Direct Antiviral Agents for Hepatitis C – New Developments

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Abstract

Numerous directly acting antiviral agents (DAAs) for the treatment of chronic hepatitis C virus (HCV) infection are currently under development. The final results of phase II clinical trials that evaluated the most advanced compounds – telaprevir and boceprevir – indicate that the addition of these NS3/4A protease inhibitors to pegylated interferon-alpha (pegIFN-α) and ribavirin strongly improves the chances of achieving a sustained virological response (SVR) in treatment-naïve HCV genotype 1 patients and in prior non-responders and relapsers. However, monotherapy with DAAs frequently results in the selection of resistant quasi-species and viral breakthrough and is therefore not suitable. Generally, NS5B polymerase inhibitors have a lower antiviral efficacy than protease inhibitors, and their ability to improve SVR rates remains to be established. Future research should elaborate on whether an SVR can be achieved with combination therapies of DAA agents without IFN-α; in addition, DAAs targeting genotypes other than HCV genotype 1 should be evaluated.

Keywords

Directly acting antiviral agents (DAAs), specifically targeted antiviral therapy for hepatitis c (STAT-C), protease inhibitor, polymerase inhibitor, hepatitis C, antiviral therapy, viral resistance, drug resistance

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The odds of achieving a sustained virological response (SVR) in patients with chronic hepatitis C with a therapy of pegylated interferon-alpha (pegIFN-α) and ribavirin are still too low, particularly in patients infected with hepatitis C virus (HCV) genotypes 1 or 4.[1–4] Therefore, intensive efforts have been made to develop directly acting antiviral agents (DAAs) against HCV.[5–12] Many of these DAAs are currently in phase I–III development and will significantly change treatment options for HCV infection in the near future. The most advanced compounds are telaprevir and boceprevir, which are both inhibitors of the HCV NS3 protease and have been shown to significantly enhance SVR rates in HCV genotype 1 patients when applied in addition to pegIFN-α and ribavirin.[13–15]

NS3/4A Protease Inhibitors

NS3/4A protease inhibitors can be divided into two chemical classes: macrocyclic inhibitors and linear tetra-peptide α-ketoamid derivatives. Ciluprevir has a macrocyclic structure and was the first protease inhibitor evaluated in patients with chronic hepatitis C. Ciluprevir, as well as subsequently developed NS3/4A protease inhibitors of both molecular classes, strongly inhibited HCV replication during monotherapy, but also frequently caused the selection of resistant mutants, which may be followed by viral breakthrough.[12,16–18] Although the development of ciluprevir was stopped because of serious cardiotoxicity observed in an animal model, the proof of principle was provided for successful suppression of HCV replication by NS3/4A inhibitors in patients with chronic hepatitis C. Subsequent studies have shown that the frequency of resistance development against protease inhibitors can be vastly reduced by the additional administration of pegIFN and ribavirin. Telaprevir and boceprevir are the most advanced NS3/4A protease inhibitors, and are currently in phase III evaluation.

Telaprevir (VX-950)

Telaprevir is an orally bioavailable NS3 protease inhibitor that belongs to the α-ketoamids and that reversibly binds the enzyme covalently, with a half-life of 58 minutes of the enzyme–inhibitor complex. An initial double-blind, randomised, placebo-controlled phase I clinical trial showed that telaprevir monotherapy >14 days in HCV genotype 1 patients was well tolerated and led to a rapid decline of HCV RNA serum levels in all dosage groups.[19] The best results were obtained in the 750mg telaprevir every eight hours (q8h) dose group with a median reduction of HCV RNA of 4.4 $\log_{10}$ after 14 days of treatment, which is the basis for telaprevir dosage in most of the subsequent clinical trials. However, viral rebound due to selected mutants occurred in all patients after treatment completion and in some patients even during therapy.[18] Subsequent phase I studies showed that the addition of pegIFN-α and ribavirin to telaprevir leads to an even

more pronounced HCV RNA decline and reduces the frequency of telaprevir-resistant mutants and viral breakthrough.[20,21] These encouraging results led to the design of phase II clinical studies to determine the impact of telaprevir in addition to pegIFN-α and ribavirin on SVR rates.

