Improved knowledge of the HCV life cycle and of structural features of HCV proteins have led to the discovery of numerous potential targets for antiviral therapy. Viral replication and polyprotein processing have been tagged as promising viral targets. Clathrin-mediated endocytosis, fusion of HCV with cellular membranes, translation of viral RNA, virus production and release as well as several host cell factors may provide alternative targets for future anti-HCV therapies. Several compounds are currently under investigation in clinical trials and showed high antiviral activity in patients with chronic hepatitis C. Recently, Phase III studies for two protease inhibitors, telaprevir and boceprevir, each given in combination with pegylated interferon (standard of care [SOC]), were completed. In HCV-genotype-1-infected patients, the addition of telaprevir or boceprevir to SOC increased sustained virological response rates from <50% to >70%. Nucleoside/nucleotide inhibitors of the HCV NS5B polymerase have shown antiviral activity against different HCV genotypes, and have a higher barrier to resistance than protease inhibitors. In addition, several allosteric binding sites have been identified for non-nucleoside inhibitors of the NS5B polymerase. Inhibitors of NS5A are potentially active against all HCV genotypes. Among the different host cell-targeting compounds, cyclophilin inhibitors have shown promising results. Future hope lies in the combination of direct-acting antiviral agents with the possibility of interferon-free treatment regimens.

Chronic hepatitis C is a major health concern. The most recent estimates indicate that >170 million people are infected worldwide, and approximately 280,000 deaths each year are attributable to chronic HCV infection [1,2]. Complications of HCV infection, such as cirrhosis and its sequelae, including liver failure and hepatocellular carcinoma, may require liver transplantation and long-term care, which consequently imposes a significant burden on healthcare systems. Eradication of HCV by antiviral therapy improves liver histology and patient outcome [3,4]. However, by current standard therapy with pegylated interferon (PEG-IFN)-α2α or -α2b in combination with weight-based ribavirin (RBV) given for 48 weeks, rates of sustained virological response (SVR) are still unsatisfying, especially for patients with HCV genotype 1 infection. Whereas, SVR can be achieved in 81–84% of patients with HCV genotype 2 or 3 infection after 24 weeks of combination therapy, the rate of SVR is much lower in patients with HCV genotype 1 infection. These patients show only an SVR rate of 34–52% after 48 weeks of combination therapy with PEG-IFN-α and RBV [5–7]. Treatment extension up to 72 weeks is possible [8] but therapy-associated side effects, such as pancytopenia or depression, are commonly observed, and may lead to early treatment discontinuation.

Improved understanding of the HCV life cycle in recent years has supported the development of direct-acting antiviral (DAA) agents that specifically target post-translational processing and HCV replication [9–12]. Inhibitors of HCV entry, HCV RNA translation and virus assembly and release are still in preclinical or very early clinical development. Several host cell-targeting compounds are also among the promising therapeutic approaches in recent anti-HCV drug discoveries. Finally, improvements have also been made in the development of new long-acting interferons (IFNs) with the potential for more favourable safety and efficacy profiles. This review will give an overview of recent developments in the HCV drug pipeline. DAA agents, host cell-targeting compounds and new IFNs that have already been evaluated in clinical trials will be discussed.
Antiviral compounds targeting polyprotein processing

NS3/4A protease inhibitors

The design of NS3/4A inhibitors is relatively difficult because the active site of the NS3/4A protease is located in a shallow groove between two β-barrels of the protease [9].

Protease inhibitors can be divided into two chemical classes, macrocyclic inhibitors and linear tetra-peptide ketoamide derivatives. NS3/4A protease inhibitors of both classes strongly inhibit HCV replication during monotherapy, but also frequently select for resistant variants, which may be followed by viral breakthrough [13,14]. The frequency of resistance development against protease inhibitors can be reduced by the additional administration of PEG-IFN and RBV.

The first NS3/4A inhibitor applied in clinical studies was ciluprevir (BLN 2061), an orally bioavailable, peptidomimetic, macrocyclic drug binding non-covalently to the active centre of the enzyme [15]. Ciluprevir monotherapy was evaluated in a double-blind placebo-controlled pilot study in treatment-naive genotype 1 patients with compensated liver disease [16]. In this study, ciluprevir, administered twice daily for 2 days at doses ranging from 25 to 500 mg, led to a mean 2–3 log₁₀ decrease of HCV RNA serum levels in most patients. Although the development of ciluprevir was stopped because of serious cardiotoxicity observed in an animal model, these studies provided the proof-of-principle for successful suppression of HCV replication by NS3/4A inhibitors in patients with chronic hepatitis C. Currently, the most advanced protease inhibitors are telaprevir and boceprevir. Both have completed Phase III evaluation and were approved in 2011.

Telaprevir

Telaprevir (VX-950), an orally bioavailable linear ketoamide protease inhibitor, was initially investigated given alone or in combination with PEG-IFN-α2a with or without RBV in patients infected with HCV genotype 1 in a number of short-term studies [17,18]. A median maximum reduction of 5.49 log₁₀ IU/ml HCV RNA from baseline was observed in patients treated with telaprevir (750 mg three times daily) plus PEG-IFN-α2a for 14 days. Variants associated with clinical resistance to telaprevir were identified at four positions close to the NS3 catalytic domain: V36A/M/L, T54A, R155K/M/S/T, A156S (all three conferring low- to medium-level resistance) and A156T/V (conferring high-level resistance) [14,19]. While viral rebound due to selected drug-resistant variants occurred in the majority of patients during monotherapy with telaprevir, a reduced frequency of resistant mutations and no viral breakthrough was observed in combination studies with PEG-IFN with or without RBV.

