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REVIEW ARTICLE

Interferon free therapy with direct acting antivirals for HCV

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Keywords
asunaprevir – daclatasvir – faldaprevir – simeprevir – NS5A inhibitors – NS5B polymerase inhibitors – protease inhibitors – ribavirin – sofosbuvir

Abbreviations
AEs adverse events; DAA direct-acting antivirals; EVR early virological response; EOT end of treatment Response; PEG-IFN pegylated interferon; RVR rapid virological response; RBV ribavirin; SVR sustained virological response.

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Abstract
The current treatment for hepatitis C virus (HCV) genotype 1 chronic infection is the addition of direct-acting antivirals (DAA) with a protease inhibitor (telaprevir or boceprevir) to the pegylated interferon (PEG-IFN) plus ribavirin (RBV) regimen. Major progress has been made in the past few years: numerous ongoing trials with different compounds, increasing sustained virological response (SVR) rates with oral regimens and shortened treatment duration. Combinations of antivirals with additive potency that lack cross-resistance and with a good safety profile may provide new regimens in the future to make HCV the first chronic viral infection to be eradicated worldwide with a finite duration of combination DAA therapy without IFN.

Hepatitis C virus (HCV) is a major cause of chronic liver disease, with an estimated 170 million people infected worldwide (1). Hepatitis C virus, identified in 1989, is an enveloped virus with a 9.6-kb single-stranded RNA genome (2), a member of the Flaviviridae family, genus Hepacivirus. The goal of treatment is to obtain a sustained virological response (SVR) defined as undetectable HCV RNA in serum after 24 weeks of post-treatment follow-up (3). Twelve-week post-treatment follow-up appears to be relevant at 24 weeks to define SVR (4). SVR results in the eradication of HCV infection and improvement of the histological outcome (5). In 2011, two direct acting antivirals (DAAs) were approved for HCV genotype 1 chronic infection, telaprevir and boceprevir and opened a new area for HCV therapy (6). These two NS3/4 protease inhibitors (PI) are given in combination with pegylated interferon (PEG-IFN) and ribavirin (RBV). Phase III clinical trials have shown that approximately 25–35% of G1 treatment-naïve patients and 50–60% of G1 HCV patients who failed to respond to a first course of treatment with PEG-IFN and RBV do not achieve SVR and are not cured of HCV infection with this triple combination. Therefore, there is a need to develop new DAAs to improve SVR. Furthermore, IFN has several side effects. The aim of this review is to describe the mechanisms of action of DAAs and summarize the results obtained with DAA combinations without IFN.

Viral cycle and targets for drug development

The HCV life cycle begins with virion attachment to its specific receptor. The HCV RNA genome serves as a template for viral replication and as a viral messenger RNA for viral production. It is translated into a polyprotein that is cleaved by proteases. Then, viral assembly occurs. Potentially, each step of the viral cycle is a target for drug development (Fig. 1). Knowledge of the structures of HCV protease and HCV polymerase has allowed structure-based drug design to develop inhibitors to these enzymes (7, 8). Several findings suggest that HCV modulation of IFN induction and signalling

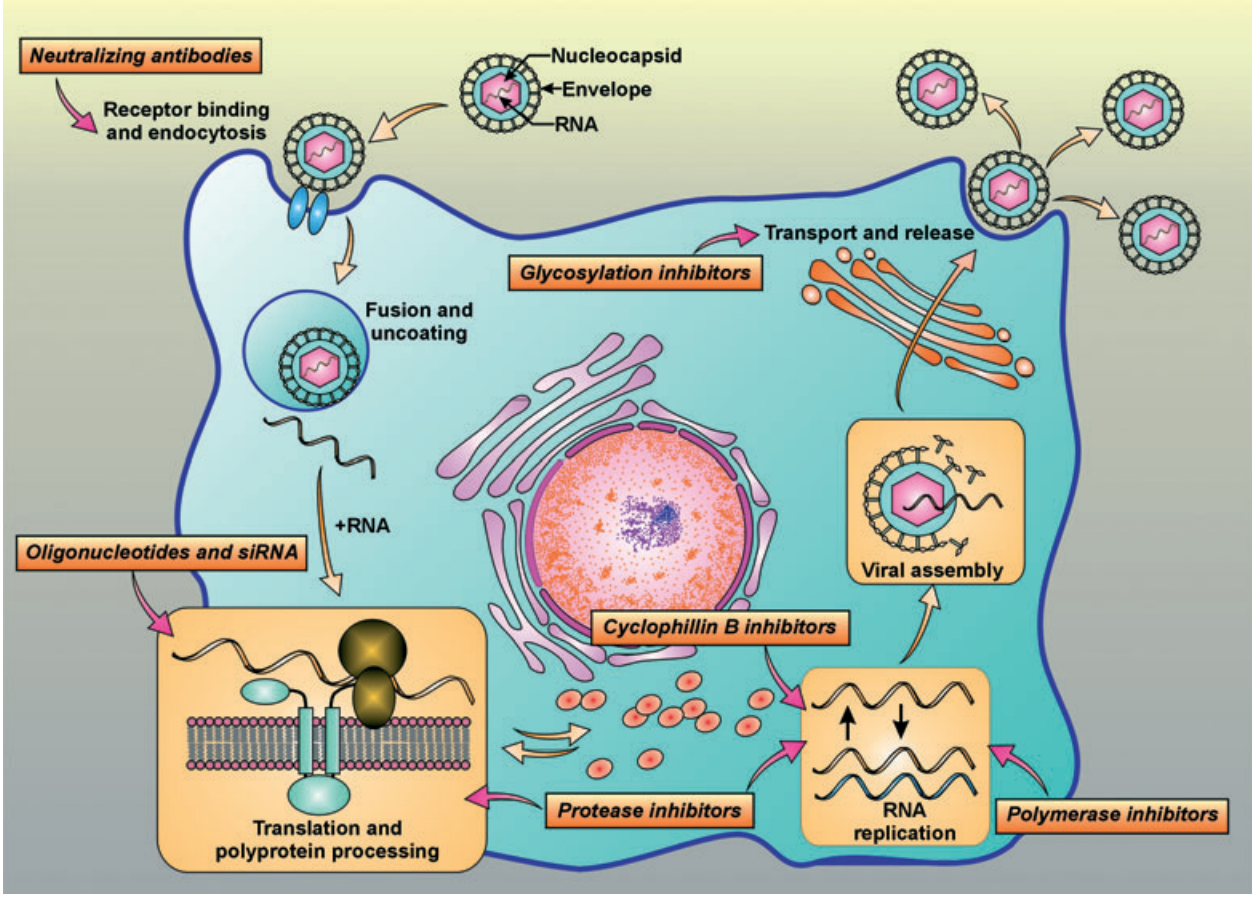


Fig. 1. Hepatitis C virus (HCV) viral cycle. The HCV lifecycle starts with virion attachment to its specific receptor (not clearly identified). The HCV RNA genome serves as a template for viral replication and as a viral messenger RNA for viral production. It is translated into a polyprotein that is cleaved by proteases. Then, viral assembly occurs. Potentially, each step of the viral cycle is a target for drug development.

attenuates the expression of IFN-stimulated genes, allowing HCV to escape the antiviral actions of the host response (9–11).

All the HCV enzymes – NS2-3 and NS3-4A proteases, NS3 helicase NS5A and NS5B RdRp – are essential for HCV replication, and are potential drug discovery targets (Fig. 2). Therefore, DAA with different viral targets, including NS3 protease inhibitors, nucleoside/nucleotide analogue and non-nucleoside inhibitors of the RNA-dependent RNA polymerase, and NS5A inhibitors are under development. General characteristics of different classes of DAA are indicated in Table 1.

