Review: Clinical Trial Outcomes

CLINICAL INVESTIGATION

Daclatasvir, an efficient inhibitor of the hepatitis C virus replication complex protein NS5A: review of virologic data, treatment rationale and clinical trials

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The treatment of hepatitis C virus (HCV) infection with pegylated interferon α (PEG-α) and ribavirin (RBV) leads to a sustained virologic response in approximately 50% of patients with HCV genotype 1. A better understanding of the HCV life-cycle has resulted in the development of several potential direct-acting antiviral drugs (DAAs), targeting viral proteins (NS3/4A protease inhibitors, NS5B polymerase inhibitors, or NS5A replication complex inhibitors). This review summarizes the clinical data for daclatasvir (DCV; BMS-790052), the first NS5A replication complex inhibitor to enter clinical development, with potent activity, broad genotypic coverage *in vitro*, and a pharmacokinetic profile supportive of once-daily dosing. DCV, either in combination with PEG-α or in interferon-free regimens with other DAAs, has demonstrated a high level of antiviral efficacy and a generally well-tolerated safety profile in treatment-naive patients, and in prior non-responders to PEG-α/RBV. DCV is likely to become a key component of new oral combinations of DAAs for chronic HCV in treatment-naive or -experienced patients.

Keywords: combination therapy • daclatasvir • direct-acting antiviral • hepatitis C virus • replication complex inhibitor

Treatment of chronic hepatitis C with pegylated interferon α (PEG-α) and ribavirin (RBV) for a duration adapted according to the early virologic response results in effective and sustained viral suppression in <50% of patients infected with hepatitis C virus (HCV) genotype 1 (the most common genotype in North America and Europe). A better understanding of the HCV life-cycle and the characterization of viral enzymes that are potential antiviral targets has led to the development of a number of potential new direct-acting antiviral drugs (DAAs) targeted against viral proteins. These include first-generation NS3/NS4A protease inhibitors, which mostly specifically target HCV genotype 1, and second-generation NS3/NS4A protease inhibitors, NS5B polymerase inhibitors, or NS5A inhibitors with a broader spectrum. Several non-specific new agents are also under development; for example, new interferons, cyclophilin inhibitors and vaccine therapy [1–5]. Recent approval of the first-generation HCV NS3/4A protease inhibitors boceprevir and telaprevir and their use in triple combinations with PEG-α/RBV, has significantly improved sustained virologic response (SVR) rates by approximately 20–30% in genotype 1-infected treatment-naive patients [6,7], including those with previous experience of prior PEG-α/RBV treatment [8,9]. However, both agents have considerable side effects (which add to those of PEG-α/RBV, including severe skin rashes/pruritus [telaprevir], anal discomfort [telaprevir], and anemia [telaprevir and boceprevir]), are dosed three-times daily, and carry a high pill burden [6–9]. Thus, there remains a need for new therapeutic strategies with simplified oral dosing, broader efficacy across HCV genotypes, minimal side effects, and improved tolerability profiles.

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Daclatasvir (DCV, BMS-790052) is a potent highly selective NS5A replication complex inhibitor with broad genotypic coverage (genotypes 1–5) and a pharmacokinetic profile supportive of once-daily dosing [10,11]. DCV is the first NS5A replication complex inhibitor to enter clinical development for the treatment of chronic hepatitis C. This review briefly describes the rationale behind the identification and development of DCV and summarizes the key safety and efficacy data from clinical studies of this agent for the treatment of chronic hepatitis C. Tables 1, 2, & 3 provide an overview of the clinical studies discussed [11–29].

The role of NS5A in replication

NS5A is a non-structural, RNA-binding phosphoprotein that appears to be pleiotropic in nature, with important roles in HCV RNA replication and assembly of new virus particles, as well as modulation of the host cells' signaling pathways [30–32]. Although the precise function of NS5A in these processes has remained enigmatic, it is clear that its key role is as part of the HCV multi-protein, membrane-bound replication complex that produces HCV RNA copies from the single-stranded positive RNA genome. The HCV replication cycle, summarized in Figure 1, involves initial translation of the positive-strand viral RNA to produce structural and non-structural viral proteins. The viral replication complex, formed from non-structural viral proteins (NS3, NS4A, NS4B, NS5A, and NS5B) associated with rearranged intracellular membranes, produces a negative-strand viral RNA intermediate to serve as a template for new progeny positive-strand RNA. The

Table 1. Overview of Phase I clinical studies with daclatasvir.

Study	Patients	Treatment	Overview of outcomes	Ref.
Phase I safety and pharmacokinetics				
SAD AI444–001	Healthy subjects (n = 48)	DCV oral solution (1, 10, 25, 50, 100, or 200 mg) vs PBO (3:1/dose)	Well tolerated with no clinically relevant AEs Dose-proportional DCV exposure	[10]
SAD AI444–002 NCT00546715	Chronic HCV GT1 (n = 18)	DCV oral solution (1, 10, or 100 mg) vs PBO (5:1/dose)	Well tolerated. No deaths, SAEs, or discontinuations due to AEs Most frequent AE = headache (four subjects) Mean $T_{1/2}$ 10–14 h Mean max decline in HCV RNA with 100 mg dose, 3.6 $\log_{10}$ IU/ml (range 3.0–4.1 $\log_{10}$ IU/ml)	[10]
MAD AI444–004 NCT00663208	Chronic HCV GT1 (n = 30)	DCV oral capsule (1, 10, 30, 60, or 100 mg QD or 30 mg BID) vs PBO (4:1/dose), 14 days	AE profile comparable to that for PBO. Most frequent AE = headache (five subjects) Median T_{max} 1–2 h; $T_{1/2}$ 12–15 h Mean max decline in HCV RNA 2.8–4.1 $\log_{10}$ IU/ml	[11]
Single dose AI444–013	Hepatic impairment (n = 30)	DCV 30 mg single dose, in patients with hepatic impairment Child–Pugh A, B or C (n = 6/group)	AUC_{INF} adjusted for unbound DCV similar to healthy controls in all groups DCV dosing adjustments in hepatic impairment are not anticipated	[12]
Drug–drug interaction studies				
AI444–020 NCT00983957	Healthy women (n = 20)	DCV tablet (60 mg QD), 10 days Ortho Tri-Cyclen® (QD), 78 days	No clinically significant pharmacokinetic drug interactions, as assessed by ethinyl estradiol, norelgestromin and norgestrel exposures	[13]
AI447–009 NCT00904059	Healthy subjects	DCV (30 mg QD) and ASV (200 mg every 12 h), 14 days	No clinically meaningful pharmacokinetic interaction	[14]
AI444032, 33 and 34	Healthy subjects (n = 14)	DCV (20 mg QD) + ATV/r (300/100 mg QD), DCV (60 or 120 mg QD) + EFV (600 mg QD), DCV (60 mg QD) + TDF (300 mg QD)	No clinically relevant pharmacokinetic interactions between DCV and TDF When administered with DCV, exposure for EFV and ATV/r were similar to historical and reported values DCV dose adjustments to 30 mg QD with ATV/r and 90 mg QD with EFV are expected to provide DCV exposure similar to that for DCV 60 mg	[15]

AE: Adverse event; ASV: Asunaprevir; ATV/r: Atazanavir plus ritonavir; BID: Twice daily; DCV: Daclatasvir; EFV: Efavirenz; GT: Genotype; HCV: Hepatitis C virus; PBO: Placebo; QD: Once daily; SAE: Serious AE; $T_{1/2}$: Half life; TDF; Tenofovir; T_{max}: Time to maximum concentration.

Table 2. Overview of Phase II clinical studies with daclatasvir in combination with pegylated interferon α/ribavirin.