Phase II Studies

Telaprevir and Pegylated Interferon with and without Ribavirin

Studies in Treatment-naïve Patients

Larger phase II clinical trials (Efficacy of Pegylated Interferon on Liver Fibrosis in Co-Infected Patient With HIV and Hepatitis C [PROVE] 1 and 2) in treatment-naïve genotype 1 patients assessed whether overall treatment duration could be reduced and/or SVR rates be improved with the addition of telaprevir to pegIFN-α-2a and ribavirin (see *Figures 1* and *2*). PROVE 1 was conducted in the US, whereas PROVE 2 was conducted in Europe. In addition, a study comparing two- versus three-times-daily administration of telaprevir in combination with either pegIFN-α-2a or -2b (C208) and studies in genotype 2-, 3- and 4- infected patients were performed (C209, C210).

In PROVE 1, telaprevir, pegIFN-α-2a and ribavirin were administered for 12 weeks in combination followed by pegIFN-α-2a and ribavirin alone for 0 (n=17), 12 (n=79) or 36 (n=79) weeks in comparison with standard treatment. SVR rates were 35, 61 and 67%, respectively, compared with 41% with standard treatment. According to the study protocol, treatment was only stopped after 12 or 24 weeks when a rapid virological response (RVR) was achieved. Serious adverse effects led to premature treatment termination in 18% of all subjects treated with telaprevir in contrast to 4% of patients with standard therapy.[14]

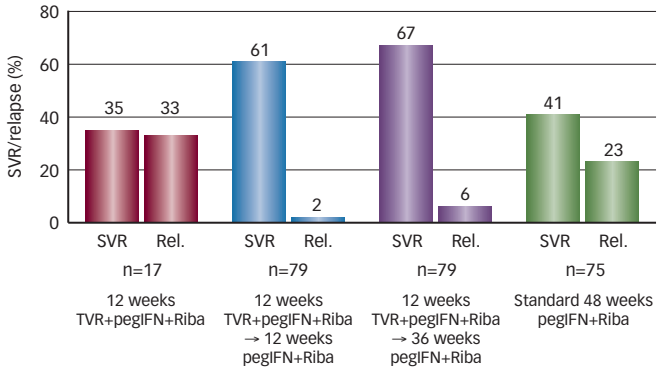
The study design of PROVE 2 was similar to that of PROVE 1, with the main difference being that treatment termination after 12 or 24 weeks was independent of achieving an RVR and one treatment arm was ribavirin-free. The recently published final results showed SVR rates of 36, 60 and 69%, respectively, for patients treated with telaprevir plus pegIFN alone for 12 weeks (n=78), telaprevir and pegIFN and ribavirin for 12 weeks (n=82) and telaprevir, pegIFN and ribavirin for 12 weeks followed by 12 weeks of pegIFN plus ribavirin alone (n=81). The SVR rate achieved by standard treatment was 46%. However, the rate of relapse in the groups treated for 12 weeks was relatively high at 30 and 48%, respectively, of all patients who were treated with and without ribavirin. Two patients who discontinued treatment at days 60 and 65 experienced a late relapse 36 and 48 weeks after the end of treatment, respectively.[13]

The results of PROVE 1 and 2 indicate that 12 weeks of triple therapy was too short because of the high rate of relapse after treatment completion. Moreover, ribavirin is necessary in therapies with telaprevir to achieve high SVR rates. However, 24–48 weeks of total therapy including 12 weeks of triple therapy with telaprevir in addition to standard treatment greatly improved SVR rates in treatment-naïve genotype 1 patients compared with the standard of care. RVR during triple therapy is an important predictor of treatment success and can be applied to define individualised treatment durations.

The most important side effects of telaprevir are rash, gastrointestinal disorders and anaemia. Although severe rash may require treatment discontinuation, moderate forms can be treated successfully with topical steroids. The median decline of blood haemoglobin concentration related to telaprevir was approximately 1g/dl. Since telaprevir was administered in most trials for only 12 weeks, the use of erythropoietin analogues was rarely necessary.