The efficacy of telaprevir (750 mg three times daily) in combination with PEG-IFN-α2a with or without RBV was studied in three large placebo-controlled Phase IIIb trials in both treatment-naive (PROVE 1, n=250 and PROVE 2, n=323) and treatment-experienced (PROVE 3, n=453) patients infected with HCV genotype 1 [20–22]. Overall, SVR rates ranged from 67% to 69% (PROVE 1/2) and 24% to 53% (PROVE 3) in the telaprevir-containing study arms compared with 41% to 46% in the placebo arms and 14% in patients receiving standard of care (SOC), respectively. Lower antiviral activities, higher virological break-through and high relapse rates (48% and 53%, respectively) were observed in the RBV-free study arms, indicating that RBV remains crucial for the achievement of SVR. Discontinuation rates due to adverse events, including severe skin rashes, were as high as 26% in patients who received telaprevir for 24 weeks. In a smaller open-label study of telaprevir plus SOC in treatment-naive patients infected with HCV genotype 1, similarly high SVR rates for telaprevir administered every 12 h versus 8 h and given in combination with PEG-IFN-α2a with RBV versus PEG-IFN-α2b with RBV were observed [23].

Telaprevir was also investigated in patients infected with HCV genotype 2, 3 or 4. While a slightly reduced antiviral activity was observed in patients with genotype 2 infection, only minimal declines of HCV RNA concentrations were observed in patients infected with HCV genotypes 3 and 4 [24,25].

Three Phase III trials, known as ADVANCE (n=1,088), ILLUMINATE (n=540), both conducted in treatment-naive patients, and REALIZE (n=662), conducted in treatment-experienced patients, evaluated the efficacy of telaprevir (750 mg three times daily) in combination with SOC for 24–48 weeks and 48 weeks overall treatment duration, respectively [26,27].

The ADVANCE trial is a randomized, placebo-controlled study to investigate the efficacy and safety of telaprevir (750 mg every 8 h) in combination with PEG-IFN-α2a (180 μg/week) and weight-based RBV (1,000–1,200 mg/day) [26]. Treatment-naive patients infected with HCV genotype 1 (n=1,095) were randomized into two telaprevir arms and one control arm. Telaprevir was administered in combination with PEG-IFN-α2a plus RBV for either 8 or 12 weeks, followed by 16 and 12 weeks of PEG-IFN-α2a plus RBV, respectively. Patients without extended rapid virological response (eRVR; undetectable HCV RNA at weeks 4 and 12) continued PEG-IFN-α2a plus RBV therapy up to week 48. Patients in the control arm received PEG-IFN-α2a plus RBV for 48 weeks. The SVR rates in both telaprevir arms were superior to standard therapy (75%, 69% and 44% in the telaprevir 12-week arm, telaprevir 8-week arm and PEG-IFN-α2a plus RBV arm, respectively). In total, 57% and
58% of the patients who received 8 or 12 weeks of triple therapy, respectively, achieved an eRVR and were thus eligible for a reduced treatment duration of 24 weeks (Table 1). In the 12-week telaprevir group, 21 of 363 (6%) patients had cirrhosis at baseline and the overall SVR in these patients was 62% (13/21). Among patients with cirrhosis, 43% (9/21) achieved an eRVR, among whom 78% (7/9) achieved SVR.

The SVR rates reported in the FDA analysis for telaprevir are slightly higher than those reported in the ADVANCE study (79% versus 75%, 73% versus 69% and 46% versus 44% in the telaprevir 12-week arm, telaprevir 8-week arm and PEG-IFN-α2a plus RBV arm, respectively). Detectable HCV RNA results throughout the follow-up period after treatment for patients who apparently achieved SVR were considered by the FDA as not clinically significant and likely to represent false-positive values.

The ILLUMINATE trial was a supplementary study to ADVANCE that aimed to investigate whether longer SOC treatment in patients with eRVR than that administered in ADVANCE increases SVR rates. In this trial, treatment-naive patients infected with HCV genotype 1 were given telaprevir (12 weeks), PEG-IFN-α2a and RBV [28]. Patients who achieved an eRVR were randomized at week 20 to continue receiving PEG-IFN-α2a plus RBV for 24 or 48 weeks of total treatment. Patients who did not achieve an eRVR were assigned to 48 weeks of treatment. The overall SVR rate of the study was 72%. Among patients who achieved an eRVR, therapy for 24 weeks was non-inferior to treatment for 48 weeks (SVR 92% compared to 88%). The study supports the use of response-guided therapy (RGT) for telaprevir-based treatment regimens. Overall, 61 (11%) patients had cirrhosis at baseline. Among patients with cirrhosis, 30 (49%) achieved an eRVR; 18 were randomized to 12-week telaprevir plus 24-weeks PEG-IFN/RBV and 12 to 12-week telaprevir plus 48-weeks PEG-IFN/RBV. The SVR rates were 67% (12/18) for the 12-week telaprevir plus 24-week PEG-IFN/RBV group and 92% (11/12) for the 12-week telaprevir plus 48-week PEG-IFN/RBV group.

Adverse events in the ADVANCE study that occurred more often in the telaprevir arms than the control arm were anaemia (the use of erythropoietin was not permitted in the Phase III telaprevir program), pruritus, rash, nausea and diarrhoea. Rash events were frequent (53–56% in the telaprevir arms compared to 41% in the control arm) and considered severe in 3–6% of patients. In the ILLUMINATE study, similar adverse events associated with telaprevir were observed: anaemia (39%) and rash (mild to moderate in 37% and severe in 7% of patients). Discontinuation of telaprevir because of rash and anaemia occurred in 7% and 2% of the study population, respectively. Rash was primarily eczematous and resolved upon cessation of therapy. Discontinuation of all study drugs because of adverse events occurred in 17% of patients in the ILLUMINATE study.