Study	Patients	Treatment	Overview of outcomes	Ref.
AI444–014 NCT00874770	Treatment-naive HCV GT1 (n = 48)	DCV (3, 10 or 60 mg) vs PBO (QD) (1:1:1:1) all + PEG-α/RBV All 48 weeks	SVR24 41.7% (5/12), 83.3% (10/12) and 83.3% (10/12) DCV 3, 10 and 60 mg, respectively, vs 25.0% (3/12) control Safety profile consistent with PEG-α/RBV treatment Most frequent AEs: headache and fatigue Treatment discontinuations due to AEs: 1, 1 and 4 patients in DCV 3, 10 and 60 mg groups, respectively and 1 patient in the PBO group; On-treatment SAEs: 1 patient in each DCV group	[16]
COMMAND-1 AI444–010 NCT01125189	Treatment-naive HCV GT1 or 4 (n = 395)	DCV (20 or 60 mg) vs placebo (QD; 2:2:1) all + PEG-α/RBV (n = 365 GT1, n = 30 GT4) 24 or 48 weeks (DCV patients with PDR re-randomized to 12 or 24 weeks DCV)	GT1: Higher rates of virologic responses (eRVR, cEVR, PDR, and SVR12) with DCV + PEG-α/RBV compared with PBO + PEG-α/RBV **(Figure 2A)** GT4: SVR12 67% (8/12), 100% (12/12) and 50.0% (3/6) in DCV 20 and 60 mg, and PBO arms, respectively AE profiles comparable between DCV and PBO groups Emergent grade 3/4 bilirubin approximately 1% across all groups Grade 3/4 ALTs: 0, 4, and 1% for DCV 20 and 60 mg arms, as well as the PBO arm, respectively On-treatment SAEs: 8% of patients in each arm; Treatment discontinuations due to AEs: 4% DCV arms, 9% PBO arm	[17,18]
COMMAND-2 AI444–011 NCT01170962	PEG-α/RBV null (n = 265) or partial (n = 154) responders HCV GT1	Null: DCV (20 or 60 mg QD; 1:1) + PEG-α/RBV Partial: DCV (20 or 60 mg) vs PBO (QD; 4:4:1) all + PEG-α/RBV	Week 12 interim analysis (study ongoing): More rapid HCV RNA suppression in null and partial responders with DCV + PEG-α/RBV compared with PEG-α/RBV RVR, eRVR and cEVR rates lower than those observed with DCV + PEG-α/RBV in treatment-naive patients **(Figure 4B)** AE profile consistent with a PEG-α/RBV background Grade 3–4 laboratory abnormalities consistent across treatment groups On-treatment SAEs 6% (12/203), 5% (10/199), and 18% (3/17), for DCV 20 and 60 mg, and PBO arms, respectively Treatment discontinuations due to AEs 3% (6/203), 5% (10/199), and 18% (3/17), for DCV 20 and 60 mg arms, as well as the PBO arm, respectively	[19]
COMMAND GT2/3 AI444–031	Treatment-naive HCV GT2 or 3 (n = 151)	DCV + PEG-α/RBV 12 wks (n = 50) or 16 wks (n = 50) vs PBO + PEG-α/RBV 24 wks (n = 51)	GT2: cEVR 91.7, 82.6 and 75% in 12- or 16-week DCV and PBO groups, respectively GT3; cEVR 80.8, 88.9 and 59.3% in DCV 12 wk, DCV 16 wk and PBO groups, respectively Most frequent AEs were those typically associated with PEG-α/RBV, including fatigue, cytopenias and depression	[20]
AI 444–021 (Japan)	Treatment-naive (n = 27) or PEG-α-2b/RBV null/partial re-sponsers (n = 18) HCV GT1b	Treatment-naive: DCV (10 or 60 mg) vs PBO (QD) + PEG-α-2b/RBV Null/partial: DCV (20 or 60 mg QD; 4:4:1) all + PEG-α-2b/RBV	In treatment-naive patients higher rates of eRVR, PDR, and SVR24 with DCV + PEG-α (2a or 2b)/RBV compared with PBO + PEG-α (2a or 2b)/RBV Lower response rates in null/partial responders AE profile consistent with a PEG-α/RBV background	[21]
AI 444–022 (Japan)	Treatment-naive (n = 25) or PEG-α-2a/RBV null/partial responders (n = 17) HCV GT1b	Treatment-naive: DCV (10 or 60 mg) vs PBO (QD) + PEG-α-2a/RBV Null/partial: DCV (20 or 60 mg QD; 4:4:1) all + PEG-α-2a/RBV	In treatment-naive patients higher rates of eRVR, PDR, and SVR24 with DCV + PEG-α (2a or 2b)/RBV compared with PBO + PEG-α (2a or 2b)/RBV Lower response rates in null/partial responders AE profile consistent with a PEG-α/RBV background	[22]

AE: Adverse events; ALT: Alanine aminotransferase; cEVR: Complete early virologic response (HCV RNA undetectable at week 12); DCV: Daclatasvir; eRVR: Extended rapid virologic response (HCV RNA undetectable at weeks 4 and 12); GT: Genotype; HCV: Hepatitis C virus; PBO: Placebo; PDR: Protocol-defined response (HCV RNA <LLOQ [25 IU/ml] at week 4 and undetectable [< 10 IU/ml] at week 10); PEG-α: Pegylated interferon α; QD: Once daily; RBV: Ribavirin; RVR: Rapid virologic response; SAE: Serious AE; SVR12/24: Undetectable hepatitis C virus RNA at follow-up at week 12/24.

Table 3. Overview of Phase II clinical studies with daclatasvir in combination with other direct-acting antiviral drugs.

Study	Patients	Treatment	Overview of outcomes	Ref.
AI447–011 Sentinel Dual vs quadruple	PEG-α/RBV null responders HCV GT1 (n = 21)	DCV (60 mg QD) + ASV (600 mg BID) ± PEG-α/RBV (n = 21)	DCV + ASV + PEG-α/RBV: SVR4 and SVR12 in 10/10 (100%), SVR24 and SVR48 in 9/10 (90%). No virologic breakthrough DCV + ASV: SVR4, SVR12 and SVR24 in 4/11 (36%) and SVR48 in 3/11 (27%) Dual and quadruple combinations were well tolerated No deaths, SAEs or treatment discontinuations for AEs Most frequent AE was diarrhea	[23,24]
AI447–011 Expansion Dual vs quadruple	PEG-α/RBV null responders HCV GT1 (n = 101)	DUAL (GT1b only) = DCV (60 mg QD) combined with ASV 200 mg BID (n = 18) or ASV 200 mg QD (n = 20), vs QUAD = DCV (60 mg QD) combined with ASV 200 mg BID + PEG-α/RBV (n = 20) or ASV 200 mg QD + PEG-α/RBV (n = 21), vs DCV (60 mg QD) plus ASV 200 mg BID and RBV (n = 22) 24 weeks of treatment	QUAD: SVR12 in 19/20 (95%) ASV BID, 20/21 (95%) ASV QD DUAL (preliminary data): SVR4 in 13/15 ASV BID, 10/16 ASV QD Dual and quadruple combinations were well tolerated with no treatment discontinuations for AEs Most frequent AEs were headache, diarrhea, asthenia, and nausea (DUAL) and headache, asthenia, diarrhea, alopecia, fatigue and irritability (QUAD)	[25]
AI447–017 Dual (Japan)	PEG-α/RBV null-responders or ineligible/intolerant, HCV GT1b (n = 43)	DCV (60 mg QD) + ASV (200 mg BID) ASV initially 600 mg BID in sentinel cohort of ten null-responders, reduced to 200 mg during treatment, 24 weeks	SVR12: all patients 33/43 (77%); null-responders 19/21 (91%); ineligible 14/22 (64%) Among ineligible patients three virologic breakthroughs and four post-treatment relapses Favorable AE profile: most frequent AE = mild headache (14/43 patients), six SAEs in five patients, three treatment discontinuations for AEs, no deaths	[26,27]
AI444–040 Dual ± RBV	Treatment-naive, HCV GT1 (n = 44) or GT2/3 (n = 44)	DCV (60 mg QD) + GS-7977 (400 mg QD) ± RBV 24 weeks	GT1: 100% SVR12 with or without RBV GT2 or 3: 86–100% SVR12 with or without RBV Most common AEs (>20%) = fatigue, headache, and nausea Most common grade 3–4 laboratory abnormality = anemia (only seen in patients receiving RBV)	[28,29]

AE: Adverse events; ASV: Asunaprevir; BID: Twice daily; DCV: Daclatasvir; GT: Genotype; HCV: Hepatitis C virus; PEG-α: Pegylated interferon α; QD: Once daily; RBV: Ribavirin; SAE: Serious AE; SVR4/12/24/48: Undetectable hepatitis C virus RNA at follow-up week 4/12/24/48.

progeny RNAs serve as templates for protein synthesis or are assembled into new virus particles. NS5A comprises 447 amino acids that can be divided into three distinct domains. The amino-terminal domain 1 includes an amphipathic alpha-helix that anchors the protein into the membrane. Domain II, the central region of the molecule, contains a cyclophilin-binding site, pointing to a role in the regulation of HCV replication. The C-terminal domain III is thought to be involved in virus assembly [33,34]. *In vitro* studies suggest that an oligomeric form of NS5A binds to viral RNA to sequester it, and protect it from degradation, while presenting it as template to the replication complex [31,34]. NS5A has also been shown to have multiple interactions with host proteins, the physiologic significance of which is not always clear [10]. A possible role in viral escape from the effects of interferon is suggested by the fact that mutations associated with sensitivity to interferon therapy can be mapped to central and C-terminal regions of NS5A [30,35,36]. The mechanism behind this observation remains controversial with several possibilities having been proposed, including interaction between NS5A and PKR protein kinase (an interferon-induced gene product that interferes with protein translation) and upregulation of IL8 (known to attenuate the antiviral properties of interferons) [35].