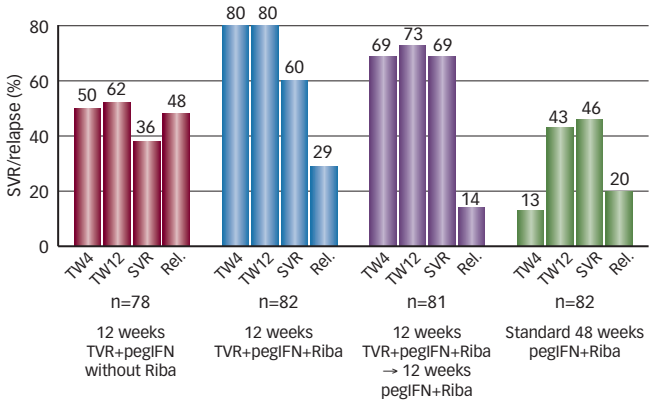
C208 was a small study (n=161) comparing three-times-daily 750mg telaprevir in combination with pegIFN-α-2a or -2b plus ribavirin versus

Figure 1: Results of PROVE 1 (US)



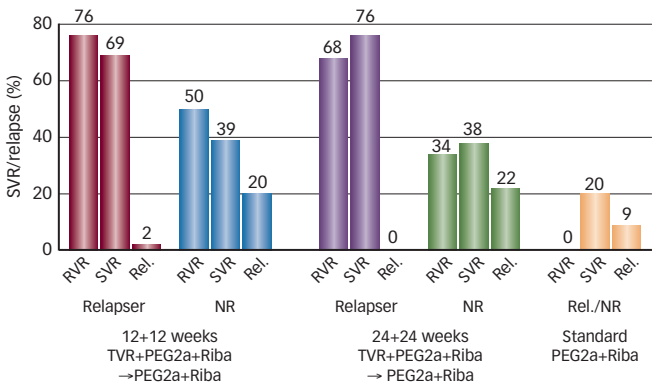
Combination therapy of telaprevir (TVR) and pegylated interferon-alpha-2a (pegIFN-α-2a) + ribavirin (Riba) in treatment-naïve genotype 1 patients.
Rel. = relapse; SVR = sustained virological response.

Figure 2: Results of PROVE 2 (Europe)



Combination therapy of telaprevir (TVR) and pegylated interferon-alpha-2a (pegIFN-α-2a) ± ribavirin (Riba) in treatment-naïve genotype 1 patients.
Rel. = relapse; SVR = sustained virological response; TW = treatment week.

Figure 3: Results of PROVE 3



Combination therapy of telaprevir (TVR) and pegylated interferon-alpha-2a (pegIFN-α-2a) ± ribavirin (Riba) in hepatitis C virus (HCV) genotype 1 patients with prior non-response (NR) or relapse (Rel.) to standard treatment.
RVR = rapid virological response; SVR = sustained virological response.

Table 1: Resistance Mutations to Hepatitis C Virus NS3 Protease Inhibitors

	V36A/M	T54S/A	V55A	Q80R/K	R155K/T/Q	A156S	A156T/V	D168A/V/T/H	V170A/T
Telaprevir (linear)	■	■	*		■	■	■		*
Boceprevir (linear)	■	■	■		■	■	*		■
SCH900518 (linear)	■	■			■	■	■		■
BILN-2061 (macrocyclic)					□		□	□	
R7227/ITMN191 (macrocyclic)					□	*	*	□	
MK-7009 (macrocyclic)					□			□	
TMC435350 (macrocyclic)				□	□			□	
BI-201335 (macrocyclic?)					□			□	□