Patients with mild to moderate rash should be followed for progression of rash or development of systemic symptoms. If rash progresses and becomes severe or if systemic symptoms develop, telaprevir should be discontinued; PEG-IFN-α and RBV may be continued. If improvement is not observed within 7 days of telaprevir discontinuation, sequential or simultaneous interruption or discontinuation of RBV and/or PEG-IFN-α should be considered. If medically indicated, earlier interruption or discontinuation of RBV and PEG-IFN-α should be considered. Patients should be monitored until the rash has resolved. Telaprevir must not be reduced or restarted if discontinued due to rash. Treatment of rash with oral antihistamines and/or topical corticosteroids may provide symptomatic relief but effectiveness of these measures has not been finally established. Treatment of rash with systemic corticosteroids is not recommended.

The REALIZE study evaluated the efficacy of a 12-week triple combination treatment regimen followed by 36 weeks SOC or 4-week lead-in with SOC, followed by 12 weeks triple combination therapy, followed by 32 weeks of SOC in HCV-genotype-1-infected previous relapers, partial non-responders (≥2 log10 decline

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Table 1. Virological response rates in treatment-naive patients chronically infected with HCV genotype 1 in the ADVANCE and SPRINT-2 trials

<table>
<thead>
<tr>
<th>Treatment response</th>
<th>ADVANCE trial</th>
<th>SPRINT-2 trial</th>
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<tbody>
<tr>
<td></td>
<td>Telaprevir +PEG-IFN/RBV</td>
<td>PEG-IFN/RBV</td>
</tr>
<tr>
<td>RVR (week 4), %</td>
<td>66–68</td>
<td>9</td>
</tr>
<tr>
<td>Week 8 (LI+ week 4), %</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>eRVR, %</td>
<td>57–58</td>
<td>8</td>
</tr>
<tr>
<td>EOT, %</td>
<td>81–87</td>
<td>63</td>
</tr>
<tr>
<td>Relapse, %</td>
<td>9</td>
<td>28</td>
</tr>
<tr>
<td>SVR, %</td>
<td>69–75</td>
<td>44</td>
</tr>
</tbody>
</table>

*1Lead-in plus 4 weeks. EOT, end of treatment; eRVR, extended rapid virological response; LI, lead-in; PEG-IFN, pegylated interferon; RBV, ribavirin; RVR, rapid virological response; SVR, sustained virological response.*

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but detectable HCV RNA at week 12 of previous treatment) and null-responders (<2 log$_{10}$ decline in HCV RNA at week 12 of previous treatment) versus SOC alone for 48 weeks ($n=662$; Figure 1A). Prior relapers achieved SVR rates of 83–88% and partial responders of 54–59%, whereas prior null responders achieved SVR rates in the range of 29–33% [27] (Figure 1B). In total, 23% ($n=122$) of telaprevir-treated patients ($n=530$) had cirrhosis at baseline. SVR rates among cirrhotic patients who received telaprevir combination treatment compared to placebo plus 48-weeks PEG-IFN with RBV were 87% (48/55) compared to 13% (2/15) for prior relapers, 34% (11/32) compared to 20% (1/5) for prior partial responders, and 14% (7/50) compared to 10% (1/10) for prior null responders. There were no significant differences between patients receiving a lead-in phase or not. Discontinuation due to adverse events occurred in 4% of the combined telaprevir study arms and in 3% of the control group. During triple therapy with telaprevir plus PEG-IFN-α plus RBV, viral breakthrough associated with the selection of resistant variants was observed in up to 25% of previous non-responders.

![Figure 1. REALIZE study design and results](image)

(A) The REALIZE study evaluated the efficacy of a 12-week triple combination treatment regimen followed by 36 weeks standard of care (SOC) or 4-week lead-in with SOC, followed by 12 weeks triple combination therapy, followed by 36 weeks of SOC in HCV-genotype-1-infected relapers, partial non-responders (<2 log$_{10}$ decline but detectable HCV RNA at treatment week 12) and null-responders (<2 log$_{10}$ decline in HCV RNA at week 12) versus SOC alone for 48 weeks ($n=662$). Dosing was telaprevir 750 mg every 8 h, pegylated interferon (PEG-IFN)-α 180 µg/week and ribavirin (RBV) 1,000–1,200 mg/day. (B) Prior relapers achieved sustained virological response (SVR) rates of 83–88% and partial responders of 54–59%, while prior null responders achieved SVR rates in the range of 29–33%. The REALIZE study showed no significant differences between patients receiving a lead-in phase or not. Discontinuation due to adverse events occurred in 4% of the combined telaprevir study arms and in 3% of the control group. During triple therapy with telaprevir plus PEG-IFN-α plus RBV, viral breakthrough associated with the selection of resistant variants was observed in up to 25% of previous non-responders.
and in 3% of the control group. The relatively low discontinuation rates due to adverse events may, in part, be explained by precise management plans for telaprevir-induced rash. During triple therapy including telaprevir plus PEG-IFN-α with RBV, viral breakthrough associated with the selection of resistant variants was observed in up to 25% of previous non-responders.

Telaprevir is an inhibitor of CYP3A4/5. Coadministration of telaprevir with drugs that are primarily metabolized by CYP3A4/5 may result in increased plasma concentrations of such drugs, which could increase or prolong their therapeutic effect and adverse reactions. Telaprevir is also an inhibitor of p-glycoprotein. Coadministration of telaprevir with drugs that are substrates for p-glycoprotein may result in increased plasma concentrations of such drugs, which could increase or prolong their therapeutic effect and adverse reactions.