Pharmacology & pharmacokinetics of DCV

DCV is the most potent HCV replication inhibitor reported to date, with *in vitro* 50% effective concentrations (EC_{50}) in the picomolar range (9–146 pM) against HCV replicons representing six major HCV genotypes (1a, 1b, 2a, 3a, 4a and 5a) [10,37,38]. The addition of 40% human serum, to adjust for the effects of protein binding, yielded a 90% effective concentration (EC_{90}) of 383 pM (0.28 ng/ml) for genotype 1a and 49 pM (0.04 ng/ml) for genotype 1b. The *in vitro* activity of DCV was specific to HCV NS5A with no

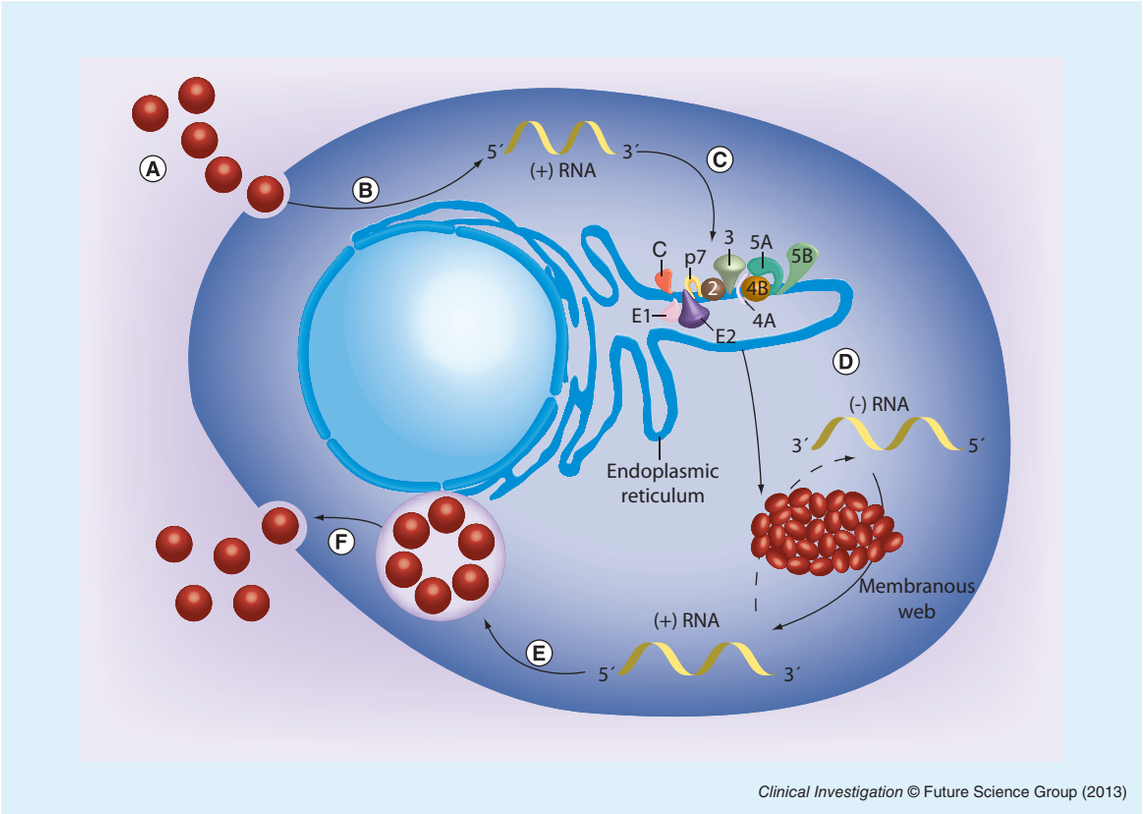

Figure 1. Lifecycle of hepatitis C virus. (A) Virus binding and internalization; **(B)** cytoplasmic release and uncoating; **(C)** translation and polyprotein processing mediated by the internal ribosome entry site; production of a negative-strand viral RNA intermediate by the viral replication complex, formed from non-structural viral proteins (NS3, NS4A, NS4B, NS5A, and NS5B) associated with rearranged intracellular membranes; **(D)** negative-strand viral RNA serves as a template for new progeny positive-strand RNA; **(E)** packaging of viral RNA and assembly of new viral particles; and **(F)** virion maturation and release.
Adapted from [61].

overt cytotoxicity against host cells *in vitro* (50% cytotoxic concentration in HuH-7 cells 17 ± 1 µM, giving a therapeutic index of $>10^5$), and no significant effects in a range of *in vitro* receptor binding and enzymatic assays [10].

The molecule was identified via replicon-based high-throughput screening for agents with high inhibitory activity against HCV replication, combined with a triaging system aimed at identifying compounds functionally distinct from those acting on the NS3 protease and NS5B RNA-dependent RNA polymerase. HCV variants resistant to DCV contained amino acid substitutions in the NS5A protein [10,39–41]. The mode of action of DCV remains to be fully characterized. One proposal is that it may alter the correct localization of NS5A into functional replication complexes [42]. The position of specific amino acid residues conferring DCV resistance suggests that the molecule binds to domain I of NS5A close to amino-terminal RNA-binding and dimerization sites. Thus, DCV could act by, either directly or allosterically, interfering with dimerization and compromising the function of oligomeric NS5A complexes [10]. However, *in vitro* NS5A dimerization studies have suggested that DCV does not block this process, suggesting a different mechanism of antiviral activity [43]. Further *in vitro* studies are in progress to better define the exact mechanism behind the potent antiviral activity of DCV.

In combination studies, DCV demonstrated additive-to-synergistic effects with PEG-α/RBV and with other DAAs including an NS3/4A protease inhibitor, a non-nucleoside NS5B polymerase inhibitor, and a nucleoside NS5B polymerase inhibitor [10,44]. Results from Phase I studies suggest a pharmacokinetic profile supportive of once-daily dosing. In single-dose studies, oral doses of 10–100 mg produced 24-h plasma concentrations above the tenfold protein binding-adjusted EC_{90} for HCV genotype 1a and 1b in all patients [10]. In multiple-dose

studies in HCV-infected patients, median peak plasma concentrations were achieved within 1–2 h post-dose, and the mean terminal half-life was between 12 and 15 h [11]. Steady state was achieved following 3–4 days of daily dosing. A dose of 100-mg daily over 14 days resulted in a mean maximum decline in HCV RNA of 3.6 $\log_{10}$ IU/ml (range 3.0–4.1 $\log_{10}$ IU/ml). DCV pharmacokinetics have been studied in patients with hepatic impairment (AI444–013) [12]. A total of 12 healthy subjects were compared with patients with mild (Child–Pugh A), moderate (Child–Pugh B) or severe (Child–Pugh C) hepatic impairment (n = 6 per group). Hepatic impairment resulted in reduced serum concentrations of total DCV; the AUC_{INF} was 43, 38 and 36% lower for subjects with Child–Pugh A, B and C, respectively, versus healthy subjects. However, subjects with moderate or severe hepatic impairment demonstrated a numerically higher unbound fraction of DCV compared with controls. As a result, exposure to active, unbound DCV was comparable between controls and subjects with moderate or severe hepatic impairment. Thus, DCV dosing adjustments in hepatic impairment are not anticipated. Studies in patients with renal impairment are planned.

Drug–drug interactions

Drug–drug interaction studies with DCV are ongoing and a complete drug-interaction profile is not yet available. However, preclinical data indicate that, although DCV is a substrate and inhibitor of P-glycoprotein and a substrate of cytochrome P450 3A4 (CYP3A4), it is not a strong inhibitor (or strong inducer) of CYP3A isozymes, suggesting it may have a low potential for drug–drug interactions, at least in studies that have been completed to date (Table 1) [15].

Three open-label studies in healthy subjects evaluated steady-state pharmacokinetic interactions between DCV with the antiretrovirals tenofovir, efavirenz and atazanavir/ritonavir (studies AI444032, 33 and 34) [15]. No clinically relevant pharmacokinetic interactions occurred between DCV and tenofovir. DCV did not appear to have any clinically significant effects on efavirenz or atazanavir/ritonavir exposure. The effects of efavirenz and atazanavir/ritonavir on DCV exposure were manageable: dose adjustments to 90 mg once a day (QD) with efavirenz (600 mg QD) and to 30 mg QD with atazanavir/ritonavir (300/100 mg QD) are expected to provide DCV exposure, similar to that for DCV 60 mg administered alone.

Another study (AI444–020) demonstrated that DCV could be coadministratered with a commonly prescribed ethinyl estradiol-based oral contraceptive (Ortho Tri-Cyclen®) [13]. Given that RBV is highly teratogenic, effective contraception is essential in women of child-bearing age who undergo PEG-α/RBV-based HCV therapy. Telaprevir and boceprevir are both substrates and significant inhibitors of CYP3A and result in a reduction of ethinyl estradiol levels when taken with hormonal contraceptives [45,46]. With DCV, no clinically significant pharmacokinetic-drug interactions were observed, as assessed by ethinyl estradiol, norelgestromin and norgestrel exposures.

Interactions between DCV and other DAAs for the treatment of HCV have also been investigated in preparation for combination therapy studies. Coadministration of DCV 30 mg QD and asunaprevir (BMS-650032, a second-generation protease inhibitor) 200 mg every 12 h for 14 days was well tolerated and did not result in a clinically meaningful pharmacokinetic interaction [14].

Safety

In Phase I single- and multiple-ascending dose studies in healthy subjects or in patients with chronic HCV infection, DCV was generally well tolerated at oral doses up to 200 mg with an adverse-event profile comparable to placebo (Table 1) [10,11]. The most frequently reported adverse event in HCV-infected patients receiving single- or multiple-dose DCV was a headache, which did not appear to be dose-related and was in all cases considered by the investigator as unrelated to the study drug [10,11].