**Mutations associated with resistance* in vitro *but not described in patients. Blocks indicate resistance mutations against linear (dark blocks) or macrocyclic (light blocks) protease inhibitors that were selected in patients during clinical studies and from* in vitro *studies.*

two-times-daily 1,125mg telaprevir in combination with pegIFN-α-2a or -2b plus ribavirin.[22] In all four treatment arms, similar SVR rates were observed (81–85%). These high overall SVR rates underline the potential of the triple-therapy approach. They are explained in part by experienced study centres with very low discontinuation rates (5%) in comparison with the PROVE studies. In addition, in this study the response-guided therapy approach was investigated. Treatment duration was shortened to 24 weeks in patients who achieved a RVR, while the remaining patients received therapy for 48 weeks. Between 80 and 83% of all patients treated with pegIFN-α-2a and 67–69% of all patients treated with pegIFN-α-2b achieved an RVR and could therefore be treated for 24 weeks.

Studies in Non-responders and Relapsers

The PROVE 3 trial was conducted to determine SVR rates of treatment with telaprevir in combination with pegIFN-α and ribavirin in treatment-experienced patients (see *Figure 3*). Telaprevir was administered in combination with pegIFN-α-2a with and without ribavirin for 12–24 weeks followed by pegIFN-α-2a and ribavirin alone for up to 24 weeks. Re-treatment of previous non-responders with 12 weeks of triple therapy followed by 12 weeks of standard treatment led to an SVR rate of 51% (69% relapser, 39% non-responder), which is significantly higher than the SVR rates achieved with the standard of care (14%). Re-treatment of non-responders with 24 weeks of triple therapy followed by 24 weeks of standard treatment led to an SVR rate of 53% (76% relapser, 38% non-responder) and re-treatment of non-responders with 24 weeks of telaprevir and pegIFN-α-2a without ribavirin followed by 24 weeks of pegIFN-α-2a alone led to a SVR rate of only 24% (42% relapser, 11% non-responder). The latter result indicates that ribavirin is required for a successful treatment of non-responders with telaprevir. As in the PROVE 1 and 2 studies, viral breakthrough was observed more frequently in patients infected with genotype 1a than in patients infected with genotype 1b.[23]

Phase III Studies

Design of Phase III Clinical Trials – Telaprevir with Pegylated Interferon-alpha and Ribavirin

Phase III clinical trials evaluating telaprevir in combination with pegIFN-α and ribavirin have been initiated. The A New Direction in HCV Care: A Study of Treatment-Naive Hepatitis C Patients with telaprevir (ADVANCE) trial enrolled more than 1,000 treatment-naïve HCV genotype 1 patients to evaluate 24 weeks of telaprevir-based therapy. Telaprevir was dosed at 750mg every eight hours and given for eight or 12 weeks in combination with pegIFN-α-2a and ribavirin followed by pegIFN-α-2a and ribavirin alone until treatment week 24. Patients who did not achieve an RVR were treated with pegIFN-α-2a and ribavirin until week 48. In the A Randomized Study of Stopping Treatment at 24 Weeks or Continuing Treatment to 48 Weeks in Treatment-Naïve Subjects With Genotype 1 Chronic Hepatitis C Who Achieve an Extended Rapid Viral Response (eRVR) While Receiving Telaprevir, Peginterferon Alfa2a (Pegasys®) and Ribavirin (Copegus®) (ILLUMINATE) trial, telaprevir was given for 12 weeks in combination with pegIFN-α-2a and ribavirin followed by pegIFN-α-2a and ribavirin alone until treatment week 24 or 28. The aim of the ILLUMINATE trial is to assess whether treatment extension beyond 24 weeks of total therapy improves SVR rates in patients with RVR or EVR. The Re-treatment of Patients with Telaprevir-based Regimen to Optimize Outcomes (REALIZE) study enrolled more than 650 patients with prior failure on standard treatment. PegIFN-α-2a and ribavirin was given for 48 weeks including 12 weeks of telaprevir at a dose of 750mg every eight hours. In one treatment arm, telaprevir treatment was initiated after a four-week lead-in phase of pegIFN-α-2a and ribavirin alone. SVR data from the ADVANCE, ILLUMINATE and REALIZE studies are expected to be published in 2010.