Boceprevir

In a Phase Ib study conducted in treatment-experienced patients infected with HCV genotype 1, administration of boceprevir (200 mg or 400 mg three times daily), a peptidomimetic α-ketoamide HCV protease inhibitor, with or without PEG-IFN-α2b resulted in mean maximum reductions of HCV RNA of up to 1.61 log₁₀ and 2.88 log₁₀ IU/ml, respectively [29]. Boceprevir in combination with PEG-IFN-α2b and RBV was subsequently tested in a larger Phase II clinical trial (SPRINT-1; n=595) in treatment-naive patients with HCV genotype 1 infection [30]. Patients received either all three drugs in combination for 28 or 48 weeks or for 24 or 44 weeks after a prior 4-week lead-in period of PEG-IFN-α2b plus RBV and RBV was subsequently administered in a larger Phase II clinical trial (SPRINT-1; n=595) in treatment-naive patients with HCV genotype 1 infection [30]. Patients received either all three drugs in combination for 28 or 48 weeks or for 24 or 44 weeks after a prior 4-week lead-in period of PEG-IFN-α2b plus RBV, or SOC for 48 weeks. SVR rates ranged from 54% for 28 weeks of triple therapy to 75% after a 4-week lead-in period with PEG-IFN-α2b plus RBV followed by 44 weeks of triple therapy, compared to 38% in the control group. Treatment discontinuations due to adverse events (mainly anaemia and gastrointestinal side effects) that occurred throughout the entire study appeared in 9–19% of patients receiving boceprevir compared to 8% in the control group.

In treatment-experienced patients with prior null-response (<2 log₁₀ IU/ml decline in HCV RNA from baseline to treatment week 12), the addition of boceprevir to PEG-IFN-α2b and RBV showed only slightly increased SVR rates compared to standard therapy in a Phase Ib study (RESPOND-1; n=357). However, many patients received suboptimal boceprevir dosages and/or no RBV (14% versus 2%) [31].

In two Phase III studies, a larger number of treatment-naive (SPRINT-2; n=1,097) and treatment-experienced (RESPOND-2; n=403, partial non-responders and relapers only) patients with HCV genotype 1 infection were enrolled [31,32]. SPRINT-2 compared a 4-week lead-in treatment period with PEG-IFN-α2b plus RBV, followed by RGT: boceprevir plus PEG-IFN-α2b with RBV for 24 weeks, with an additional 20 weeks of PEG-IFN-α2b with RBV only if HCV RNA was detectable during weeks 8–24 in the first arm of the study (RGT arm), or boceprevir plus PEG-IFN-α2b with RBV for 44 weeks in the second arm (44-week triple therapy arm) or PEG-IFN-α2b with RBV plus placebo for 44 weeks in the third arm (control arm). The 4-week lead-in approach with PEG-IFN-α2b and RBV was chosen for the design of this Phase III trial as findings from the Phase II clinical development suggested that this approach may produce better SVR results and lower relapse and/or breakthrough rates compared to triple therapy without a lead-in phase. Furthermore, the lead-in phase might differentiate responders and non-responders to SOC, thereby assisting in the personalization of treatment durations with subsequent triple therapy.

The overall SVR rates were superior in the boceprevir arms compared with the control arm (66%, 63% and 37% for the RGT, 44-week triple therapy and control arms, respectively; Table 1). In patients with cirrhosis at baseline, SVR rates were higher in those who received treatment with the combination of boceprevir with PEG-IFN and RBV for 44 weeks after lead-in therapy with PEG-IFN and RBV (10/24, 42%) compared with those who received RGT (5/16, 31%). The SVR rates were further distinguished according to ethnicity. In total, 938 patients were not African-American or Black and 159 patients were African-American or Black. Black patients with chronic hepatitis C have repeatedly been shown to be less responsive to PEG-IFN-α-based treatment regimens [33]. SVR rates among the patients who were not African-American or Black were higher than SVR rates in African-American or Black patients (68%, 67% and 40% versus 53%, 42% and 23% for patients in the 44-week triple-therapy arm, the RGT arm and the control arm, respectively).

A total of 44% of the patients were considered for reduced treatment duration (those who had undetectable HCV RNA from week 8–24). In SPRINT-2, boceprevir was well tolerated; the most frequent adverse events were anaemia (49%) and dysgeusia (37–43%). Dose modifications (generally of PEG-IFN and RBV) due to anaemia occurred twice as often in patients treated with the combination of boceprevir with PEG-IFN and RBV (26%) compared to PEG-IFN and RBV (13%). The proportion of patients who discontinued study drug due to anaemia was 1% in patients treated with the combination of boceprevir with PEG-IFN and RBV and 1% in patients who received PEG-IFN and RBV. The proportion of patients who received an erythropoiesis stimulating agent was 43% in the boceprevir-containing arms compared to 24% in the PEG-IFN with RBV arm. The proportion of patients who received a transfusion for the management of anaemia was 3% of patients in

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the boceprevir-containing arms compared to <1% in patients who received PEG-IFN with RBV alone. Complete study drug discontinuation because of any adverse event occurred in 12–16% of the study population.

In the second Phase III study (RESPOND-2; n=403), treatment-experienced patients with HCV genotype 1 infection (partial non-responders and relapers only) were enrolled to receive a 4-week lead-in with PEG-IFN-α2b plus RBV alone, followed by either 44 weeks of triple combination therapy or a response-guided schedule with the possibility of stopping therapy at week 36 of overall treatment duration, respectively [32] (Figure 2A). SVR rates ranged from 40–52% in previous partial responders to 69–75% in previous relapers (Figure 2B). In patients with cirrhosis at baseline, SVR rates were higher in those patients who received treatment with the combination of boceprevir with PEG-IFN-α2b and RBV for 44 weeks after 4 weeks of lead-in therapy with PEG-IFN-α2b and RBV (17/22, 77%) compared with those who received RGT (6/17, 35%). Overall, 46% of patients in the RGT arm were eligible to undergo a shortened treatment duration, based on early rapid virological response results.