In Phase II studies of DCV at doses up to 60-mg per day in combination with PEG-α/RBV, no exposure–safety relationships have been observed and no relevant dose-dependent hematological- or liver-related abnormalities have been reported in patients receiving DCV (Tables 2 & 3) [47]. In study AI444–014 (Phase IIa) in 48 treatment-naive patients, the safety profile for escalating doses of DCV (3–60 mg) combined with PEG-α/RBV over 48 weeks was consistent with the typical profile of PEG-α/RBV treatment and no additional or unique adverse events were attributable to DCV (Table 4) [16]. The most frequent adverse events among patients receiving DCV were moderate headache and fatigue. Incidences of hematologic (anemia, neutropenia, lymphopenia or thrombocytopenia), dermatologic and hepatic events, as well as rates of dose reductions, dose interruptions and use of granulocyte colony-stimulating factor or erythropoietin were similar between DCV and control groups. Six patients discontinued DCV for adverse events; one each in the 3- and 10-mg groups and four in the 60-mg group (for fatigue, headache, attention disturbance, auditory hallucinations, and ageusia in the 3-mg group, asthenia in the 10-mg group, and anxiety rash, alopecia, and lymphopenia in the 60-mg group). Serious adverse events were reported for one patient in each DCV group (bronchitis at 60 mg, syncope at 10 mg, and anemia, chest pain, syncope, and epistaxis at 3 mg) [16]. Similarly, in the Phase IIb COMMAND-1 (treatment naive) and COMMAND-2 (null or partial responders)

Table 4. Adverse events (any grade) on-treatment occurring in ≥4 (33.3%) patients in any cohort among patients receiving 48 weeks of treatment with daclatasvir or placebo in combination with pegylated interferon α/ribavirin (study AI444–014).

Adverse event	No. of patients (%)			
	DCV 3 mg + PEG-a/RBV (n = 12)	DCV 10 mg + PEG-a/RBV (n = 12)	DCV 60 mg + PEG-a/RBV (n = 12)	Placebo + PEG-a/RBV (n = 12)
Fatigue	7 (58.3)	6 (50.0)	6 (50.0)	9 (75.0)
Anemia	3 (25.0)	5 (41.7)	6 (50.0)	5 (41.7)
Insomnia	4 (33.3)	4 (33.3)	5 (41.7)	6 (50.0)
Asthenia	1 (8.3)	3 (25.0)	5 (41.7)	1 (8.3)
Nausea	5 (41.7)	4 (33.3)	4 (33.3)	6 (50.0)
Decreased appetite	3 (25.0)	2 (16.7)	4 (33.3)	3 (25.0)
Pruritus	3 (25.0)	5 (41.7)	4 (33.3)	3 (25.0)
Vomiting	2 (16.7)	1 (8.3)	4 (33.3)	0
Headache	7 (58.3)	9 (75.0)	3 (25.0)	3 (25.0)
Irritability	6 (50.0)	3 (25.0)	3 (25.0)	2 (16.7)
Alopecia	1 (8.3)	4 (33.3)	3 (25.0)	2 (16.7)
Influenza-like illness	6 (50.0)	3 (25.0)	2 (16.7)	4 (33.3)
Neutropenia†	3 (25.0)	4 (33.3)	2 (16.7)	5 (41.7)
Rash	4 (33.3)	4 (33.3)	2 (16.7)	3 (25.0)
Cough	2 (16.7)	5 (41.7)	1 (8.3)	3 (25.0)

†Grade 3–4 neutropenia occurred in two patients: 60-mg (one) and placebo (one).

DCV: Daclatasvir; PEG-α: Pegylated interferon α; RBV: Ribavirin.

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studies, the adverse-event profiles for the combination of DCV 20- or 60-mg with PEG-α/RBV were those commonly reported for PEG-α/RBV alone [17–19]. Rates of treatment discontinuations due to adverse events in the DCV arms of these studies ranged from 3–5%. Combinations of DCV with asunaprevir, or with the nucleotide analog GS-7977 (sofosbuvir), have been generally well tolerated in studies to date **(Table 3)** [23–29].

Resistance

In vitro resistance selection studies have been conducted with HCV genotype 1a and 1b replicons cultured over 4–5 weeks in the presence of DCV concentrations of 50–500 pM (10–100× the initial EC_{50}) for genotype 1b and 200 pM–1 nM (4–20× the initial EC_{50}) for genotype 1a [48]. Genotypic analysis of selected DCV-resistant variants identified DCV resistance-associated mutations that map to the N-terminal region of NS5A [48]. In genotype 1b replicons, the primary resistance-associated amino acid substitutions were L31F/V, P32L and Y93H with secondary substitutions identified as L23F, R30Q, and P58S. Reductions in susceptibility to DCV were modest with multiple substitutions needed to increase the EC_{50} to nano-Molar levels. In genotype 1a replicons, primary DCV resistance-associated amino acid substitutions were M28T, Q30E/H/R, L31M/V, P32L, and Y93C/H/N. These mutations were generally associated with higher levels of resistance than those seen in genotype 1b replicons. Further studies using replicon elimination assays confirmed the role of the NS5A mutations identified and showed that higher doses of DCV (up to 10,000 × EC_{50}) and longer culture times were required to select multiple mutations conferring high levels of resistance [49].

To date, *in vivo* data have also confirmed the role of these substitutions in reducing susceptibility to DCV. In a Phase I multiple-ascending dose study in 24 patients chronically infected with HCV genotype 1, 14 days of monotherapy with DCV at doses of 1–100 mg QD generally resulted in rapid viral load declines over the treatment period, but viral breakthrough was frequently observed during the second week of treatment, particularly in patients infected with HCV genotype 1a, and was associated with the detection of resistant virus [11,50]. Most DCV-resistant substitutions observed were those previously identified *in vitro* **(Table 5)**. To assess the impact of the substitutions selected *in vivo*, the observed mutations were introduced into replicons representing HCV genotypes 1a and 1b [50]. In genotype 1b replicons, all of the single and some of the double amino acid substitutions (e.g., Q54H-Y93H) resulted in a mild reduction in DCV antiviral potency (reduction to 28-fold), while some of the double amino acid

substitutions (e.g., L31V-Y93H) conferred a high level of resistance (reduction to 14,789-fold). In genotype 1a replicons, major substitutions conferred greater changes in DCV antiviral potency than single substitutions in genotype 1b; this might explain why viral breakthrough in the multiple-ascending dose study was more common among patients with genotype 1a [11].

NS5A polymorphisms associated with resistance to DCV have been detected in DCV-naive patients, raising the question of whether these may reduce the response rate to DCV [51]. The reported frequency of NS5A polymorphisms potentially associated with DCV resistance appears to vary and some studies suggest that they may be rare in patients infected with HCV genotypes 1a and 3

Table 5. *In vitro* analysis of the daclatasvir resistance and replicative capacity of genotype 1a and 1b replicons carrying NS5A amino acid substitutions observed in hepatitis C virus variants emerging in patients treated with 14 days of daclatasvir monotherapy.

Replicon	Average replication level, % (SD)[†]	Average EC_{50}, ng/ml (SD)[‡]	Resistance (fold)
Genotype 1a[§]			
WT	100	0.0044 (0.0028)	1
M28A	27 (25)	20.2 (13.3)	4591
M28T	31 (23)	3.0 (0.3)	682
M28V	16 (11)	0.0055 (0.0019)	1.3
Q30E	130 (56)	110.9 (66.0)	25,205
Q30H	75 (31)	6.5 (1.4)	1477
Q30R	41 (16)	5.4 (0.8)	1227
Q30K	19 (9)	108 (52)	24,545
L31M	55 (15)	1.5 (0.5)	341
L31V	117 (29)	14.9 (4.4)	3386
H58D	92 (9)	2.2 (0.3)	500
H58P	266 (261)	0.0053 (0.0006)	1.2
Y93C	11 (7)	8.2 (3.0)	1864
Y93H	18 (11)	23.9 (7.0)	5432
Y93N	13 (8)	208.9 (47.9)	47,477
M28V-Q30R	147 (55)	1.4 (0.013)	350
Q30H-Y93H	20 (6)	409.8 (153.6)	93,136
Q30R-H58D	60 (12)	1867 (46)	424,318
Genotype 1b[¶]			
WT	100	0.0019 (0.0007)	1
L31M	99 (23)	0.0062 (0.0014)	3
L31V	158 (54)	0.053 (0.015)	28
Q54H	83 (18)	0.0024 (0.0003)	1
Q54N	83 (29)	0.0027 (0.0006)	1
Y93H	27 (16)	0.046 (0.018)	24
L31M-Y93H	70 (68)	13.5 (12.2)	7105
L31V-Y93H	50 (38)	28.1 (24.7)	14,789
Q54H-Y93H	22 (7)	0.018 (0.005)	9
L31V-Q54H-Y93H	189 (25)	36.1 (7.7)	19,000

[†]Replicative capacity of variant replicons expressed as percent relative to WT.