Viral Resistance to Telaprevir

Due to the high replication rate of HCV and the poor fidelity of its RNA-dependent RNA polymerase, numerous variants (quasi-species) are continuously produced during HCV replication. Among them, variants carrying mutations altering the conformation of the binding sites of DAA compounds can develop. During treatment with specific antivirals, these pre-existing drug-resistant variants have a fitness advantage and can be selected to become the dominant viral quasi-species with the consequence of viral breakthrough. To date, mutations conferring telaprevir resistance have been identified at four positions: V36A/M/L, T54A, R155K/M/S/T and A156S/T18[24–27] (see *Table 1*). The A156 mutation was shown by *in vitro* analyses in the replicon assay, while the other mutations could only be detected *in vivo* by a clonal sequencing approach during telaprevir administration in patients with chronic

hepatitis C. The incidence of breakthrough of resistant mutants was much lower during combination therapy of telaprevir and pegIFN-α-2a in a 14-day study compared with telaprevir monotherapy. It is important to note that up to three years after telaprevir treatment, low to medium levels of V36 and R155 variants were still observed in single patients.[28]

As shown for other NS3/4A protease inhibitors as well (e.g. ITMN-191), the genetic barrier to telaprevir resistance differs significantly between HCV subtypes. In all clinical studies of telaprevir alone or in combination with pegIFN-alfa and ribavirin, viral resistance and breakthrough occurred much more frequently in patients infected with HCV genotype 1a compared with genotype 1b. This difference was shown to result from nucleotide differences at position 155 in HCV subtype 1a (aga, encodes R) versus 1b (cga, also encodes R). The mutation most frequently associated with resistance to telaprevir is R155K; changing R to K at position 155 requires one nucleotide change in HCV subtype 1a and two nucleotide changes in subtype 1b isolates.[29]

Boceprevir (SCH 503034)

Boceprevir is another peptidomimetic orally bioavailable α-ketoamid HCV protease inhibitor that forms a covalent but reversible complex with the NS3 protein.[30] Phase I clinical trials in HCV genotype 1 patients with prior failure on standard therapy revealed that boceprevir (100–400mg daily) monotherapy and combination therapy with pegIFN-α-2b resulted in mean maximum reductions in HCV RNA load of up to 1.61 $\log_{10}$ and 2.88 $\log_{10}$, respectively.[31] Boceprevir was well-tolerated alone and in combination with pegIFN-α-2b. However, viral breakthrough due to selection of pre-existing resistant mutants was observed in some patients, in particular during boceprevir monotherapy.[32]