Protease inhibitors

The NS3 serine protease is located in the N-terminal region of NS3. The NS3 serine protease domain associates with the NS4A cofactor to cleave four specific sites.

This enzyme has been characterized at the biochemical level and its structure is known (7, 8). The serine protease activity of NS3 is an attractive target for new drugs that could effectively block viral replication. The NS3/4A protease inhibitors can be divided into two chemical classes: macrocyclic inhibitors and linear tetrapeptide a-ketoamid derivatives. In 2003, a protease inhibitor (BILN 2061) that blocks HCV replication in the replicon model was shown to be effective in humans (12–14).

Although protease inhibitors have a high antiviral efficacy, they have several potential limitations. Protease inhibitors are highly specific and as the amino acid sequence of the NS3 protease domain differs significantly between HCV genotypes, their antiviral efficacy differs among genotypes. Indeed, telaprevir is less effective in non-1 genotypes. Furthermore, as HCV has a high mutation replication rate, with a lack of proof reading, resistance is an important issue.

The genetic barrier to resistance is defined as the number of amino acid substitutions required to confer full resistance to a drug. Usually, DAA with a low genetic barrier to resistance require only one or two amino acid substitutions for high resistance. DAA with a high barrier of resistance usually require three or more amino acid substitutions in the same region.

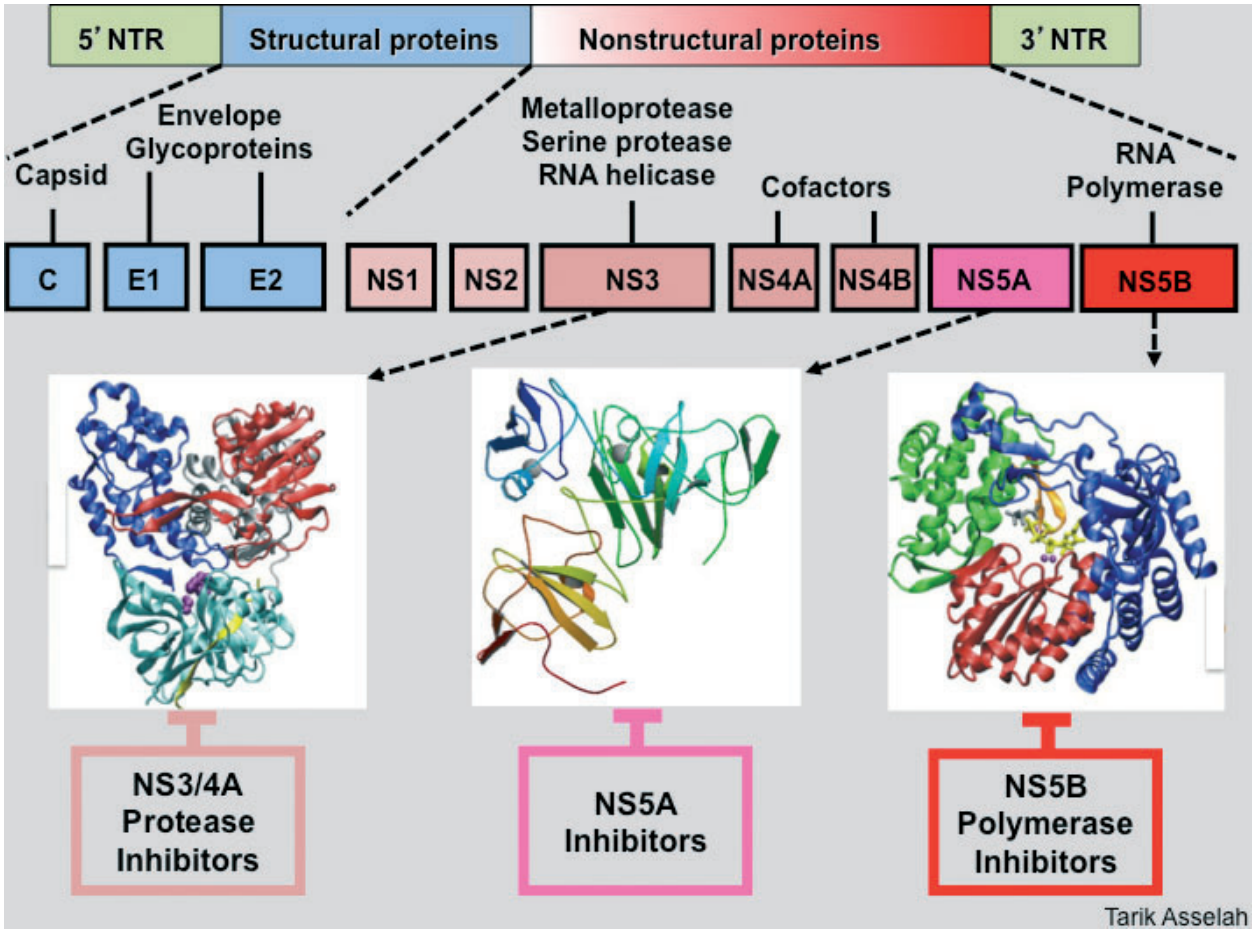


Fig 2. Hepatitis C virus (HCV) genome and potential drug discovery targets. The HCV RNA genome serves as a template for viral replication and as a viral messenger RNA for viral production. It is translated into a polyprotein that is cleaved by proteases. All the HCV enzymes – NS2-3 and NS3-4A proteases, NS3 helicase, NS5A and NS5B RdRp – are essential for HCV replication, and are therefore potential drug discovery targets.

Table 1. General characteristics of different classes of DAAs

	Efficacy	Genotype dependency	Barrier to resistance
NS3/4A (protease inhibitors)	+++	+++	++
NS5A	+++	+++	++
NS5B (nucleos(t) ides)	+++	+++	+++
NS5B (nonnucleosides)	++	+	+

The genetic barrier to protease inhibitors is usually low. Resistance differs significantly between HCV subtypes. Viral resistance to telaprevir occurred much more frequently in genotype 1a compared with genotype 1b. This result of nucleotide differences at position 155 in HCV subtype 1a (AGA, encodes R) vs. 1b (CGA, also encodes R). The mutation most frequently associated with resistance to telaprevir was R155K; changing R–K at position 155 requires one nucleotide change in HCV subtype 1a and two nucleotide changes in subtype 1b isolates (15).

As illustrated with the R155K mutation, which reduces replication capacity in the replicon model (16), resistance mutations frequently impair viral fitness.

However, under antiviral pressure, during therapy, second site mutations are selected that restore fitness, explaining why the R155K primary mutation is frequently found in association with V36M in genotype 1a viruses. Therefore, it is recommended to stop rapid treatment in patients with viral breakthrough and good adherence to therapy.

Polymerase inhibitors

Polymerase inhibitors interfere with viral replication by binding to the NS5B RNA-dependent RNA polymerase. NS5B RNA polymerase inhibitors can be divided into

two distinct categories – nucleoside inhibitors and non-nucleotide inhibitors.

Nucleoside analogue inhibitors mimic the natural substrates of the polymerase and are incorporated into the RNA chain causing direct chain termination (17). Nucleoside analogue polymerase inhibitors are compounds that require conversion to an active triphosphate form. As the active site of NS5B is highly conserved, nucleoside analogue inhibitors are potentially effective against all the different genotypes. Moreover, single amino acid substitutions in every position of the active site may result in loss of function. Resistance to nucleoside analogue inhibitors is usually low.