[‡]1 ng/ml daclatasvir = 1.35 nM dalcatasvir.

[§]Genotype 1a replicon: H77C with cell culture replication-enhancing mutations P1496L and S2204I.

[¶]Genotype 1b replicon: Con1 with cell culture replication-enhancing mutation S2204I.

EC_{50}: 50% effective concentration; WT: wild type.

Data taken from [50].

[52] and in null-responders [53]. In Phase II clinical studies of triple therapy with DCV in combination with PEG-α/RBV, polymorphisms at amino acid positions associated with DCV resistance were detected in more than a third of patients infected with HCV genotype 1a or 1b; however, to date, the presence of these substitutions has not shown any clear correlation with treatment failure [16,54]. Virologic failure among treatment-naive patients treated with DCV at 10 or 60 mg QD is uncommon, suggesting that an appropriate dose of DCV combined with PEG-α/RBV is sufficient to suppress the development of HCV drug resistance in these patients [16,54]. In the Phase IIa study AI444–014 DCV-resistant variants emerged in two out of 12 patients in the DCV 10-mg plus PEG-α/RBV group (one associated with detectable HCV RNA at end-of-treatment [EOT] and one with virologic relapse occurring 24 weeks post-treatment) and in two out of 12 patients in the DCV 60 mg plus PEG-α/RBV group (one associated with virologic breakthrough at week 24 of treatment and one associated with virologic relapse occurring 4 weeks post-treatment). Emerging variants were consistent with those previously identified *in vitro*. Persistence of resistance variants was assessed in isolates from two patients in the DCV dose groups who experienced breakthrough. Emerging variants detected at breakthough in these patients remained detectable by population sequencing at 24 weeks post-treatment, the last time point evaluated in this study [16,54].

In *in vitro* studies, DCV-resistant variants remained fully sensitive to asunaprevir and an NS5B inhibitor (BMS-791325), supporting the use of DCV in combination with other DAAs. Dual DCV and asunaprevir resistance has been observed *in vitro* [55] and in patients treated with the dual combination of these two agents without PEG-α/RBV, primarily in patients infected with HCV genotype 1a [23,54]. In the study AI447–011, on-treatment emergence of resistant variants associated with viral breakthrough or relapse in prior null responders treated with DCV and asunaprevir dual therapy occurred in seven of nine patients with genotype 1a infection, and in neither of the two patients with HCV genotype 1b infection (Figure 2A) [23,56]. No DCV resistance-associated variants were detected at baseline, but resistant variants emerging after virologic breakthrough carried substitutions associated with both asunaprevir and DCV resistance (NS5A variants: Q30R, L31M/V, Y93C/N; NS3 variants: R155K, D168A/E/V/Y). Phenotypic analysis confirmed reductions in susceptibility of 30- to 525-fold for asunaprevir and 3400- to >330,000-fold for DCV [56]. In study AI447–017, which included only patients with HCV genotype 1b infection, the emergence of resistance during dual therapy was less frequent: seven out of 22 PEG-α/RBV-ineligible or -intolerant patients receiving 24 weeks of treatment with DCV plus asunaprevir experienced viral breakthrough (three patients, after 12–20 weeks of treatment) or post-treatment relapse (four patients, within 4–12 weeks after EOT) associated with the emergence of dual resistance to DCV and asunaprevir [26,57]. The most frequent DCV resistance-associated substitutions were L31M and Y93H. The addition of PEG-α/RBV to the combination of DCV and asunaprevir appears to provide sufficient antiviral activity to suppress the emergence of dual resistance to the two DAAs, regardless of HCV subtype. Among ten null responders receiving the quadruple regimen of DCV, asunaprevir and PEG-α/RBV in study AI447–011, nine of whom had HCV genotype 1a, no virologic breakthrough occurred [23].

Efficacy in clinical studies

Initial clinical studies of DCV have focused mainly on HCV genotype 1 infection, as this is the most prevalent genotype in North America and Europe. More than 700 patients with HCV genotype 1 infection have been treated with DCV in clinical studies to date. Since DCV demonstrated broad genotypic activity *in vitro*, some studies have also been conducted in patients infected with HCV genotype 2, 3 (44 patients in AI444–040 [28,29] and 151 patients in AI444–031 [20]) or 4 (30 patients in study AI444–010) [17,18], although data in these populations are currently limited to small numbers of patients (Tables 2 & 3).

DCV monotherapy

The potent antiviral activity of DCV observed *in vitro* translates into substantial viral suppression *in vivo* [10,11]. Single 10- and 100-mg doses of DCV in HCV-infected patients gave mean 3.2 and 3.3 $\log_{10}$ reductions in serum HCV RNA levels, respectively, 24 h after drug administration. In a multiple-ascending dose study in 30 patients chronically infected with HCV genotype 1, 14 days of DCV monotherapy (1–100 mg QD) resulted in mean maximum HCV RNA declines from baseline of between 2.8 and 4.1 $\log_{10}$ IU/ml. A greater and more sustained decline in HCV RNA was observed in patients with genotype 1b than for patients infected with genotype 1a [11].

Triple therapy: DCV combined with PEG-α/RBV

■ Treatment-naive patients

DCV in combination with PEG-α/RBV resulted in greater virologic response rates than PEG-α/RBV in treatment-naive patients in Phase IIa and IIb studies (Table 2). In the Phase IIa study (AI444–014), 48 weeks of treatment with DCV (3, 10 or 60 mg), in combination with PEG-α/RBV, acheived higher rates of extended rapid virologic response (eRVR) and SVR24 than the control group (PEG-α-2a/RBV alone) in treatment-naive,