Figure 2. RESPOND-2 study design and results

(A) The RESPOND-2 study was conducted in 403 patients with chronic HCV genotype 1 infection. Only previous partial responders and relapers enrolled (n=403). Partial responders or relapers following pegylated interferon (PEG-IFN)-α2b plus ribavirin (RBV) treatment were randomized into three treatment arms. Each patient received a 4-week lead-in with PEG-IFN-α2b plus RBV. Patients in the control arm then received PEG-IFN-α2b plus RBV for 44 weeks. Patients in experimental arm 1 received boceprevir and PEG-IFN-α2b plus RBV for 32 weeks, and those with detectable HCV RNA at week 8 and/or at subsequent HCV RNA testing received an additional 12 weeks of PEG-IFN-α2b plus RBV. Patients in experimental arm 2 received boceprevir plus PEG-IFN-α2b plus RBV for 44 weeks. (B) The RESPOND-2 study showed sustained virological response rates ranged from 40–52% in previous partial responders to 69–75% in previous relapers. Overall, 46% of patients in the response-guided therapy (RGT) arm (experimental arm 1) were eligible to undergo a shortened treatment duration, based on early rapid virological response results.

Treatment discontinuations due to adverse events occurred in 8–12% of treatment-experienced patients compared to 3% in the control group. TW8, treatment week 8.
based on eRVR results. Treatment discontinuations due to adverse events occurred in 8–12% of treatment-experienced patients, compared to 3% in the control group. Erythropoietin was given to 25–43% of patients in boceprevir-containing regimens.

Boceprevir is a strong inhibitor of CYP3A4/5. Drugs metabolized primarily by CYP3A4/5 may have increased exposure when administered with boceprevir, which could increase or prolong their therapeutic and adverse effects. Plasma concentrations of cyclosporine, sirolimus and tacrolimus, for example, are significantly increased during coadministration with boceprevir. Close monitoring of immunosuppressant blood level is recommended.

Other protease inhibitors
Danoprevir (RG7227), TMC435, vaniprevir (MK-7009), BI201335, narlaprevir (SCH900518), BMS-650032, ACH-1625, ABT-450, MK-5172, GS-9256 and GS-9451 have been tested in Phase I and/or Phase II clinical trials [34–43]. Overall, these compounds exhibit a high antiviral activity in HCV-genotype-1-infected patients and recently reported Phase II results were comparable to, or even surpassed, SVR results obtained with telaprevir and boceprevir [39,44–46] (Table 2).

Potential advantages of some of these second-generation protease inhibitors include improved pharmacokinetics (once or twice daily dosing), broader genotypic activity, coverage of first generation protease-inhibitor-resistant variants and better tolerability and safety. Ritonavir boosting is being investigated in a number of protease inhibitors, including narlaprevir, ABT-450 and danoprevir, to reduce side effects and to enhance patient exposure to the agents, thereby potentially overcoming resistance issues and allowing less frequent dosing.

Resistance to direct-acting antiviral agents
Numerous variants (quasi-species) are continuously produced during HCV replication because of the high replication rate of HCV and the poor fidelity of its RNA-dependent RNA polymerase. Among these quasi-species, variants carrying mutations that alter the conformation of the binding sites of DAA compounds can develop. During treatment with DAAs, these preexisting drug-resistant variants are selected and might become the dominant strain, which may subsequently lead to viral breakthrough.

To date, drug-related mutations that confer resistance to boceprevir and telaprevir have been identified at several positions in the NS3/4A gene [13]. Patients infected with HCV subtype 1a are more likely to develop drug-related mutations to protease inhibitors compared to patients with subtype 1b as, for example, the frequently occurring mutation Arg155Lys of the NS3/4A gene requires only one nucleotide change in HCV subtype 1a but two nucleotide changes in subtype 1b isolates.

Although the emergence of drug-related mutations is frequently observed during DAA monotherapy, the addition of PEG-IFN-α and RBV can prevent the development of drug-resistant mutations in many patients and there is evidence that drug-resistant HCV strains remain sensitive to PEG-IFN-α and RBV treatment [19]. Consequently, PEG-IFN-α and RBV responsiveness is crucial in DAA treatment. The development of drug-related mutations is more likely to occur in patients who previously responded poorly to PEG-IFN-α plus RBV than those who responded well to PEG-IFN-α plus RBV.

Antiviral compounds targeting the HCV RNA polymerase

NS5B polymerase inhibitors
The NS5B RNA-dependent RNA polymerase enzyme has a highly conserved structure across all hepatitis C genotypes. There are two classes of polymerase inhibitors: nucleoside/nucleotide analogues and non-nucleoside RNA-dependent RNA polymerase inhibitors. Nucleoside inhibitors mimic the natural substrates of the RNA-dependent RNA polymerase and are incorporated into the elongated RNA, where they act as chain terminators. Non-nucleoside inhibitors are allosteric inhibitors (for example, HCV-796, ABT-072/333 and GS-9190) making them more susceptible to select for resistant variants.

