate and inhibitor of P-gp. Additionally, it inhibits organic anion transporting polypeptide (OATP) 1B1, organic cation transporter

(OCT) 1 and breast cancer resistance protein (BCRP). Therefore, attention must be paid during concomitant administration of substrates of P-gp, OATP 1B1, OCT1 or BCRP. In fact, they may prolong their therapeutic effect and adverse reactions. Notably, DCV dose should be reduced to 30 mg once daily when co-administered with strong inhibitors of CYP3A4 (e.g. telaprevir, boceprevir, atazanavir/ritonavir, cobicistat, clarithromycin, telithromycin, and ketoconazole). Similarly, DCV dose should be increased to 90 mg/day when it is administered along with moderate CYP3A inducers (e.g. bosentan, dexamethasone, and nafcilin). Its co-administration with strong inducers of CYP3A4 is contraindicated. Drug-drug interactions and dose recommendations of DCV with commonly used medications are summarized in Table 3.

### **Onset of resistance: A real clinical problem?**

So far, HCV NS5A-targeting molecules are amongst the most potent antivirals with broad activity against HCV genotypes and subtypes.<sup>40</sup> However, the first generation NS5A-inhibitors showed a moderately low genetic barrier, leading to emergence of drug-resistant mutants.<sup>41</sup> Such mutations have been described in the N-terminal region of HCV NS5A.<sup>41</sup> The *in vitro* resistance profile of DCV is well known, thanks to the development of HCV replicon system, cell culture-adaptive virus complex and human hepatocyte chimeric mice. Importantly, the resistance seems to give cross-resistance to other NS5A-inhibitors, while DCV-resistant variants remained completely sensitive to IFN-alpha and to other classes of DAAs, such as HCV NS3/4A and HCV NS5B-inhibitors.

Regarding Gt 1, the genetic barrier to resistance is significantly lower for subtype 1a in comparison with 1b. In Gt 1a, Q30E and Y93N determined the highest levels of resistance.<sup>41</sup> In Gt 2a, HCV-NS5A F28S, L31M, C92R, and Y93H are the major resistance mutations described.<sup>42</sup> Zhou *et al.*<sup>43</sup> evaluated NS5A polymorphisms and their impact on response rates in patients with Gt 2 infection who were treated with DCV-based regimens. In particular, the authors performed 426 Gt 2 NS5A sequencing and concluded that high SVR rates were obtained in treated patients regardless of viral subtype or baseline NS5A polymorphisms. In Gt 3a, the position residues 31 and 93 have been identified as locations for DCV-resistance, through which DCV has sub-nanomolar potency, with EC50 ranging from 120 to 870 pmol/L.<sup>44</sup>

A typical example of DCV-resistance can be found in the COMMAND-1 study.<sup>12</sup> In this trial, significantly lower rates of SVR and higher percentages of on-treatment virologic failure were observed in Gt 1a infection. Of note, in a study involving Gt 1b subjects treated with DCV in combination with the NS3 protease inhibitor ASN, NS3 resistance variants quickly disappeared. Conversely, NS5A resistance variants were detectable throughout the 48 weeks of observation.<sup>45</sup>

The impact of pre-existing drug-resistant substitutions on virologic response to DCV and ASN combination therapy was recently evaluated in 31 patients with Gt 1b who were treated for 24 weeks.<sup>46</sup> Three subjects experienced virologic breakthrough, and two patients relapsed. Virologic failure was associated with the onset of both NS5A-L31/Y93 and NS3-D168 variants. NS5A-L31/Y93 variants continued to occur at high frequency, through post-treatment week 103 and up to 170, while NS3-D168 variants were replaced by wild-type in all considered subjects. Recently, Kinugasa *et al.*<sup>47</sup> conducted a study that indicated the possible implications of low frequency RAVs in Gt 1b patients who had been treated