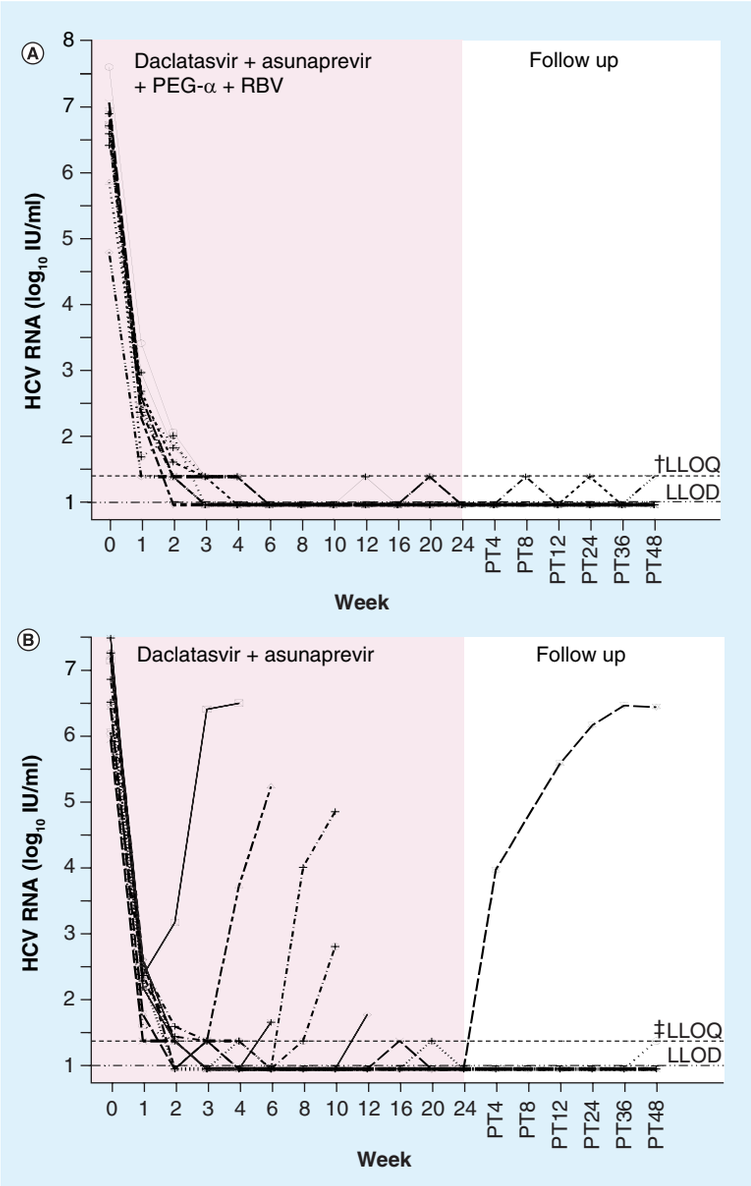

Figure 2. HCV RNA levels with daclatasvir plus asunaprevir (A) with PEG-α/RBV; or (B) without PEG-α/RBV in prior null-responders to PEG-α/RBV through 24 weeks of treatment and 48 weeks PT follow up.
For patients who had viral breakthrough HCV RNA levels are shown up to the initiation of rescue treatment.
†In one patient HCV RNA levels were <LLOQ at week 48 PT and undetectable 43 days later.
‡In one patient HCV RNA levels were <LLOQ at week 48 PT and undetectable 13 days later.
HCV: Hepatitis C virus; LLOQ: 25 IU/ml; PEG-α: Pegylated interferon α; PT: Post-treatment; RBV: Ribavirin.
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non-cirrhotic patients with chronic HCV genotype 1 infection (Figure 3) [16]. Among patients treated with DCV, 96% (25/26) of those who achieved SVR12 also achieved SVR24. The efficacy of the triple combination of DCV with PEG-α/RBV in treatment-naive patients is under further evaluation in a Phase IIb study in 395 patients with chronic HCV genotype 1 (n = 365) or genotype 4 (n = 30) infection (the COMMAND-1 study, AI444–010) [17,18]. Rates of early virologic responses and SVR12 were higher in patients treated with DCV (20 or 60 mg) plus PEG-α-2a/RBV than with placebo plus PEG-α-2a/RBV (Table 2 & Figure 4A). In patients with HCV genotype 1 infection, virologic response rates were higher for patients with genotype 1b versus 1a in both DCV groups, and for patients with *IL28B* genotype CC (favourable for antiviral response) versus non-CC (unfavourable for antiviral response) in all treatment groups. HCV RNA below the lower limit of quantitation (25 IU/ml) at week 4 and undetectable at week 10 (protocol-defined response [PDR]) was achieved by 71% (104/147) and 72% (105/146) of DCV 20 and 60 mg recipients, respectively; these patients were assigned to 24 weeks of therapy and rerandomized to receive either 12 or 24 weeks of DCV. In these patients, SVR12 rates were comparable (75–87%) across DCV dose groups (20 vs 60 mg) and across different durations of triple therapy (12 or 24 weeks). These results suggest that treatment-naive patients with genotype 1 HCV, particularly those with the *IL28B* CC genotype, can be treated successfully with a reduced duration of the DCV component of the triple combination. Preliminary results from a Phase II study suggest that the *in vitro* activity of DCV against HCV genotype 2/3 also translates into clinical efficacy *in vivo* [19]. Treatment lasting 12 weeks with DCV plus PEG-α/RBV resulted in higher virologic response rates than 12 weeks of PEG-α/RBV alone in patients with chronic HCV genotype 2/3 infection [19].

■ Patients previously treated with PEG-α/RBV

Patients who have failed to respond to initial treatment with PEG-α/RBV can be classified as null-responders, who have a <2 $\log_{10}$ decline in HCV RNA over 12 weeks of treatment or partial responders, who achieve a ≥2 $\log_{10}$ decline in HCV RNA but do not reach undetectable levels of HCV RNA. In these patients, re-treatment with triple therapy with a protease inhibitor and PEG-α/RBV results in a low rate of SVR: approximately 14–33% for null-responders and 40–59% for partial responders [9,58,59]. Although the triple combination of DCV with PEG-α/RBV appears to offer some improvement on these response rates in previously treated patients, responses are greatly reduced in comparison with those observed in treatment-naive patients.

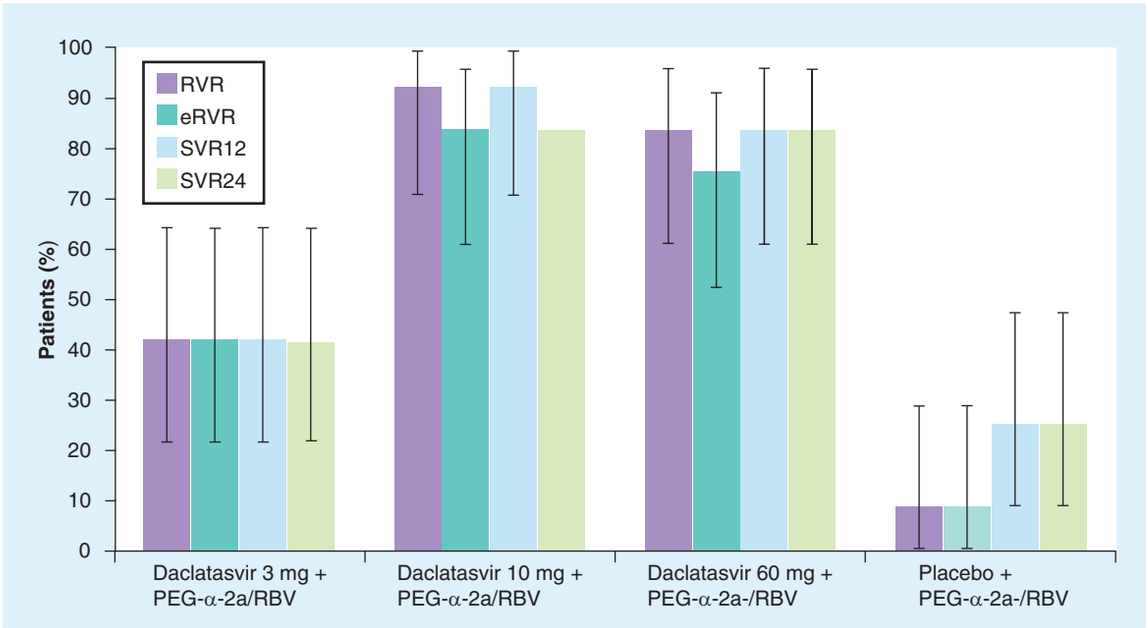

Figure 3. Virologic responses in treatment-naive patients with hepatitis C virus genotype 1 infection treated with daclatasvir + pegylated interferon α-2a/ribavirin for 48 weeks (modified intent-to-treat analysis, n = 12 per treatment arm) [16].

Error bars represent 80% confidence intervals. Hepatitis C virus RNA assay LLOQ: 25 IU/ml.
eRVR: Undetectable HCV RNA at weeks 4 and 12; PEG-α-2a: Pegylated interferon α-2a; RBV: Ribavirin; RVR: Undetectable hepatitis C virus RNA at week 4; SVR12/24: Undetectable hepatitis C virus RNA at follow-up weeks 12/24.

Week 12 results from the COMMAND-2 Phase II study (AI444–011) demonstrated that DCV (20 or 60 mg) plus PEG-α-2a/RBV resulted in more rapid HCV RNA suppression in prior null (n = 265) and partial (n = 137) responders than PEG-α-2a/RBV alone (Figure 4B) [19].

Two studies carried out in Japan evaluated the combination of DCV with either PEG-α-2a/RBV (AI 444–022) or PEG-α-2b/RBV (AI444–021), in treatment-naive and -experienced patients with genotype 1 chronic HCV infection [21,22]. In both studies, results in treatment-naive patients were comparable with those seen in previous Phase II studies, with significantly higher rates of SVR with the triple combination than with PEG-α/RBV alone. Among null or partial responders receiving DCV with PEG-α-2b/RBV (AI444–021), eRVR and PDR rates were both 56% (5/9) in the DCV 10-mg arm, and 22 (2/9) and 33% (3/9) in the DCV 60-mg arm, respectively. Among patients who achieved a PDR, 40 (2/5; 10 mg) and 67% (2/3; 60 mg) of patients achieved SVR24 [21]. Among null or partial responders receiving DCV with PEG-α-2a/RBV (AI444–022), eRVR and PDR rates were 63 (5/8) and 88% (7/8) in the DCV 10-mg arm, and 78 (7/9) and 78% (7/9) in the DCV 60-mg arm. Among patients who achieved a PDR, 57 (4/7; 10 mg) and 86% (6/7; 60 mg) achieved SVR24 [22].

Quadruple therapy with DCV plus asunaprevir plus PEG-IFN/RBV

Although response rates achieved with DCV plus PEG-α/RBV are significantly greater than rates achieved with PEG-α/RBV alone in null and partial responders, there are still many patients who do not respond or who experience virologic breakthrough. It is possible that the addition of a second DAA could sufficiently increase the potency of the regimen to allow these patients to achieve an SVR. Preliminary results from a Phase IIa study assessing the quadruple regimen of DCV plus asunaprevir plus PEG-α/RBV in null responders are very promising, with SVR4 rates >90% achieved after 24 weeks of treatment.