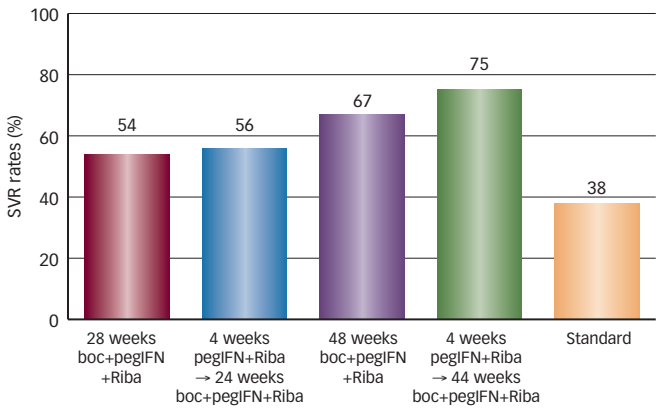
Phase II Studies

Boceprevir and Pegylated Interferon with and without Ribavirin

Treatment-naïve Phase II Study

The aim of the HCV Serine Protease Inhibitor Therapy-1 (SPRINT 1) trial was to investigate the safety, tolerability and antiviral efficacy of boceprevir (800mg three times a day) in combination with pegIFN-α-2b and ribavirin in treatment-naïve HCV genotype 1 patients.[15] Treatment with boceprevir in combination with pegIFN-α-2b and ribavirin was performed either continuously for 28 or 48 weeks or for 24 or 44 weeks after a previous four-week lead-in phase of pegIFN-α-2b and ribavirin alone. The lead-in design was chosen to determine a potential benefit of pre-treatment with pegIFN-α-2b and ribavirin in terms of avoiding resistance development. The control group was treated with pegIFN-α-2b and ribavirin for 48 weeks. SVR rates after 28 weeks of triple treatment were 54%, and 56% after 24 weeks with an additional four weeks of pre-treatment lead-in with pegIFN-α-2 and ribavirin (see *Figure 4*). SVR rates after 48 weeks of triple treatment were 67%, and 75% after 44 weeks with an additional four weeks of pre-treatment lead-in with pegIFN-α-2b and ribavirin. After triple therapy for four weeks with boceprevir, pegIFN and ribavirin, 38% of patients achieved an RVR. The most common side effects related to boceprevir were anaemia, nausea, vomiting and dysgeusia. In general, SPRINT-1 has proved a higher antiviral efficacy of combination therapy with boceprevir in comparison with the standard of care with slightly better results after a four-week lead-in phase. However, RVR rates of only 38% during boceprevir triple therapy indicate that boceprevir is potentially less potent than telaprevir, which, during triple therapy with pegIFN-α-2b, led to an RVR rate of approximately 70%.

Figure 4: Results of SPRINT-1



Combination therapy of boceprevir (boc) and pegylated interferon-alpha-2a (pegIFN-α-2a) + ribavirin (Riba) in treatment-naïve genotype 1 patients. SVR = sustained virological response.

Studies in Non-responders and Relapsers

In a complex study of HCV genotype 1 non-responders, the addition of boceprevir to pegIFN-α-2b and ribavirin resulted in only slightly increased SVR rates compared with standard treatment (14% versus 2%).[33]

Design of Phase III Studies

A phase III clinical trial (SPRINT-2) evaluating boceprevir in treatment-naïve patients was recently initiated and has enrolled >1,000 patients. Equivalent to the SPRINT-1 study design, patients received 800mg boceprevir three times daily in combination with pegIFN-α-2b and weight-based ribavirin for 28 or 48 weeks. The SCH 503034 Plus Peg-Intron, With and Without Added Ribavirin, in Patients With Chronic Hepatitis C, Genotype 1, Who Did Not Respond to Previous Treatment With Peginterferon Alfa Plus Ribavirin (RESPOND-2) study evaluated boceprevir in combination with pegIFN-α-2b and ribavirin at the same doses, but for 36 and 48 weeks in relapsers and partial responders. In all investigational arms, a lead-in strategy with pegIFN-α-2b and ribavirin is subsequently administered.

Viral Resistance to Boceprevir

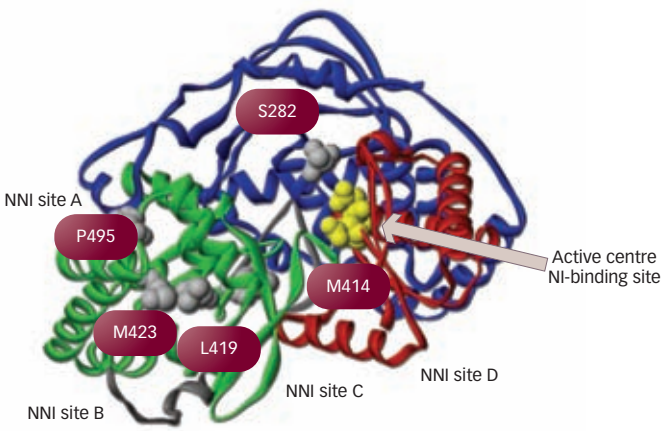
In the replicon system, mutations at three positions conferring boceprevir resistance were discovered (see *Table 1*). T54A, A156S and V170A confer low-level resistance to boceprevir, whereas A156T, which also confers telaprevir and ciluprevir resistance, exhibited greater levels of resistance.[34] In patients with chronic hepatitis C, three additional mutations were detected during boceprevir monotherapy (V36G/M/A, V55A and R155K).[32]