Non-nucleoside inhibitors bind to several discrete sites on the HCV polymerase, which results in conformational protein change before the elongation complex is formed (17). NS5B is structurally organized in a characteristic 'right-hand motif' containing finger, palm and thumb domains, and offers at least four NNI-binding sites, a benzimidazole (thumb 1)-binding, thiophene (thumb 2)-binding, benzothiadiazine (palm1)-binding and benzofuran-(palm 2)-binding site. Resistance is more frequent with non-nucleoside inhibitors. Furthermore, mutations at non-nucleoside inhibitor-binding sites do not necessarily lead to impaired function of the enzyme.

NS5A inhibitors

The NS5A is a membrane-associated phosphoprotein present in basally phosphorylated (p56) and hyperphosphorylated (p58) forms (18–20). It was previously reported that only p58-defective mutants could be complemented in trans, and NS5A is involved in HCV virion production, suggesting that different forms of NS5A exert multiple functions at various stages of the viral life cycle. The N terminus of NS5A (domain I) has been crystallized in alternative dimeric forms and contains both zinc- and RNA-binding domains, properties that have been demonstrated in vitro. NS5A has been shown to interact with a number of host proteins and plays a role in interferon resistance in vivo (19). Daclatasvir (previously BMS 790052) is active at picomolar concentrations in vitro towards replicons expressing a broad range of HCV genotypes and acts in an additive to synergistic fashion with IFN and other DAAs (18–20). The resistance profile of daclatasvir reveals inhibitor sensitivity maps to the N terminus of domain 1 of NS5A (19). It has been demonstrated that NS5A inhibitors could block hyperphosphorylation of NS5A, which is believed to play an essential role in the viral life cycle.

Interferon-free combination trials

Several IFN-free combination trials are ongoing with different DAAs: NS3/4a protease Inhibitor, HCV polymerase complex – non-nucleoside NS5B, nucleoside NS5B or NS5A inhibitor. Major progress has been made in the past few years: numerous ongoing trials with different DAAs; increasing SVR rates with oral regimens and shortened treatment duration. The priorities for future combination are listed in Table 2. Among the unmet needs is the treatment of genotype 4 infected patients. Approximately 20% among the 170 millions, HCV-infected patients worldwide are genotype 4 (approximately 34 millions). The standard treatment for HCV G4 is PEG-IFN/RBV for 48 weeks. Naive G4 IL28B non-CC patients have SVR rates below 50% with the standard PEG-IFN/RBV for 48 weeks (21). Furthermore, previous relapsers or non-responders G4 patients have very low chance to cure with the same PEG-IFN/RBV regimen.

Table 2. High priorities for HCV drug development

High efficacy (potency)
Favourable safety profile
High barrier to resistance
Oral regimen (IFN free)
Pan-genotypic
Favourable pill burden (once or twice a day)
Short duration
Few drug–drug interactions
Available for ELD, cirrhosis and HIV-HCV
Price affordable

Hepatitis C Virus drug development is nowadays faster because of short treatment durations, no need for a control arm with IFN-based regimens, and also because 12-week post-treatment follow-up is as relevant as 24 weeks to determine the SVR (4). At present, several studies of DAA combinations are ongoing in treatment-naïve HCV patients (Table 3).

Sound-C2 study: faldaprevir, BI 207127 with or without ribavirin (Boehringer-Ingelheim)

Sound-C2 is an open-label, randomized, Phase IIb study with 362 treatment-naïve HCV genotype-1 patients in one of five treatment arms (Fig. 3A). This study evaluated the safety and efficacy of faldaprevir (protease inhibitor) and BI 207127 (polymerase inhibitor), with and without ribavirin (22). Final results from the Phase IIb study, Sound-C2, showed that up to 85% of GT-1b HCV patients achieved SVR (Fig. 3B). The optimal regimen was 28 weeks of faldaprevir (once a day), and BI 207127 (BID). The full results from the largest IFN-free trial to date include patients with cirrhosis and have confirmed early data (23).

SVR was achieved for 70%, compared with 85% in the prevalent GT-1b patient subgroup. Nine per cent of the total population had cirrhosis and achieved SVR rates of up to 67 per cent.

The most common adverse events (AEs) were mild skin changes (itchy skin, rash or photosensitivity) or gastrointestinal disorders and transient indirect hyperbilirubinaemia, which sometimes presented as jaundice.

Table 3. IFN free ongoing clinical trials

First drug (company)	Second drug	Third drug	Fourth drug
Boehringer Ingelheim			
Faldaprevir (BI201335)	BI207127	Ribavirin	
Protease inhibitor	NS5B NNI		
Abbott			
ABT-450/r	ABT 267	ABT 333	Ribavirin
Protease inhibitor	NS5A inhibitor	NS5B NNI	
Gilead			
Sofosbuvir (GS 7977)	Ribavirin		
Sofosbuvir (GS 7977)	GS 5885		
NS5B NI	NS5A inhibitor		
Gilead/BMS			
Sofosbuvir (GS 7977)	Daclatasvir	± Ribavirin	
NS5B NI	NS5A inhibitor		
BMS			
Asunaprevir	Daclatasvir		
Protease inhibitor	NS5A inhibitor		
Vertex			
Telaprevir	VX 222		
Protease inhibitor	NS5B NNI	Ribavirin	

Thirty-six per cent of patients experienced some form of side effect, 12% of these were considered severe and 8% led to discontinuation of treatment. IFN-free phase III studies with Faldaprevir, BI 207127 and RBV are ongoing.

Aviator study: ABT-450/r, ABT-267, ABT-333 and ribavirin (Abbott)

The Aviator phase 2b study assesses the safety and efficacy of ABT-450/r (dosed 100/100–200/100 mg QD), ABT-267 (25 mg QD), ABT-333 (400 mg BID) and RBV in non-cirrhotic treatment-naïve patients and prior peg-interferon/ribavirin null responders for 8, 12 or 24 weeks (Fig. 4A) (24).

ABT-450 is a protease inhibitor, boosted by ritonavir; ABT-267 is an NS5A inhibitor; and ABT-333 is a NS5B polymerase NNI. Enrolment was open to GT1-infected patients regardless of IL28B host genotype. Ribavirin dosing was weight-based. Results from the treatment groups are summarized in Fig. 4B.

The SVR12 in treatment-naïve GT1 patients was 97.5% (77 of 79), and 93.3% (42 of 45) in GT1 null responder patients.

In GT1a patients, SVR12 was achieved in 96% (52 of 54) of treatment-naïve patients and 89% (25 of 28) of null responder patients.

In GT1b patients, SVR12 was achieved in 100% of treatment-naïve (25 of 25) and null responder patients (17 of 17).

In addition, results from the 12-week triple-DAA regimen without RBV in treatment-naïve patients showed:

- SVR12 in 87.3% (69 of 79) of GT1 patients.
- SVR12 in 83% (43 of 52) of GT1a patients.
- SVR12 in 96% (24 of 25) of GT1b patients.

The treatment was well tolerated. Four of 448 patients (1%) in the 8- and 12-week arms discontinued treatment because of adverse events. Of five serious AEs (1%), one (arthralgia or joint pain) was possibly study drug-related. In the trial, the most common adverse events were fatigue (28 and 27%) and headache (28 and 31%) for treatment-naïve and null responders respectively.