In the sentinel cohort of study AI447–011, 21 genotype-1 infected patients with prior null-response to PEG-α/RBV received 24 weeks treatment with DCV (60 mg QD) plus the NS3 protease inhibitor asunaprevir (600 mg twice a day [BID]) with or without PEG-α/RBV [23]. The quadruple combination was received by ten patients (nine with genotype 1a). HCV RNA levels declined rapidly and remained suppressed throughout

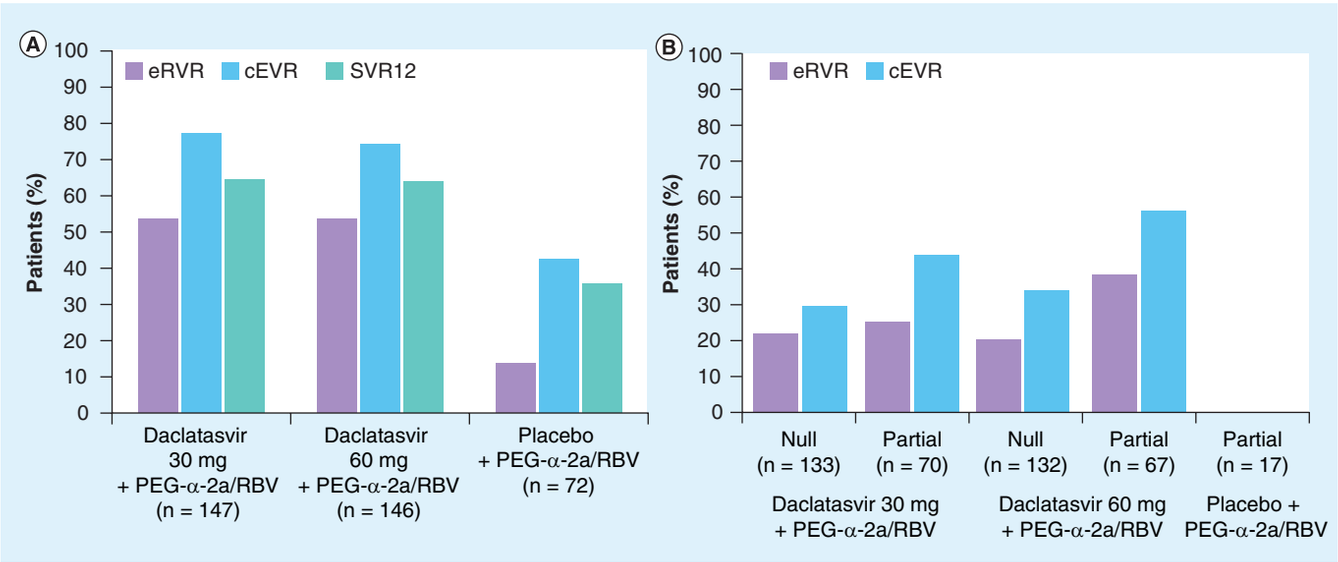

Figure 4. Virologic responses at weeks 4 and 12 in patients infected with hepatitis C virus genotype 1 treated with daclatasvir and pegylated interferon α-2a/ribavirin. (A) Treatment-naive patients (COMMAND-1) [17,18]; **(B)** Patients with null or partial response to previous pegylated interferon α-2a/RBV (COMMAND-2) [19] . Error bars represent 80% confidence intervals. Hepatitis C virus RNA assay LLOQ: 25 IU/ml.

cEVR: Undetectable hepatitis C virus RNA at week 12; eRVR: Undetectable hepatitis C virus RNA at weeks 4 and 12; PEG-α-2a: Pegylated interferon α-2a; RBV: Ribavirin; SVR12: Undetectable hepatitis C virus RNA at follow-up week 12.

the study period in all patients (Figure 2A). At week 2, the median decrease in HCV RNA from baseline was -5.3 $\log_{10}$ IU/ml. All ten patients achieved SVR4 and SVR12, and nine of ten achieved SVR24 and SVR48. No patient experienced virologic breakthrough. An expansion cohort of this study assessed the efficacy of the quadruple regimen DCV plus asunaprevir plus PEG-α/RBV using a lower dose of asunaprevir (200 mg QD; n = 21; or 200 mg BID; n = 20) in 41 prior null responders (36 with genotype 1a HCV) [25]. After 24 weeks of therapy, 18 out of 20 (90%) and 21 out of 21 (100%) patients receiving asunaprevir 200 mg BID or QD, respectively, had undetectable HCV RNA. SVR12 was achieved by 18 out of 20 (95%) and 20 out of 21 (95%) patients receiving asunaprevir 200 mg BID or QD, respectively. The quadruple combination was well tolerated with no treatment discontinuations for adverse events. The most frequent adverse events were headache, asthenia, diarrhea, alopecia, fatigue and irritability [25].

Interferon-free dual therapy with DCV plus DAAs

The low SVR rate achieved with PEG-α/RBV treatment for patients with HCV genotype 1, coupled with the poor tolerability and high rates of adverse effects, has prompted the study of regimens that exclude PEG-α or RBV, or both. Such regimens will be particularly important for patients who are ineligible for or intolerant to PEG-α/RBV and, thus, have no current treatment options. Encouraging results have been obtained in preliminary clinical studies of DCV in interferon-free combinations with other DAAs in treatment-naive and -experienced patients without cirrhosis.

The first dual DAA oral combination to be studied was DCV plus asunaprevir in the dual combination arm of study AI447–011; in the sentinel cohort 11 patients (nine infected with HCV genotype 1a) received only DCV (60 mg) and asunaprevir (600 mg BID) [23]. As with the quadruple combination, HCV RNA levels declined rapidly after initiation of treatment (Figure 2B); at week 2 the median decrease in HCV RNA from baseline was -5.1 $\log_{10}$ IU/ml and seven out of 11 (64%) patients had undetectable HCV RNA at week 4 (RVR). However, only four out of 11 (36%; two out of nine with genotype 1a and two out of two with genotype 1b) patients achieved SVR12 and SVR24. Of these, three patients also had SVR48; the fourth patient had HCV RNA <25 IU/ml at week 48 post-treatment and undetectable HCV RNA 43 days later. Viral relapse occurred in one patient infected with subtype 1a at week 4 after cessation of treatment. In this patient the pre-existing NS3 resistance variant was detectable at baseline and at time of relapse, and the NS5A resistance variant Q30E was detected at relapse. Viral breakthrough occurred in six out of 11 patients (55%, all with HCV genotype 1a), between weeks 3 and 12. In all six patients, HCV variants with resistance to both antiviral agents were

detected in post-breakthrough samples (but not at baseline). Addition of PEG-α/RBV rescue therapy resulted in initial HCV RNA reduction in all six patients. In four out of six patients, HCV RNA became undetectable with rescue therapy; two experienced relapse after treatment cessation, and two sustained undetectable HCV RNA up to the most recent testing. The remaining two patients did not achieve undetectable HCV RNA (treatment was discontinued). The dual combination was well tolerated with no serious adverse events or discontinuations. Transient elevations of alanine aminotransferase levels were observed in six patients; these elevations were not associated with clinically significant increases in bilirubin level. Dose-ranging studies with asunaprevir suggest that these elevations may be related to the protease inhibitor component of the combination [60], thus subsequent studies have used a lower dose of asunaprevir. The DCV–asunaprevir combination is currently under further evaluation in an expansion cohort of study AI447–011, in which 20 patients have received DCV 60 mg QD plus asunaprevir 200 mg BID and 21 patients received DCV 60 mg QD plus asunaprevir 200 mg QD. Preliminary results report an SVR4 in 13/15 patients with BID asunaprevir and 10/16 patients with QD asunaprevir [25].

Another open-label, Phase IIa study (AI447–017) assessed the combination of DCV and asunaprevir in 43 Japanese patients who were intolerant or ineligible to PEG-α/RBV (n = 20) or with previous null-response to PEG-α/RBV (n = 21). All patients in this study were infected with HCV genotype 1b, the predominant subtype in Japan. Patients received DCV (60 mg QD) plus asunaprevir (initially 600 mg BID in a sentinel cohort of ten null-responders, subsequently reduced to 200 mg BID) for 24 weeks [26,27]. Among null-responders, 18/21 (86%) had undetectable HCV RNA at week 24 (end of dosing), and 19/21 (90%) had SVR12 (Figure 5). There was no viral breakthrough during treatment, and no case of relapse after treatment was stopped. In some patients, variants carrying NS5A and NS3 resistance-associated amino-acid substitutions were detected at baseline. Most had no apparent effect on virologic response; however, the resistance variant Y93H was present at baseline in 5/7 failures, and may have been associated with failure where the pharmacokinetics for both drugs was also low [57]. Among ineligible or intolerant patients, 16/22 (73%) had undetectable HCV RNA at week 24 (EOT), and 14/22 (64%) achieved SVR12 (Figure 5). Treatment failure (three viral breakthroughs and four post-treatment relapses) was associated with the emergence of HCV variants with resistance to DCV and asunaprevir. The plasma trough concentrations in these patients were below the median level for both agents, suggesting a role for non-adherence in treatment failure.

DCV has also been studied in an interferon-free regimen in combination with an NS5B polymerase inhibitor. In study AI444–040, treatment-naive patients with HCV genotype 1, 2 or 3 received DCV (60 mg QD) plus the nucleotide analog NS5B inhibitor GS-7977 (sofosbuvir; 400 mg QD, with or without lead-in), with or without RBV, over 24 weeks [28,29]. This was the first study to assess the combination of an NS5A inhibitor and a nucleotide NS5B inhibitor. In patients with genotype 1a or 1b, 86–87% (12/14 and 13/15) of patients treated with the dual combination DCV plus GS-7977 and 93% (14/15) of those receiving the triple combination DCV plus GS-7977 and RBV had undetectable HCV RNA at week 24 (EOT). All patients achieved SVR4 and SVR12. In patients with HCV genotype 2 or 3, 94–100% (15/16 and 14/14) of patients treated with the dual combination DCV plus GS-7977, and 86% (12/14) of those receiving the triple combination DCV plus GS-7977 and RBV, had undetectable HCV RNA at week 24 (EOT); 88–100% (14/16 and 14/14) of patients treated with the dual combination DCV and GS-7977, and 86% (12/14; two patients were lost to follow up) of those receiving the triple combination of DCV plus GS-7977 plus RBV achieved SVR4 and SVR 12. Thus, with an all-oral combination of DCV plus GS-7977, SVR12 rates of >95% were achieved independent of HCV genotype. Addition of RBV had no effect on virologic response but increased the frequency of anemia (which was absent in the RBV-free arms).