Nucleoside/nucleotide inhibitors
Nucleoside/nucleotide polymerase inhibitors target the active binding site of NS5B, with potential activity against all HCV genotypes. Despite promising results from early clinical studies, further development of the first two nucleoside polymerase inhibitors, valopicitabine and R1626, was halted due to insufficient antiviral activity and severe adverse events, respectively [47,48]. Mericitabine, a prodrug of the cytidine nucleoside analogue PSI-6130, is currently under investigation in an ongoing placebo-controlled Phase IIb study in treatment-naïve patients with HCV genotype 1 or 4 (n=408). Interim results showed that RG7128 plus PEG-IFN

Table 2. Basic characteristics of direct-acting antiviral agents

<table>
<thead>
<tr>
<th>DAAs</th>
<th>Efficacy</th>
<th>Genotype dependency</th>
<th>Barrier to resistance</th>
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<tbody>
<tr>
<td>NS3/4A</td>
<td>+++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>(protease inhibitors)</td>
<td></td>
<td></td>
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<tr>
<td>NS5A</td>
<td>+++</td>
<td>++</td>
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<tr>
<td>NS5B</td>
<td>++</td>
<td>++</td>
<td>+++</td>
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<tr>
<td>(nucleosides)</td>
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</tr>
<tr>
<td>NS5B</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>(non-nucleosides)</td>
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DAAs, direct-acting antiviral agents; +, mild; ++, moderate; ++++, strong.
with RBV achieved high rates of virological suppression (91%) with SVR12 rates of 76% in patients with eRVR (60%) [49].

PSI-7977, a chirally pure isomer of PSI-7851, has yielded promising early data with high RVR rates (88–98%) across different dose groups plus SOC in treatment-naive patients infected with HCV genotype 1 [50–52]. The nucleotide analogue PSI-938 is still in the early stages of clinical development [51,53].

Non-nucleoside inhibitors

The structure of the NS5B polymerase resembles a characteristic ‘right hand motif’, consisting of finger, palm and thumb domains. At least four different allosteric binding sites have been identified for the inhibition of the NS5B polymerase by non-nucleoside inhibitors: benzimidazole (thumb 1), thiophene (thumb 2), benzothiazidazine (palm 1) and benzofuran (palm 2) [53]. As non-nucleoside inhibitors bind more distantly to the active site of NS5B, resistant variants are rapidly selected with these compounds.

**Non-nucleoside site 1 inhibitors: benzimidazole (thumb 1)**

BI 207127, BILB1941 and MK-3281 are non-nucleoside site 1 inhibitors that have been shown to exhibit low to medium antiviral activity in Phase I clinical trials [54–56]. Four weeks of BI 207127 (600 mg three times daily) in combination with PEG-IFN-α-2a plus RBV resulted in a median reduction of 5.6 log10 HCV RNA in treatment-naive genotype-1-infected patients and no viral breakthrough was observed [54]. Results of further studies have to be awaited. The further development of BILB1941 and MK-3281 was halted due to gastrointestinal adverse events.

**Non-nucleoside site 2 inhibitors: thiophene (thumb 2)**

Interim results from a Phase II trial investigating different doses of filibuvir (PF-00868534), a non-nucleoside site 2 inhibitor, in combination with SOC for 4 weeks followed by SOC for 44 weeks have shown similar SVR (at 12 week follow-up) results for the different filibuvir groups compared to the SOC group, indicating that longer dosing of filibuvir may be necessary [57]. Other site 2 inhibitors include VX-759, VX-916 and VX-222 [58–60]. However, at this point, only VX-222 has progressed to Phase II development.

**Non-nucleoside site 3 inhibitors: benzothiazidazine (palm 1)**

A Phase II trial of ANA598 in combination with PEG-IFN-α-2a plus RBV in treatment-naive patients infected with HCV genotype 1 is currently ongoing. It was recently reported that 73% of patients who received 400 mg of ANA598 twice daily achieved undetectable HCV RNA levels at treatment week 12 [61]. Other site 3 palm 1 inhibitors include ABT-072 and ABT-333, both of which have entered Phase II clinical trials [62].

**Non-nucleoside site 4 inhibitors: benzofuran (palm 2)**

Monotherapy with HCV-796 (nbesuvir) showed low antiviral activity in patients infected with HCV genotype 1. In addition, selection of resistant variants and viral breakthrough was observed in several patients [63]. Further drug development was suspended due to liver enzyme elevations in a subsequent Phase II study [64]. GS-9190 (tegobuvir) displayed low antiviral activity in a Phase I clinical study and drug-resistant variants were observed. However, GS-9190 has entered Phase II clinical trials, both in combination with SOC and with the protease inhibitor GS-9256 with or without RBV [65–68]. Finally, IDX375 also binds to the palm pocket and is currently in Phase I clinical trials [68].

**NS5A inhibitors**

Inhibitors of NS5A are potentially active against all HCV genotypes. BMS-790052 binds to domain I of the NS5A protein, which is crucial for the regulation of HCV replication, assembly and release. Following the promising results from a Phase I study [69], BMS-790052 is currently under investigation in a number of Phase II clinical trials. Recently, interim data were released from a Phase II study investigating different doses of BMS-790052 in combination with SOC. RVR rates were 83% and 92% in patients who received 10 mg and 60 mg BMS-790052 once daily, respectively. In addition, undetectable HCV RNA levels at week 12 (complete early virological response) were observed in 83% of patients [70]. Other NS5A inhibitors include BMS-824393, AZD7295 and PPI-461 [71–73].

Direct-acting antiviral combination therapies

In the placebo-controlled INFOrm-1 study, combinations of different doses of a nucleoside polymerase inhibitor (RG7128) and a NS3/4A protease inhibitor (danoprevir) were tested in 87 treatment-naive and -experienced patients infected with HCV genotype 1 for up to 2 weeks [74]. At the highest doses tested (1,000 mg RG7128 and 900 mg danoprevir twice daily), 63% of treatment-naive patients and 25% of treatment-experienced patients achieved undetectable HCV RNA after 2 weeks of combination therapy and only one patient experienced viral rebound without exhibiting resistant variants.