Electron study: sofosbuvir (GS-7977), GS-5885 and ribavirin (Gilead)

Interim data from the ongoing Phase 2 Electron study examining a 12-week course of therapy with the NS5B nucleotide inhibitor Sofosbuvir (formerly GS-7977), the NS5A inhibitor GS-5885 and RBV in patients with genotype 1 chronic HCV infection were reported (25). Among treatment-naïve patients receiving this combination, 100% (n = 25/25) remained HCV RNA undetectable 4 weeks after the end of treatment (SVR4) (Table 4). Among the nine genotype 1 previous null responders who were treated with Sofosbuvir, GS-5885 and ribavirin for 12 weeks, three of nine patients have reached the 4-week post-treatment time point and all three remain HCV negative. Both Sofosbuvir in combination with ribavirin and sofosbuvir in combination with GS-5885 and ribavirin were well tolerated. The most common AEs were headache, fatigue, upper respiratory tract infection and nausea. The most common clinically significant grade 3/4 laboratory abnormality was a haemoglobin reduction.

Interestingly, important results were reported with a dual therapy of Sofosbuvir plus ribavirin in a genotype 1 naïve patient population (26). In 60 HCV-G1 treatment-naïve patients, Sofosbuvir with RBV for 24 weeks resulted in:

- Full dose RBV: SVR4 of 77% (ITT), 82% (mITT).
- Low dose RBV: SVR4 of 56% (ITT), 64% (mITT).

Gilead recently initiated the first Phase 3 trial (ION-I) evaluating a fixed-dose combination of sofosbuvir and GS-5885 in treatment-naïve genotype 1 patients. This four-arm study is evaluating the fixed-dose combination with and without ribavirin for 12- and 24-week durations in 800 patients; 20 per cent of whom have evidence of cirrhosis.

Daclatasvir (BMS) plus sofosbuvir (Gilead) with or without ribavirin

This trial was designed to test the combination of daclatasvir (NS5A inhibitor) and sofosbuvir (NS5B nucleotide inhibitor) in three genotypes of the virus (1, 2 and 3), with or without RBV, for 12 or 24 weeks of therapy, and with or without a week-long run-in with sofosbuvir (27).

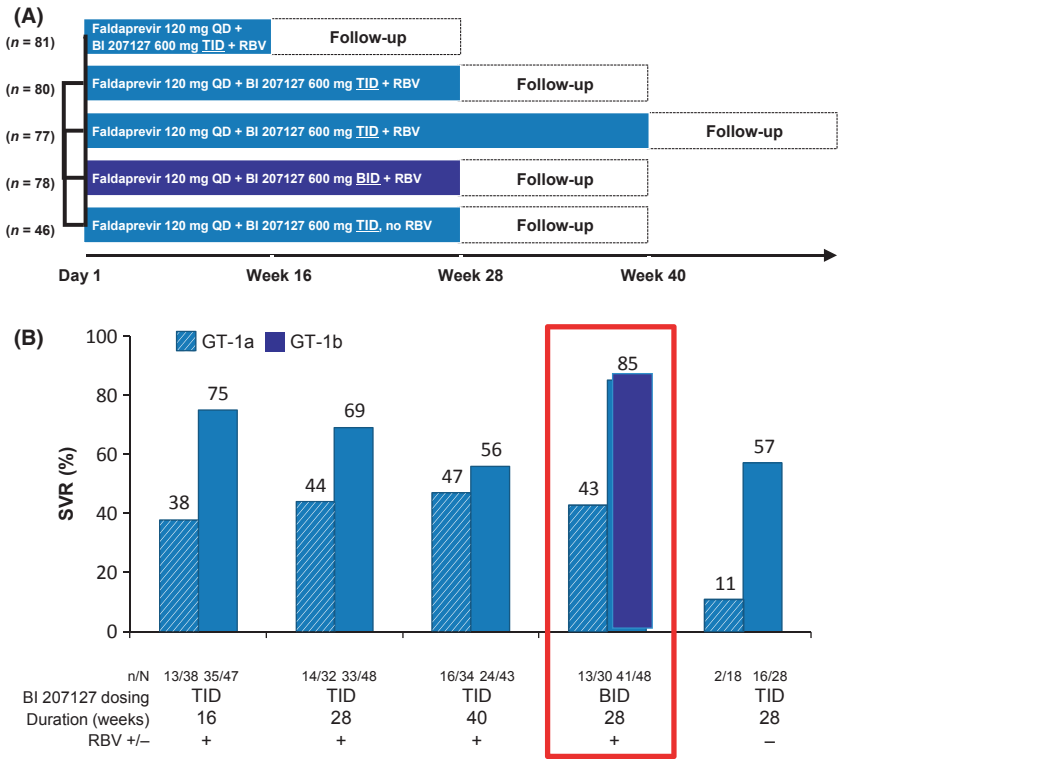


Fig. 3. (A) Sound C2 Trial Design : Faldaprevir (BI 201335) and BI 207127 (Boehringer-Ingelheim). Three hundred and sixty-two treatment-naïve patients with chronic genotype-1 HCV infections were randomized into five IFN-free treatment arms, each with 120 mg BI 201 335 once daily (QD), but with different dosings of BI 207 127 and RBV. (B) Results from the Sound C2 trial : Faldaprevir (BI 201 335) and BI 207 127 (Boehringer-Ingelheim) 85 per cent of HCV patients infected with genotype-1b (GT-1b) achieved sustained virological response with the optimal regimen of 28 weeks of faldaprevir (BI 201 335, once a day), and BI 207 127 (BID) (22).

A total of 44 patients with the viral genotypes 2 and 3 were enrolled in three arms – one with a 7-day sofosbuvir run-in followed by 23 weeks of the two together, one with the combination for 24 weeks and one with the combination plus ribavirin for 24 weeks. Eighty-eight per cent of patients in the first group reached an SVR*12*, compared with 100% in the second group, and 86% in the third group.

In genotype 1, the trial had three arms, with a total of 44 patients with the same regimens as in the genotype 2/3 patients. They also tested the combination with and without RBV for 12 weeks in a total of 82 patients. All patients receiving the first three regimens achieved an SVR12 and, all but one remained undetectable at SVR24. Sulkowski *et al.* reported that of the 82 patients in the 12-week arms, 68 had reached 12 weeks post-treatment and all had SVR12.

Daclatasvir, asunaprevir and BMS-791325 (BMS)

Daclatasvir is the first NS5A replication complex inhibitor to be investigated in HCV clinical trials and is currently in Phase III development. Asunaprevir is an NS3 protease inhibitor in Phase III development with daclatasvir. BMS-791325 is a non-nucleoside inhibitor of the NS5B polymerase, currently in Phase II development as a component of daclatasvir-based treatment regimens.

This Phase II study combines these three different classes of DAAs – daclatasvir, asunaprevir and BMS-791325 – in HCV G1 treatment-naïve patients (Fig. 5A). Data reported here are from an interim analysis of Part 1 of this study (28).

Group 1 (24-week treatment): 94% (15/16) achieved undetectable viral load by the EOT and SVR4. Two patients discontinued the study drugs prior to the protocol-defined last treatment visit, one because of inability to comply with study procedures (poor venous access) who achieved SVR4, and one who withdrew consent and was lost *to* follow-up.

Group 2 (12-week treatment): 100% (16/16) achieved undetectable viral load by the EOT and 94% (15/16) achieved SVR12. The remaining one patient was lost to follow-up after completing treatment, but did return approximately 24 weeks post-treatment and in preliminary data, has achieved SVR24. One patient discontinued study drugs prior to the protocol-defined last treatment visit (because of poor/non-compliance) and achieved SVR12.