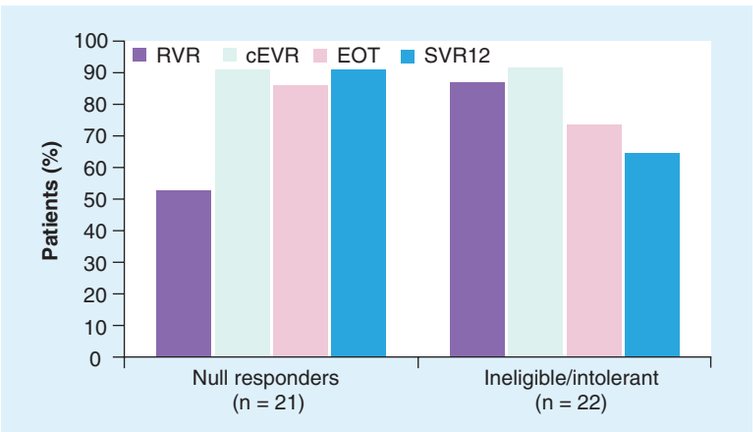

Figure 5. Virologic responses with daclatasvir plus asunaprevir in Japanese hepatitis C virus genotype 1b-infected patients ineligible/intolerant to pegylated interferon α/ribavirin or with previous null response to pegylated interferon α/ribavirin (intention-to-treat, missing = failure analysis) [26,27].
Hepatitis C virus RNA assay LLOQ: 15 IU/ml.
cEVR: Undetectable hepatitis C virus RNA at week 12; EOT: End of treatment at week 24 or last on-treatment visit for patients who discontinued early; RVR: Undetectable hepatitis C virus RNA at week 4; SVR12: Undetectable hepatitis C virus RNA at follow-up week 12.

Conclusion

SVR rates achieved in patients chronically infected with HCV genotype 1 have improved with the introduction of the first-generation protease inhibitors, but unmet needs remain [4,5]. Boceprevir and telaprevir are dosed three-times daily and are associated with significant adverse events, in addition to those associated with PEG-α/RBV. New antiviral treatment options are needed with different mechanisms of action to improve SVR rates, QD oral dosing, low pill burden good tolerability to improve adherence and limited drug–drug interactions to simplify patient management.

DCV is the first HCV NS5A replication complex inhibitor to enter clinical development. With once-daily dosing, potent antiviral activity against HCV genotypes 1–5, fair tolerability, as well as low potential for drug–drug interactions, DCV has many features to suggest that it is a good candidate to evaluate in combination with other antivirals for the treatment of chronic HCV. In the studies summarized in this review, DCV has shown a consistent strong antiviral effect in various different antiviral combinations (both with and without PEG-α/RBV) and various different patient populations (treatment-naive, non-responder and HCV genotypes 1a, 1b, 2, 3 and 4). Initial studies evaluated 48 weeks of DCV with PEG-α/RBV; subsequent studies have used response-guided treatment designs to assess potential for shorter treatment. Most data suggest that 24 weeks may be sufficient for most patients receiving DCV-containing regimens; further studies are needed to determine whether even shorter treatment durations are feasible in certain patient populations.

DCV has a relatively low barrier to resistance as a monotherapy and HCV variants associated with DCV resistance have been detected in several studies at baseline. However, clinical study data suggest that treatment with an appropriate dose of DCV in combination with other agents provides sufficient potency to prevent the emergence of resistance in most patient populations [23].

Some DCV-containing regimens without PEG-α/RBV have achieved high rates of SVR, with the improved tolerability that can be anticipated by eliminating PEG-α/RBV, offering the promise of simplified and better-tolerated oral regimens for some patients. Most recently, the all-oral, interferon-sparing regimen of DCV plus the NS5B polymerase inhibitor, GS-7977 (sofosbuvir), demonstrated SVR4 rates of >95% in treatment-naive patients with genotypes 1, 2 or 3 infection [25]. If findings are confirmed in further studies with a longer follow-up, DCV plus GS-7977 may represent a significant advance in the first-line treatment of HCV.

Patients with prior null or partial response to PEG-α/RBV represent a particularly difficult-to-treat population; in this case, the additional potency offered by DAA combinations may offer a better solution than PEG-α/RBV-based triple therapy. DCV plus asunaprevir resulted in a rapid decline in HCV RNA in treatment-experienced patients, but in patients with genotype 1a infection, the longer-term efficacy of this combination was compromised by the emergence of resistance [23]. Addition of PEG-α/RBV to the dual combination therapy appeared to limit the emergence of resistance in this population [23,35]. Thus, at least for patients with genotype 1a infection, PEG-α/RBV may be needed to maintain the response and achieve an SVR. This may not be the case for patients with HCV genotype 1b [23,30,35]. In the Japanese study AI447–017, low rates of virologic breakthrough were observed during treatment of PEG-α/RBV null-responders or ineligible/intolerant patients with HCV genotype 1b infection with DCV plus asunaprevir [24,30].

In summary, the data presented in this review demonstrate that DCV is a potent, NS5A replication complex inhibitor that can be combined with a range of other anti-HCV therapies, including interferons, NS3 protease inhibitors, or NS5B polymerase inhibitors, to achieve high SVR rates. Together with a good tolerability profile and a convenient once-daily dosing schedule, the results from clinical studies to date demonstrate that DCV is an encouraging new candidate for the treatment of chronic HCV infection. In addition to DCV, further new anti-HCV agents are being developed and investigated, including NS3/4A protease inhibitors, nucleoside and non-nucleoside inhibitors of the HCV NS5B polymerase, cyclophilin inhibitors and new interferons such as PEG-IFN-λ [36]. The development of multiple therapies targeting different aspects of the HCV life-cycle may allow further new combination regimens aimed at maximizing viral suppression, minimizing resistance, and demonstrating better tolerability than the currently approved therapies.

Future perspective

After more than 15 years with the combination of PEG-α/RBV as the standard treatment for chronic HCV infection, this field is now dramatically changing with the rapid entry of numerous new antivirals into clinical development, including DAAs and agents with non-viral targets (e.g., cyclophilin inhibitors, IFN-λ and vaccine therapy). It is likely that combinations of these agents, in interferon-free regimens, will soon become the standard of care for HCV infection, tailored to individual patients according to the degree of disease progression (e.g., fibrosis, cirrhosis, hepatocellular carcinoma), HCV genotypes and subtypes, resistance profiles, and prior therapeutic history. These regimens have a better safety profile, and greater antiviral potency compared with the combination of PEG-α/RBV and a first-generation protease inhibitor. By analogy with previous results achieved with interferon-based treatments, given the extended follow-up of patients treated

with oral combinations of DAAs, including DCV, it is reasonable to assume that that patients achieving SVR12 or SVR24 with these treatments have indeed achieved a cure, although further clinical evidence will be needed to confirm this assumption. Why the same difference of SVR and resistance profile between genotype 1a and genotype 1b is observed with DCV than with protease inhibitors is unclear and warrants further investigation. Future challenges to be addressed, over and above the already increased efficacy, will be needed to further improve the safety, adherence and costs of these new oral combinations.

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Executive summary

Pharmacology & pharmacokinetics

- Daclatasvir (DCV; BMS-790052), the first hepatitis C virus (HCV) NS5A replication complex inhibitor to enter clinical development, has a pharmacokinetic profile that supports once-daily dosing.
- DCV is the most potent HCV replication inhibitor reported to date, with *in vitro* EC_{50} in the picomolar range against HCV replicons representing six major HCV genotypes (1a, 1b, 2a, 3a, 4a and 5a).

Safety

- DCV has demonstrated a generally well-tolerated safety profile in clinical studies to date.

Resistance

- *In vitro* resistance selection studies (with genotype 1a and 1b replicons) have identified DCV resistance-associated mutations that map to the N-terminal region of NS5A and reduced susceptibility to DCV (low to medium barrier to resistance). However, treatment with an appropriate dose of DCV in combination with other agents provides sufficient potency to prevent the emergence of resistance in most patient populations.

Efficacy in clinical studies

- DCV significantly increases the antiviral potency of the pegylated interferon α and ribavirin (RBV) combination in treatment-naive and -experienced patients.
- The oral combination of DCV with the nucleotide analog GS-7977 (sofosbuvir), with or without RBV, resulted in a sustained virologic response of more than 95% in treatment-naive HCV genotype 1a- or 1b-infected patients.
- The quadruple regimen of DCV with asunaprevir and pegylated interferon α/RBV resulted in a sustained virologic response of 90% in HCV genotype 1a- and 1b-infected null-responders.
- Further study is needed, but in all populations tested to date, at least one DCV-containing regimen has provided high rates of virologic response and a generally well-tolerated safety profile, suggesting that successful outcomes can be achieved with DCV in a majority of patients by tailoring therapy based on patient and disease characteristics.

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