Other NS3 Protease Inhibitors

Other NS3 protease inhibitors are currently in phase I–II development (R7227/ITMN191, MK7009, BI201335, TMC435350, SCH900518, BMS-650032, PHX1766, ACH-1625).[12,35,36] In general, they exhibit a high antiviral activity in HCV genotype 1 patients, similar to telaprevir and boceprevir. Potential advantages of these second-wave protease inhibitors might be better tolerability, broader genotypic activity and/or improved pharmacokinetics, which may allow a once-daily dosage (e.g. TMC435).[35]

Triple-therapy studies for a number of compounds have been initiated and confirm that resistance development is significantly reduced by combination with pegIFN and ribavirin. Whereas linear

Figure 5: Structure of the Hepatitis C Virus NS5B RNA Polymerase and Binding Sites



NNI = Non-nucleoside inhibitor; NI = Nucleoside inhibitor.

tetrapeptide and macrocyclic inhibitors do not differ in general in terms of their antiviral activity, their resistance profile differs significantly. However, R155 is an overlapping position for resistance, and different mutations at this amino acid site within the NS3 protease confer resistance to all protease inhibitors that are currently in advanced clinical development.[12]

Compounds Targeting Hepatitis C Virus Replication

NS5B Polymerase Inhibitors

NS5B RNA polymerase inhibitors can be divided into two distinct categories. Nucleoside analogue inhibitors (NAIs) such as valopicitabine (NM283), R7128, R1626, PSI-7851 and IDX184 mimic the natural substrates of the polymerase and are incorporated into the growing RNA chain, thus causing direct chain termination by tackling the active site of NS5B37-48. As the active centre of NS5B is a highly conserved region of the HCV genome, NAIs are potentially effective against all different genotypes, in contrast to NS3/4A inhibitors. Moreover, single amino acid substitutions in every position of the active centre may result in loss of function. Thus, there is a relatively high genetic barrier in the development of resistance to NAIs.

In contrast to NAIs, the heterogeneous class of non-nucleoside inhibitors (NNIs) bind to different allosteric enzyme sites, which results in conformational protein change before the elongation complex is formed.[49] NS5B is structurally organised in a characteristic 'right-hand motif' (see *Figure 5*) containing finger, palm and thumb domains, and offers at least four NNI-binding sites, a benzimidazole (thumb 1)-binding, thiophene (thumb 2)-binding, benzothiadiazine (palm 1)-binding and benzofuran-(palm 2)-binding site.[49,50] Theoretically, NNIs targeting different binding sites can be used in combination or in sequence to manage the development of resistance. As NNIs bind distantly to the active centre of NS5B, their application results more frequently in resistance development than during treatment with NAIs. In addition, mutations at the NNI-binding sites do not necessarily lead to impaired function of the enzyme.

Nucleoside Analogues

Valopicitabine (NM283, 2'-C-methylcytidine/NM107) was the first nucleoside inhibitor investigated in patients with chronic hepatitis C. The antiviral activity of valopicitabine was low.[51] The clinical development of valopicitabine was stopped due to gastrointestinal side effects and an insufficient risk–benefit profile.

The second nucleoside inhibitor investigated in patients with chronic hepatitis C was R1626 (4'-azidocytidine/PSI-6130). A phase I study showed high antiviral activity at high doses of R1626 in patients infected with HCV genotype 1.[44–46] No viral breakthrough with selection of resistant variants was reported from monotherapy or combination studies with pegIFN ± ribavirin. However, severe lymphopenia and infectious disease adverse events led to R1626 development being stopped.

R7128 is another nucleoside polymerase inhibitor with potent antiviral activity during monotherapy in HCV genotype 1 patients. Currently, R7128 is being investigated in phase II clinical trials in HCV genotype 1-, 2- and 3-infected patients in combination with pegIFN and ribavirin.[39] No resistance development against R7128 was observed during both monotherapy and combination therapy with pegIFN and ribavirin. Other NAIs of the NS5B polymerase (PSI-7851 and IDX184) are being evaluated in phase I clinical trials in patients with chronic hepatitis C and many compounds are in pre-clinical development.[12]

Non-nucleoside Analogues

Non-nucleoside Inhibitors – Site 1 Inhibitors (Thumb 1/Benzimidazole Site)

BILB1941, BI207127 and MK-3281 are NNI-site 1 inhibitors that have been investigated in clinical phase I trials and exhibit low to medium antiviral activities.[12,52,53] No selection of resistant variants and viral breakthrough has been observed during five days of treatment with BILB1941 or BI207127.