Viral load declined rapidly in both groups and was below LLOQ in all patients (32/32) by week 4. There

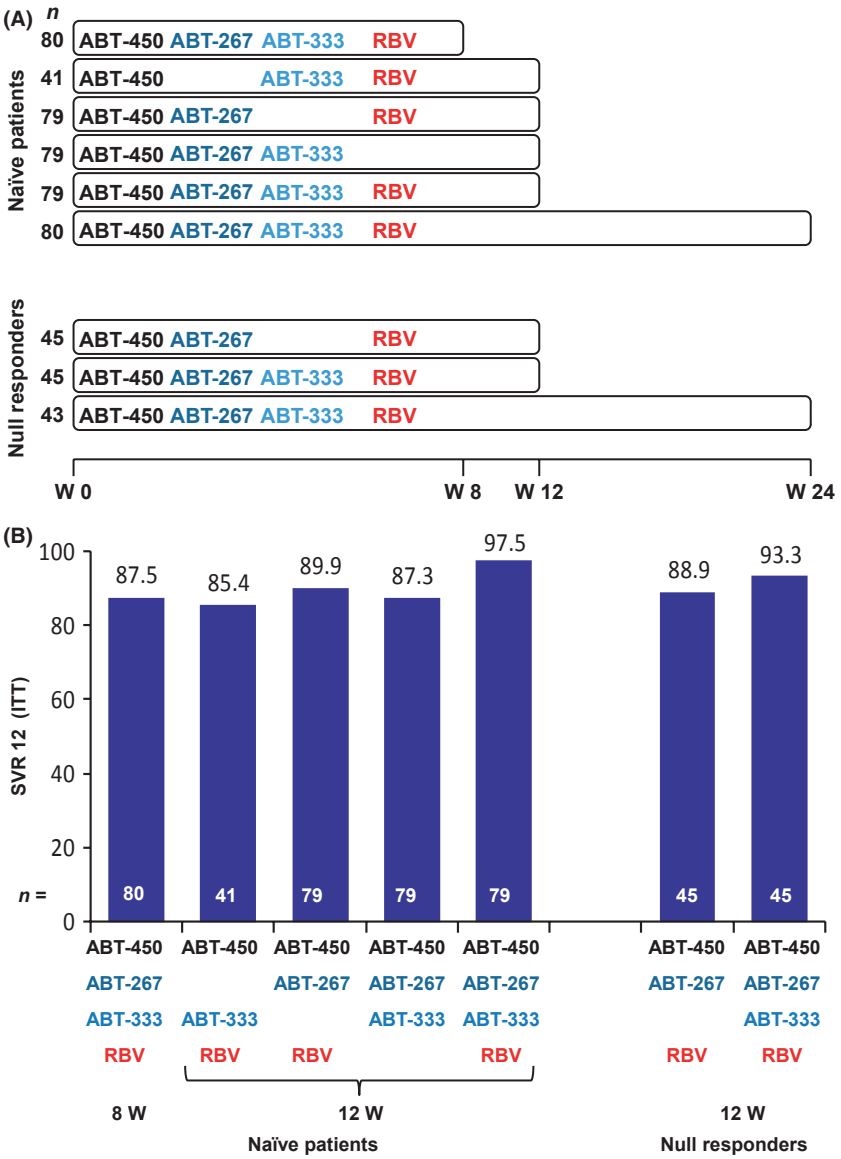


Fig 4. (A) Aviator Study trial design: ABT-450/r, ABT-267, ABT-333 and ribavirin (Abbott). This phase 2b study assesses the safety, and efficacy of ABT-450/r (dosed 100/100 mg to 200/100 mg QD), ABT-267 (25 mg QD), ABT-333 (400 mg BID) and ribavirin in non-cirrhotic treatment-naïve patients and prior peg-interferon/ribavirin null responders for 8, 12 or 24 weeks. (B) Results from the Aviator Study: ABT-450/r, ABT-267, ABT-333 and ribavirin (Abbott) sustained virological response (SVR)12 in treatment-naïve genotype 1 (GT1) patients was 97.5 per cent (77 of 79), and 93.3 per cent (42 of 45) in GT1 null responder patients. In GT1a patients, SVR12 was achieved in 96 per cent (52 of 54) of treatment-naïve patients and 89 per cent (25 of 28) of null responder patients. In GT1b patients, SVR12 was achieved in 100 per cent of treatment-naïve (25 of 25) and null responder patients (17 of 17). In addition, results from the 12-week triple-DAA regimen without RBV in treatment-naïve patients showed: SVR12 was achieved in 87.3 per cent (69 of 79) of GT1 patients; SVR12 in GT1a patients was 83 per cent (43 of 52); SVR12 in GT1b patients was 96 per cent (24 of 25) (24).

was no viral breakthrough during treatment and no post-treatment relapse.

There were no discontinuations because of adverse events. Headache was the most common adverse event in this study (31%, 10/32). There were no deaths, discontinuations owing to AEs, or serious AEs owing to study drugs. Most AEs were mild to moderate in severity. The most common AEs ($\geq$10% total) were headache, diarrhoea and asthaenia. No grade 3–4 elevations in liver enzymes (ALT/AST) or bilirubin were observed. One grade 3 AE (headache) resolved after 7 days with continued study treatment and one grade 3–4 laboratory abnormality (lymphopaenia) was recorded in Group 2 at a single study visit concomitant with influenza. All other AEs were grade 1 or 2.

Table 4. Sofosbuvir, GS-5885 and Ribavrin (Results) (25)

	SOF + RBV		SOF + GS-5885 + RBV	
HCV RNA <15 IU/mL	Treatment-naïve (n = 25)	Null responder(n = 10)	Treatment-naïve (n = 25)	Null responder (n = 9)
Week 1	8/25 (32)	1/10 (10)	11/25 (44)	0/9 (0)
Week 2	17/25 (68)	7/10 (70)	22/25 (88)	4/9 (44)
Week 4	25/25 (100)	10/10 (100)	25/25 (100)	8/9 (89)
EOT	25/25 (100)	10/10 (100)	25/25 (100)	9/9 (100)
SVR4	22/25 (88)	1/10 (10)	25/25 (100)*	3/3 (100)†
SVR12	21/25 (84)	1/10 (10)	–	–

Daclatasvir and asunaprevir (BMS) in genotype 1b prior null responders

Previous data on daclatasvir and asunaprevir have reported exciting results in genotype 1b null responders (29, 30).

A new Phase II study demonstrated that the dual regimen of daclatasvir and asunaprevir, without IFN or RBV, achieved high rates of SVR12 in GT1b patients who were prior null responders to IFN alfa and RBV (31).

Trial design is presented in Fig. 6A. In Group A1 (daclatasvir + asunaprevir 200 mg BID), 78% (14/18) of patients achieved SVR12. Of the four patients who did not achieve SVR12, one patient was missing a viral load measurement at 12 weeks post-treatment and one had transient viraemia (detectable viral load). Both of these patients had undetectable viral load on subsequent visits.