**Table 3. Precautions and interactions of daclatasvir with commonly used medications**

No Dose Adjustment	Administer with Caution	Contraindicated
Antivirals, HCV <ul style="list-style-type: none"> <li>• Sofosbuvir</li> <li>• Simeprevir</li> <li>• Peg-IFN and ribavirin</li> </ul>	Antibacterials <ul style="list-style-type: none"> <li>• Erythromycin</li> </ul>	Anticonvulsants <ul style="list-style-type: none"> <li>• Carbamazepine</li> <li>• Oxcarbazepine</li> <li>• Phenobarbital</li> <li>• Phenytoin</li> </ul>
Antivirals, HIV or HBV <ul style="list-style-type: none"> <li>• Tenofovir disoproxil fumarate</li> <li>• Lamivudine</li> <li>• Zidovudine</li> <li>• Emtricitabine</li> <li>• Abacavir</li> <li>• Didanosine</li> <li>• Stavudine</li> </ul>	Anticoagulants <ul style="list-style-type: none"> <li>• Dabigatran etexilate (not recommended in specific renal impairment groups)</li> </ul>	Antimycobacterial <ul style="list-style-type: none"> <li>• Rifampicin</li> </ul>
Acid reducing agents <ul style="list-style-type: none"> <li>• Famotidine</li> </ul>	Cardiovascular agents <ul style="list-style-type: none"> <li>• Digoxin (initiate treatment using the lowest appropriate digoxin dosage)</li> <li>• Nifedipine</li> <li>• Amlodipine</li> </ul>	Corticosteroids <ul style="list-style-type: none"> <li>• Systemic dexamethasone</li> </ul>
Proton pump Inhibitors <ul style="list-style-type: none"> <li>• Omeprazole</li> </ul>	Lipid lowering agents (monitor for HMG-CoA reductase inhibitor associated side effects, i.e. myopathy) <ul style="list-style-type: none"> <li>• Rosuvastatin</li> <li>• Atorvastatin</li> <li>• Fluvastatin</li> <li>• Simvastatin</li> <li>• Pitavastatin</li> <li>• Pravastatin</li> </ul>	Herbal supplements <ul style="list-style-type: none"> <li>• St. John's wort (<i>Hypericum perforatum</i>)</li> </ul>
Antibacterials <ul style="list-style-type: none"> <li>• Azithromycin</li> <li>• Ciprofloxacin</li> </ul>		
Anticoagulants <ul style="list-style-type: none"> <li>• Warfarin</li> </ul>		
Antidepressants <ul style="list-style-type: none"> <li>• Escitalopram</li> </ul>		
Antifungal <ul style="list-style-type: none"> <li>• Fluconazole</li> </ul>		
Hormonal contraceptives <ul style="list-style-type: none"> <li>• Ethinyl estradiol/norgestimate</li> </ul>		
Immunosuppressants <ul style="list-style-type: none"> <li>• Cyclosporine</li> <li>• Tacrolimus</li> <li>• Sirolimus</li> </ul>		
Narcotic analgesics <ul style="list-style-type: none"> <li>• Buprenorphine/naloxone</li> <li>• Methadone</li> </ul>		

for 24 weeks with ASV and DCV. Viral sequences in regions 3 and 5A before treatment were examined with direct sequencing, next-generation sequencing and the PCR-invader method. The authors concluded that the presence of RAVs at a low frequency might not alter the outcomes of antiviral therapy. Moreover, while direct sequencing may not detect RAVs for ASV plus DCV therapy that occur at a low frequency (<12%), deep sequencing and PCR-invader methods can reveal such RAVs.

DCV resistance-associated variants were recently described in Gt 4 subjects. Bartolini *et al.*<sup>48</sup> assessed the NS5A variability

in 5 Gt 4 treatment-naïve patients to analyse the resistance-associated variants in virologic failure; the patients were treated with standard therapy plus DCV. Among the patients who experienced virologic breakthrough, multiple substitutions associated with DCV-resistance were observed at the NS5A amino acid positions 28, 31 and 93.