Several other drug combinations are actively being investigated (Table 3). In a Phase II study of the NS3 protease inhibitor GS-9256 (75 mg twice daily) plus the non-nucleoside NS5B polymerase inhibitor tegobuvir (40 mg twice daily) alone or in combination with RBV or PEG-IFN-α with RBV for up to 28 days, viral breakthrough was observed for the combination of the
two direct antiviral compounds. This clearly shows that two drugs with a low barrier to resistance are insufficient for continuous suppression of virus replication. Interestingly, the addition of RBV enhanced antiviral activity and reduced viral breakthrough rates, even in the absence of PEG-IFN-a [67].

An IFN-sparing triple combination therapy with the NS3 protease inhibitor BI 201335 (120 mg once daily) and the non-nucleoside polymerase inhibitor BI 207127 (400 mg or 600 mg 3 times daily) plus RBV resulted in residual or even undetectable HCV RNA levels after 4 weeks in all patients treated with the higher dose of BI 207127 (Table 3).

Finally, in a trial of HCV-genotype-1-infected null responders treated with the NS5A inhibitor BMS-790052 plus the NS3 protease inhibitor BMS-650032 alone or in combination with PEG-IFN-α plus RBV for 24 weeks, SVR12 was achieved in 4/11 and 9/10 patients, respectively. All patients with viral breakthrough in the DAA-only combination regimen (6/11) were genotype 1a patients [75].

Preventing drug resistance will be the primary challenge in DAA combination therapies. Nucleoside/nucleotide analogues with a high barrier to resistance and/or drug combinations that have a genetic barrier of four or more mutations may be required. Future trials need to address the tolerability and safety of long-term DAA administration and how SVR can be achieved by various DAA combinations without the addition of PEG-IFN-α plus RBV.

**Cyclophilin inhibitors**

Cyclophilins are ubiquitous proteins in human cells that are involved in protein folding. Moreover, cyclophilins participate in HCV replication as functional regulators of the HCV NS5B polymerase.

The cyclophilin inhibitor Debio-025 (alisporivir), a cyclosporine A analogue, showed antiviral activity in patients infected with different HCV genotypes (1–4) during monotherapy and in combination studies with PEG-IFN-α. Maximum log_{10} changes in HCV RNA of

**Table 3. Overview of ongoing and recently completed direct-acting antiviral combination studies with or without PEG-IFN plus RBV**

<table>
<thead>
<tr>
<th>NS3 protease inhibitor</th>
<th>NS5A inhibitor</th>
<th>NS5B nucleoside/nucleotide inhibitor</th>
<th>NS5B non-nucleoside/nucleotide inhibitor</th>
<th>Duration of DAA therapy</th>
<th>DAAs alone</th>
<th>DAAs plus RBV</th>
<th>DAA plus PEG-IFN/RBV</th>
<th>Study population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Danoprevir (100 mg 3 times daily)</td>
<td>–</td>
<td>RG 7128 (500 mg or 1,000 mg twice daily)</td>
<td>–</td>
<td>1–2 weeks</td>
<td>X</td>
<td>–</td>
<td>–</td>
<td>HCV-1, treatment-naive and -experienced</td>
</tr>
<tr>
<td>Telaprevir (1,125 mg twice daily)</td>
<td>–</td>
<td>–</td>
<td>VX-222 (100 mg or 400 mg twice daily)</td>
<td>12 weeks</td>
<td>X</td>
<td>–</td>
<td>X</td>
<td>HCV-1, treatment-naive</td>
</tr>
<tr>
<td>BMS-650032 (600 mg twice daily)</td>
<td>BMS-790052 (60 mg once daily)</td>
<td>–</td>
<td>–</td>
<td>24 weeks</td>
<td>X</td>
<td>–</td>
<td>X</td>
<td>HCV-1, treatment-experienced, null-responders</td>
</tr>
<tr>
<td>BI 201335 (120 mg once daily)</td>
<td>–</td>
<td>–</td>
<td>BI 207127 (400 mg or 600 mg 3 times daily)</td>
<td>4 weeks</td>
<td>–</td>
<td>X</td>
<td>–</td>
<td>HCV-1, treatment-naive</td>
</tr>
<tr>
<td>GS-9256 (75 mg twice daily)</td>
<td>–</td>
<td>–</td>
<td>Tegobuvir (40 mg twice daily)</td>
<td>4 weeks</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>HCV-1, treatment-naive</td>
</tr>
<tr>
<td>ABT-450/r (50/100 mg once daily)</td>
<td>–</td>
<td>–</td>
<td>ABT-072 (100 mg once daily)</td>
<td>12 weeks</td>
<td>–</td>
<td>X</td>
<td>–</td>
<td>HCV-1, treatment-naive</td>
</tr>
<tr>
<td>–</td>
<td>–</td>
<td>PSI-7977 (400 mg once daily) and PSI-938 (300 mg once daily)</td>
<td>–</td>
<td>1–2 weeks</td>
<td>X</td>
<td>–</td>
<td>–</td>
<td>HCV-1, treatment-naive</td>
</tr>
<tr>
<td>IDX320 (400 mg once daily)</td>
<td>–</td>
<td>IDX184 (100 mg once daily)</td>
<td>–</td>
<td>1–2 weeks</td>
<td>X</td>
<td>–</td>
<td>–</td>
<td>HCV-1, treatment-naive</td>
</tr>
</tbody>
</table>

DAAs, direct-acting antiviral; PEG-IFN, pegylated interferon; r, ritonavir-boosted; RBV, ribavirin; X, treatment regime.
up to 4.75 were observed in patients with HCV genotypes 1 or 4 who received 1,000 mg Debio-025 in combination with PEG-IFN-α2a plus RBV for 4 weeks [76]. SVR24 rates in the Debio-025 containing group were significantly higher than in the control group (76% versus 55%; \( P=0.008 \)) [77]. No viral breakthrough has been observed in clinical studies so far.