In Group A2 (daclatasvir + asunaprevir 200 mg QD), 65% (13/20) of patients achieved SVR12. With daclatasvir and asunaprevir Dual therapy, eight patients experienced

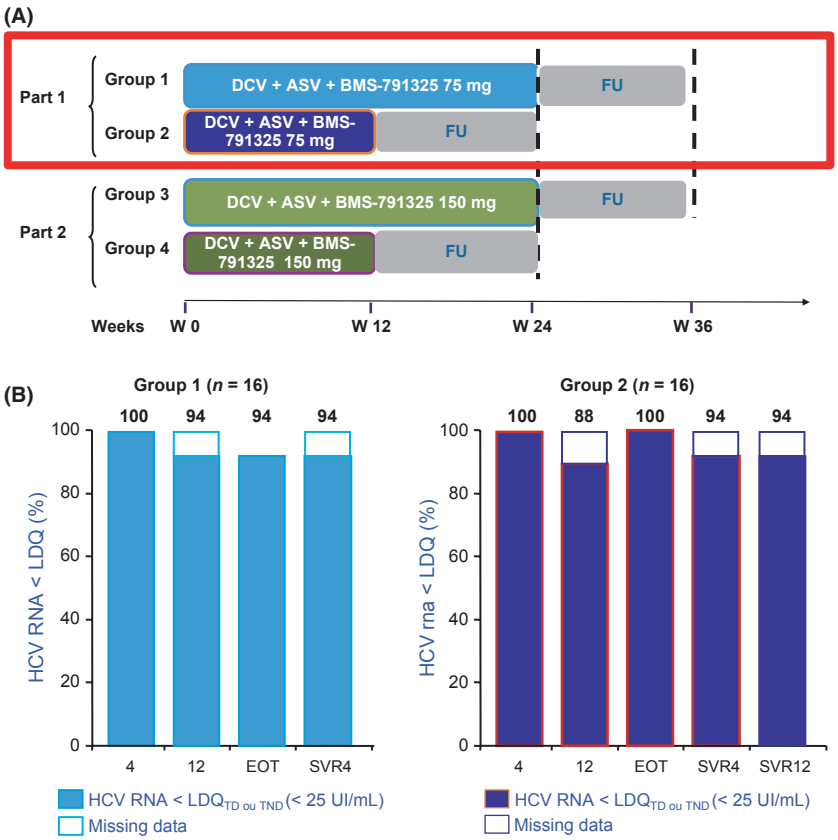


Fig. 5. (A) Trial Design: Daclatasvir, Asunaprevir and BMS-791325 (BMS) In Part 1, 32 patients were randomized 1:1 (n = 16/arm) into two groups with 24 weeks (group 1) or 12 weeks (group 2) of the triple therapy with Daclatasvir, Asunaprevir and BMS-791 325. (B) Results from the trial : Daclatasvir, Asunaprevir and BMS-791 325 (BMS) Group 1 (24-week treatment): 94% (15/16) achieved undetectable viral load by the end of treatment and sustained through sustained virological response (SVR)4. Group 2 (12-week treatment): 100% (16/16) achieved undetectable viral load by the end of treatment and 94% (15/16) achieved SVR12 (28).

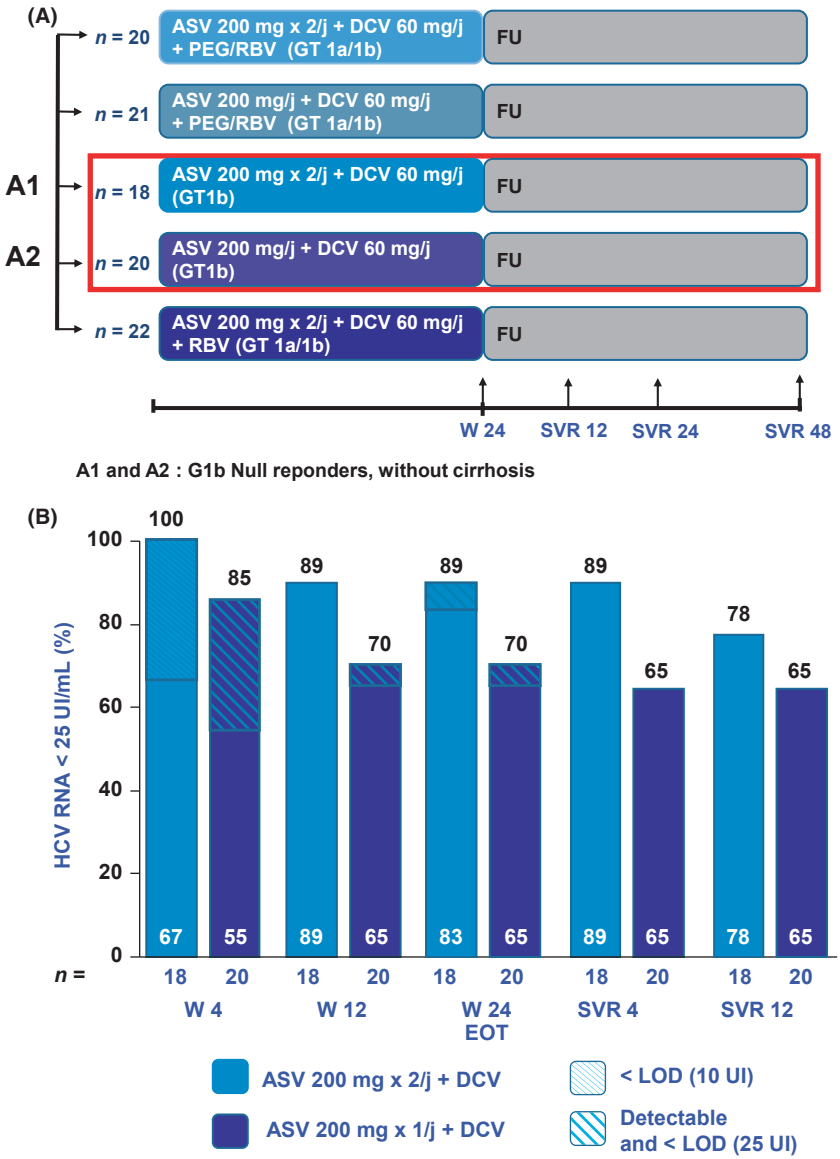


Fig. 6. (A) Trial Design: Daclatasvir and Asunaprevir Patients received one of five treatment regimens for 24 weeks. Genotype 1b infected patients were randomized to receive one of four treatment regimens for 24 weeks (two DCV/ASV Dual treatment groups, two DCV/ASV/Alfa/RBV Quad treatment groups). Genotype 1a infected patients were randomized to receive one of two treatment regimens for 24 weeks (two DCV/ASV/Alfa/RBV Quad treatment groups). A fifth group (DCV/ASV/RBV Triple therapy) included both GT1a and GT1b infected patients and enrolled separately. The DCV/ASV Dual treatment groups received DCV 60 mg once daily and ASV 200 mg either twice daily (Group A1) or once daily (Group A2). (B) Results from the trial: Daclatasvir and Asunaprevir. In Group A1 (DCV + ASV 200 mg BID), 78% (14/18) of patients achieved SVR12. In Group A2 (DCV + ASV 200 mg QD), 65% (13/20) of patients achieved SVR12 (31).

virological breakthrough – 2 patients in Group A1 and 6 in Group A2. All received rescue therapy with the addition of IFNalfa/RBV to their regimen. One patient in Group A2 relapsed at week 4 post-treatment.

An analysis of HCV sequences confirmed that 5/6 Group A2 patients with a virological breakthrough had baseline polymorphisms that confer Daclatasvir resistance (NS5A domain). In addition, at breakthrough, seven patients had confirmed resistance to both Daclatasvir and Asunaprevir.

There were no serious adverse events owing to study drug in the patients treated with daclatasvir and asunaprevir combination therapy, no deaths and no treatment discontinuations owing to AEs. Most AEs were mild to moderate. The most common AEs were headache, diarrhoea, asthaenia, and insomnia.

Grade 3–4 ALT/AST elevations were infrequent and none were accompanied by elevated total or direct bilirubin. All AST/ALT elevations improved without intervention.