HCV NS5A-resistant variants exist naturally and appear frequently after virologic response failure following suboptimal treatment, including with HCV NS5A inhibitors. DCV-resistant variants tend to persist after the drug termination,

and cross-resistance has been observed to all HCV NS5A inhibitors.<sup>39</sup> Nevertheless, DCV-resistant variants remained fully sensitive to IFN-alpha and other classes of DAAs, such as NS3/4A and NS5B inhibitors. Notably, clinical costs of previously selected resistant variants are still to be defined.

### Indications and current approval

On the 24th of July 2015, the US-FDA approved DCV (60 mg) for use with SOF to treat CHC Gt 3 infection in adult patients.<sup>1,49,50</sup> This approval has been recently expanded to include: Gt 1-infected patients, HCV/HIV-co-infected patients, and patients with advanced cirrhosis or post-transplant recurrence of HCV.<sup>49</sup>

### Conclusions and future perspectives

DCV/BMS-790052 is the first-in-class HCV NS5A replication complex with potent antiviral activity. It is a new, oral, DAA with a pan-genotypic action against HCV. It determines not only a rapid and strong HCV-RNA decline but also a favourable safety profile. Like all NS5A inhibitors, DCV should be administered in association with another potent DAA of a different class in order to minimize the risk of resistance. According to the recently published HCV Recommendations of the European Association for the Study of Liver (EASL),<sup>50</sup> DCV at the maximum dosage (60 mg) plus SOF represents a good IFN-free strategy for Gt 1-, Gt 2-, Gt 3- and Gt 4-infected patients. Although the above-cited EASL recommendations suggest that RBV inclusion is still questionable, even for treatment-experienced patients, the aforementioned trial by Poordad<sup>19</sup> concludes that RBV does not seem to improve the rates of SVR; rather, it worsens the safety profile.

Regarding Gt 1, DCV has provided quite high rates of SVR (>90%) in all available trials and in different combinations (with SOF or ASN alone, ASN with BEC, and with or without RBV).<sup>51</sup> In Gt 2 and Gt 3 treatment-naïve patients, the SVR of DCV plus SOF is again very good (92% and 90–92%, respectively). Likewise, excellent results were reported for the historically difficult-to-treat Gt 3 patients who failed a previous anti-HCV treatment, with rates reaching up to 100%,<sup>26</sup> including in patients with advanced liver disease. Indeed, the recently published data suggest that a 24-week treatment course is the optimal duration of treatment with DCV in patients with cirrhosis.<sup>26,27</sup> Pertaining to Gt 4 patients, the combination of DCV and ASN plus BEC and DCV plus SOF with or without RBV provided relatively high SVR rates (90% and 91%, respectively). Still, no data are available regarding Gt 2 and Gt 4 treatment-experienced patients. Notably, all DCV-based therapeutic regimens have provided very high rates of SVR in unique patient populations, such as: HIV/HCV co-infected, liver transplant recipients, and patients with CHC who are on chronic haemodialysis, including patients with advanced liver disease.<sup>33,35–37</sup> In regards to real-world experience, Welzel *et al.* has recently evaluated 485 Gt 1 and Gt 3 CHC patients, of whom 42% had cirrhosis and CPT B/C. Interestingly, SVR12 (modified intention-to-treat) was achieved by 91% of the patients.

Regarding the safety profile, SAEs and AEs leading to treatment discontinuation were rather rare in all of the DCV IFN-free trials. Obviously, the inclusion of RBV in such IFN-free regimens would only worsen the safety profile without adding real benefit in terms of SVR.<sup>19</sup>

To conclude, DCV is a potent HCV NS5A protein inhibitor with high pan-genotypic virologic efficacy and a favourable safety profile, with low rates of discontinuations. Indeed, the promising results concerning the new DCV-containing regimens will lead to relevant changes to the treatment algorithm for CHC. Future large-scale trials are warranted to assess all DCV-containing regimens in patients with Gt 2 and Gt 4 infection and in patients with advanced hepatic disease.

### Conflict of interest

None

### Author contributions

Manuscript writing (NG, SG), data collection (SG, NG, PA), discussion and approval of the manuscript (PA, NG, SG), and critical revision (PA, NG, SG).

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