SCY-635 is another non-immunosuppressive analogue of cyclosporine A that exhibits potent suppression of HCV. Different doses of SCY-635 were investigated in patients infected with HCV genotype 1 and a mean maximum decline of 2.3 log10 IU/ml in viral load was observed after 15 days of 900 mg SCY-635 monotherapy [78]. No viral rebound was observed during SCY-635 therapy. However, minimal evidence for selection of resistant variants was observed within NS5B [78].

**Silibinin**

Silibinin, an extract of milk thistle (*Silybum marianum*) with antioxidant activity has been used as self-medication for liver diseases over centuries [79]. Though its mechanism of action is not yet fully understood, it was recently reported that silibinin at high doses is a direct inhibitor of the NS5B polymerase [80]. Other findings suggest that silymarin blocks virus entry, possibly by targeting the host cell [81].

**Ribavirin analogues**

Adverse haematological effects associated with RBV are commonly observed and have prompted the development of taribavirin, a produrg that is converted only by hepatocytes to RBV. In two Phase III trials, administration of taribavirin (600 mg twice daily) plus PEG-IFN-α2a or -α2b failed to achieve non-inferiority to administration of weight-based RBV plus PEG-IFN-α2a or -α2b [82,83]. In a recently completed Phase IIb trial, weight-based taribavirin was non-inferior compared to weight-based RBV, with fewer haematological side effects [84]. However, anaemia rates increased with higher taribavirin dosing and the dropout rates for anaemia did not differ between the two study drugs.

**New interferons**

Albinterferon-α2b (Joulferon) is a recombinant protein consisting of IFN-α2b fused to human albumin that can be administered every 2 or 4 weeks due to a longer half-life compared to the currently marketed PEG-IFNs. However, albinterferon was neither better tolerated nor did it show superior efficacy in patients infected with HCV genotype 1 or 2/3 in two large Phase III studies, respectively [85,86].

Locteron is a controlled release formulation of IFN-α2b that can be administered every 2 weeks. Week 12 interim results from a Phase Ib study have shown a similar reduction in HCV RNA and a reduction of flu-like symptoms by 57% for locteron at a dose of 480 μg compared to PEG-IFN-α2b in treatment-naive patients infected with HCV genotype 1 [87].

PEG-IFN-λ (PEG-rIL-29) is a type III IFN that binds to a unique receptor with more limited distribution than the type I IFN receptor used by IFN-α. In a Phase Ib trial, 526 treatment-naive patients were enrolled to receive different doses of PEG-IFN-λ (ranging from 120, 180 to 240 μg/subcutaneously) administered weekly with daily RBV. PEG-IFN-λ provided higher RVR and complete early virological response rates than PEG-IFN-α2a (5.8% versus 14.7% and 37.9% versus 55.9% in genotype 1 and 4 patients, respectively) and was associated with fewer haematologic toxicities, flu-like and musculoskeletal symptoms (15.2% versus 0.8%, 42.9% versus 9.9% and 46.6% versus 14.5%, respectively) [88]. The incidence of elevations in serum bilirubin level was highest at the two higher doses of PEG-IFN-λ, and especially at the highest dose (7.6% of patients with direct bilirubin above 1.2 mg/dl).

**Conclusions**

SVR rates in patients infected with HCV genotype 1, the most prevalent genotype in Europe and the US are approximately half the rates attained in patients infected with HCV genotype 2 or genotype 3 with the current SOC. Recently completed pivotal Phase III studies have shown SVR rates in the telaprevir trials of 69–73% for treatment-naive patients, 83–88% for previous relapers, 54–59% for previous partial responders and 29–33% for previous null responders. SVR rates in the boceprevir trials were 63–66% for treatment-naive patients, 69–75% for previous relapers and 40–52% for previous partial responders.

Several nucleoside/nucleotide and non-nucleoside inhibitors of the HCV NS5B polymerase have shown promising antiviral efficacy in early clinical studies. Unlike first-generation protease inhibitors, which are mostly active in patients with HCV genotype 1 and 2 only, nucleoside/nucleotide polymerase inhibitors bind to the highly conserved centre of the HCV polymerase, with the potential advantage of equal effectiveness across all different HCV genotypes.

At present, a number of studies focus on different combinations of HCV protease inhibitors, NS5A inhibitors and polymerase inhibitors with or without the addition of SOC. While a rapid decline in HCV RNA was observed in most of these studies, SVR rates are not available for the majority of these studies.
With the addition of new antiviral compounds to standard therapy, a number of additional side effects unrelated to SOC have been observed, and increased treatment-discontinuation rates have been reported. Consequently, successful management of these side effects will gain importance. In addition, frequent dosing intervals (that is, every 8 h) may lead to increased non-adherence outside of clinical trials and more patient-friendly formulations have to be developed.

One of the major challenges of future anti-HCV treatment regimens will be the emerging field of DAA drug resistance. Relatively low barriers to resistance have been reported for HCV protease inhibitors and non-nucleoside polymerase inhibitors as compared to nucleoside/nucleotide HCV polymerase inhibitors that appear to have a high barrier to resistance. Pre-existing resistant variants and their potential long-term persistence have to be taken into account for the selection of optimal treatment and re-treatment strategies. Finally, the potential drug–drug interactions, especially in patients with HIV coinfection who are on antiretroviral therapy and patients after organ transplantation taking immunosuppressive therapy will have major importance. Therapeutic drug monitoring may play a role in the management of these patients [89].

Disclosure statement

SZ is a clinical investigator, member of the speaker’s bureau and consultant for Abbott, Achillion, Anadys, BMS, Boehringer, Gilead, Merck, Novartis, Pharmasset, Pfizer, Roche, Santaris, Tibotec and Vertex. HF has no competing interests.

References


New antiviral therapies for HCV