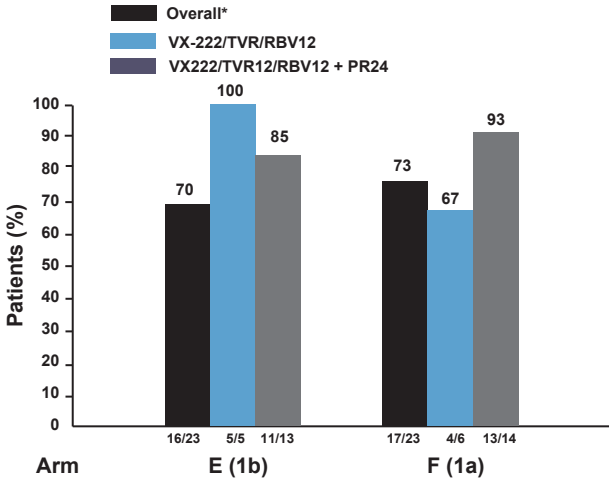


Fig. 7. Results from the Zenith trial sustained virological response (SVR)12 rates in the overall cohort and in 2 subgroups. Overall, SVR occurred in 70% of patients in arm E and 73% in arm F. Patients who discontinued treatment before week 12 are counted as failures here. In the blue bars are shown the patients who completed 12 weeks of total therapy, (the all-oral regimen), we see that among genotype 1b patients who met criteria for discontinuation of all therapy at week 12, 5 of 5 had SVR compared with 4 of 6 of the genotype 1a patients. In the grey bars, we see the patients who were assigned to PR for an additional 24 weeks, and had SVR rates of 85 and 93% (32).

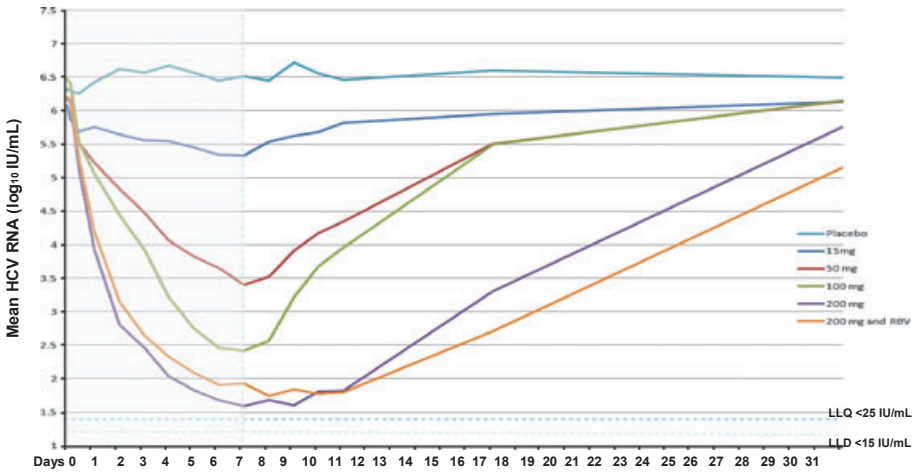
The daclatasvir and asunaprevir dual regimen is part of a global registrational programme and a registrational programme specific to Japan, where the majority of HCV patients have GT1b.

Zenith study: VX-222, telaprevir and ribavirin (Vertex)

VX-222 is a selective, non-nucleoside, non-competitive inhibitor of the hepatitis C virus (HCV) NS5B polymerase. Telaprevir is a selective, HCV NS3/4A protease inhibitor. ZENITH is assessing the safety and efficacy of two dose levels of VX-222 with TVR either alone (DUAL), or with RBV (TRIPLE), or with PR (QUAD) in chronic HCV genotype 1 treatment-naïve patients (32) (Fig. 7).

The two arms being presented represent arms E and F of the overall study in treatment-naïve genotype 1 patients without cirrhosis. Patients received VX -222 400 mg bid plus telaprevir 1125 mg bid plus ribavirin for 12 weeks. Arm E consisted of 23 genotype 1b patients, Arm F 23 genotype 1a patients.

Patients with undetectable HCV RNA at weeks 2 and 8, with no evidence of breakthrough during the first 12 weeks of treatment were assigned to stop treatment after 12 weeks. In contrast, patients with detectable HCV RNA at weeks 2 and/or 8 received an additional 24 weeks of PR therapy starting after week



HCV RNA after 7 days treatment	PLB	15 mg	50 mg	100 mg	200 mg	200 mg + RBV
HCV RNA < LLQ, N (%) HCV RNA <LLD, N (%)	0 (0%) 0 (0%)	0 (0%) 0 (0%)	0 (0%) 0 (0%)	1 (12.5%) 1 (12.5%)	4 (50%) 0 (0%)	5 (62.5%) 2 (25%)
Mean HCV RNA reduction (min, max)	0.11 (–0.28, 0.66)	–0.97 (–0.17, –1.59)	–3.02 (–2,21, –3.57)	–3.95 (–3.39, –4.51)	–4.54 (–3.81,–5.08)	–4.18 (–3.6, –5.2)

Fig. 8. Results obtained with ALS-200 Rapid, dose-related reductions in HCV RNA observed after 7 days treatment with ALS-2200. Near maximal reduction in HCV RNA observed in high-dose groups. Median 4.54 $\log_{10}$ reduction after 7 days with 200 mg dose, 5 of 8 subjects below LLQ after 7 days with 200 mg dose + RBV (33).

12. Patients with vBT during the first 12 weeks had the option to enter the extension phase and receive 48 weeks of PR.

Overall, SVR occurred in 70% of patients in arm E and 73% in arm F. The most common adverse events during oral therapy are shown here, and included diarrhoea, rash, pruritus, nausea, fatigue. Also, anaemia was reported in 13%.

There were no serious adverse events, but grade ¾ Aes occurred in 7%. Haemoglobin less than 10 occurred in 7 or 15%, but there were no cases of haemoglobin < 8.5.

ALS-2200 (VX 135), nucleotide HCV polymerase inhibitor (Alios, Vertex)

ALS-2200 is a novel uridine-base nucleoside analogue, which has demonstrated potent, highly specific inhibition of NS5B-directed HCV RNA replication. ALS-2200 showed activity in HCV replicon cell lines that show resistance to other DAAs. The compound has pan-genotypic activity in replicon cell lines and a long half-life of NTP in human hepatocytes making ALS-2200 potentially suitable for once-daily dosing.

Rapid, dose-related reductions in HCV RNA observed after 7 days of treatment with ALS-2200 (33). No viral breakthrough observed during dosing period. ALS-2200 was well tolerated. Fig. 8.

Conclusion

Several trials with DAA combinations have reported increased SVR, low resistance and a good safety profile. There is realistic hope for an oral regimen against HCV in the near future, as several compounds with different mechanisms of action are in advanced drug development. Some of these drugs or drug combinations have pan-genotypic activity. This rapid progress strongly suggests that in the near future, IFN-free short duration DAA combinations will make HCV the first chronic viral infection to be eradicated worldwide.

Disclosure

Tarik Asselah is a speaker and investigator for BMS, Boehringer-Ingelheim, Tibotec, Janssen, Gilead, Roche and Merck. Patrick Marcellin is a speaker and investigator for BMS, Boehringer-Ingelheim, Tibotec, Janssen, Gilead, Roche and Merck.

References

1. Marcellin P, Asselah T, Boyer N. Fibrosis and disease progression in hepatitis C. *Hepatology* 2002; **36**: S47–56.
2. Choo QL, Kuo G, Weiner AJ, *et al.* Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. *Science* 1989; **244**: 359–62.
3. EASL Clinical Practice Guidelines. management of hepatitis C virus infection. *J Hepatol* 2011; **55**: 245–64.
4. Martinot-Peignoux M, Stern C, Maylin S, *et al.* Twelve weeks posttreatment follow-up is as relevant as 24 weeks to determine the sustained virologic response in patients with hepatitis C virus receiving pegylated interferon and ribavirin. *Hepatology* 2010; **51**: 1122–6.
5. Maylin S, Martinot M, Moucari R, *et al.* Eradication of hepatitis C virus in patients successfully treated for chronic hepatitis C. *Gastroenterology* 2008; **135**: 821–9.
6. Asselah T, Marcellin P. Direct acting antivirals for the treatment of chronic hepatitis C: one pill a day for tomorrow. *Liver Int* 2012; **32**: 88–102.
7. Kim JL, Morgenstern KA, Lin C, *et al.* Crystal structure of the hepatitis C virus NS3 protease domain complexed with a synthetic NS4A cofactor peptide. *Cell* 1996; **87**: 343–55.
8. Yao N, Hesson T, Cable M, *et al.* Structure of the hepatitis C virus RNA helicase domain. *Nat Struct Biol* 1997; **4**: 463–7.
9. Estrabaud E, Vidaud M, Marcellin P, Asselah T. Genomics and HCV infection : progression of fibrosis and treatment response. *J Hepatol* 2012; **57**: 1110–25.
10. Asselah T. Interferon stimulated genes and hepatitis C virus infection. *J Interferon Cytokine Res*. 2012; **26**: 455–62.
11. Asselah T, Bieche I, Narguet S, *et al.* Liver gene expression signature to predict response to pegylated interferon plus ribavirin combination therapy in patients with chronic hepatitis C. *Gut* 2008; **57**: 516–24.
12. Lamarre D, Anderson PC, Bailey M, *et al.* An NS3 protease inhibitor with antiviral effects in humans infected with hepatitis C virus. *Nature* 2003; **426**: 186–9.
13. Hinrichsen H, Benhamou Y, Wedemeyer H, *et al.* Short-term antiviral efficacy of BILN 2061, a hepatitis C virus serine protease inhibitor, in hepatitis C genotype 1 patients. *Gastroenterology* 2004; **127**: 1347–55.
14. Asselah T, Marcellin P. BILN 2061: a major step toward new therapeutic strategies in hepatitis C. *J Hepatol* 2004; **41**: 178–81.
15. McCown MF, Rajyaguru S, Kular S, *et al.* GT-1a or GT-1b subtype-specific resistance profiles for hepatitis C virus inhibitors telaprevir and HCV-796. *Antimicrob Agents Chemother* 2009; **53**: 2129–32.
16. Zhou Y, Muh U, Hanzelka BL, *et al.* Phenotypic and structural analyses of hepatitis C virus NS3 protease Arg155 variants: sensitivity to telaprevir (VX-950) and interferon alpha. *J Biol Chem* 2007; **282**: 22619–28.
17. Koch U, Narjes F. Recent progress in the development of inhibitors of the hepatitis C virus RNA-dependent RNA polymerase. *Curr Top Med Chem* 2007; **7**: 1302–29.
18. Gish RG, Meanwell NA. The NS5A replication complex inhibitors: difference makers ? *Clin Liver Dis* 2011; **15**: 627–39.
19. Gao M, Nettles RE, Belema M, *et al.* Chemical genetics strategy identifies an HCV NS5A inhibitor with a potent clinical effect. *Nature* 2010; **465**: 96–100.
20. Asselah T. NS5A inhibitors: a new breakthrough for the treatment of chronic hepatitis C. *J Hepatol* 2011; **54**: 1069–72.
21. Asselah T, De Muynck S, Broet P, *et al.* IL28B polymorphism is associated with treatment response in patients with genotype 4 chronic hepatitis C. *J Hepatol* 2011; **3**: 527–32.
22. Zeuzem S, Soriano V, Asselah T, *et al.* Interferon (IFN)-free combinationtreatment with HCV NS3/4A protease inhibitor BI 201335 and the non-nucleoside NS 5B inhibitor BI 207127 + /- ribavirin (R): final results of

SOUND-C2 and predictors of response. *Hepatology* 2012; **56**(Suppl): 308–9A.
23. Zeuzem S, Asselah T, Angus P, *et al.* Efficacy of the protease inhibitor BI201335, polymerase inhibitor BI 207127, and ribavirin in patients with chronic HCV infection. *Gastroenterology* 2011; **141**: 2047–55.
24. Kowdley KV, Lawitz E, Poordad F, *et al.* A 12-week interferon-free treatment regimen with ABT-450/r, ABT-267, ABT-333 and ribavirin achieves SVR rates (observed data) of 99% in treatment-naive patients and 93% in prior null responders with HCV genotype 1 infection. *Hepatology* 2012; **56**(Suppl): LB1.
25. Gane EJ, Stedman CA, Hyland RH, *et al.* Once daily sofosbuvir (GS-7977) plus ribavirin in patients with HCV genotypes 1, 2, and 3: the ELECTRON trial. *Hepatology* 2012; **56**(Suppl): 306A.
26. Osinusi A, *et al.* High efficacy of GS-7977 in combination with low or full dose Ribavirin for 24 weeks in difficult to treat HCV infected genotype 1 patients. *Hepatology* 2012; **56**(Suppl): LB4.
27. Sulkowski MS, Gardiner DF, Rodriguez-Torres M, *et al.* High rate of sustained virologic response with the all-oral combination of daclatasvir (NS5A inhibitor) plus sofosbuvir (nucleotide NS5B inhibitor), with or without ribavirin, in treatment-naive patients chronically infected with HCV genotype 1, 2, or 3. *Hepatology* 2012; **56**(Suppl): LB2.
28. Everson GT, Sims KD, Rodriguez-Torres M, *et al.* An interferon-free, ribavirin-free 12-week regimen of daclatasvir (DCV), asunaprevir (ASV), and BMS-791325 yielded SVR4 of 94% in treatment-naive patients with genotype (GT) 1 chronic hepatitis C virus (HCV) infection. *Hepatology* 2012; **56**(Suppl): LB3.
29. Lok AS, Gardiner DF, Lawitz E, *et al.* Preliminary study of two antiviral agents for hepatitis C genotype 1. *N Engl J Med* 2012; **366**: 216–24.
30. Asselah T. A revolution in HCV treatment with direct-acting antivirals: from non-response to eradication. *J Hepatol* 2012; **57**: 455–7.
31. Lok AS, Gardiner DF, Hezode C, *et al.* Sustained virologicresponse in chronic HCV genotype (GT) 1-infected null responders with combination of daclatasvir (DCV; NS5A inhibitor and asunaprevir (ASV; NS3 inhibitor) with or without peginterferon alfa-2a/ribavirin (PEG/RBV) *Hepatology* 2012; **56**(Suppl): 230A.
32. Jacobson IM, Sulkowski MS, Gane EJ, *et al.* VX-222, telaprevir and ribavirin in treatment-naïve patients with genotype 1 chronic hepatitis C: results of the ZENITH study interferon-free regimen. *Hepatology* 2012; **56**(Suppl): 308A.
33. Marcellin P, Popa S, Berliba A, *et al.* ALS-2200, a novel once-daily nucleotide HCV polymerase inhibitor, demonstrates potent antiviral activity over 7 days in treatment-naïve genotype 1 (GT1) patients. *Hepatology* 2012; **56**(Suppl): 235A.