Non-nucleoside Inhibitors – Site 2 Inhibitors (Thumb 2/Thiophene Site)

Filibuvir (PF-00868554) is a NNI-site 2 inhibitor with medium antiviral activity in a phase I study. In a subsequent trial, viral breakthrough was observed in five out of 26 patients during combination therapy with pegIFN-α-2a and ribavirin for four weeks.[53]

Other NNI-site 2 inhibitors that were evaluated in phase I trials are VCH-759, VCH-916 and VCH-22212.[54] As with treatment with filibuvir, VCH-759 and VCH-916 application resulted in viral breakthroughs with selection of resistant variants, indicating a low genetic barrier to resistance of these agents.

Non-nucleoside Inhibitors – Site 3 Inhibitors (Palm 1/Benzothiadiazine Site)

ANA598 is a NNI-site 3 inhibitor that displayed antiviral activity during treatment of HCV genotype-1-infected patients. No viral breakthrough was observed during a short-term monotherapy trial.[55]

Non-nucleoside Inhibitors – Site 4 Inhibitors (Palm 2/Benzofuran Site)

Monotherapy with the NNI-site 4 inhibitor HCV-796 showed low antiviral activity in HCV genotype-1-infected patients and resulted in selection of resistant variants and viral breakthrough in several patients.[56] GS-9190 displays a low antiviral activity in a clinical study and variants conferring resistance were identified in the beta-hairpin of the polymerase.

NS5A Inhibitors

In a single ascending-dose study, it was shown that inhibition of the NS5A protein with BMS-790052 leads to a sharp initial decline of HCV RNA concentrations.[57] BMS-790052 binds to domain I of the NS5A protein, which was shown to be important for regulation of HCV replication. No clinical data on resistance to this class of drugs have been presented yet and the results of multiple-dose and combination therapy studies are awaited.

Directly Acting Antiviral Agents and Hepatitis C Virus Genotype

As the amino acid sequence of the NS3 protease domain varies significantly between HCV genotypes, protease inhibitors may have a different antiviral efficacy in patients infected with different HCV genotypes. Indeed, telaprevir was less effective in treatment-naïve patients infected with genotypes other than genotype 1. For HCV genotype 2, a somewhat weaker antiviral activity in comparison with HCV genotype 1 was observed with a mean viral decline of 3.9 $\log_{10}$ IU/ml during 14 days of monotherapy with telaprevir.

In genotype-3- and 4-infected patients, no significant antiviral activity was detectable (0.5–0.9 $\log_{10}$ decline).[58,59] In contrast to NS3, the active centre of NS5B is a highly conserved region of the HCV genome. Thus, NAIs of NS5B have been shown to be effective across different HCV genotypes.[60]

Combination Therapies of Specific Antivirals

It is a fundamental question whether SVR can be achieved by combination therapies of different specifically targeted antiviral therapy for hepatitis C (STAT-C) compounds without pegIFN-α and ribavirin A first clinical trial (INFORM-1 study) evaluated the combination of a polymerase inhibitor (R7128) and an NS3 inhibitor (R7227/ITMN191). In this proof-of-principle study, patients were treated with both compounds for up to two weeks. HCV RNA concentrations decreased up to 5.2 $\log_{10}$ IU/ml, no viral break-through was observed and HCV RNA was undetectable at the end of dosing in up to 63% of treatment-naïve patients.[61] Future clinical trials need to address whether long-term suppression of HCV replication or even SVR can be achieved with such direct antiviral combination therapies. Currently, combination studies with several compounds are being conducted (R7128+R7227, VX-950+VCH222, BMS790052+ BMS650032, BI201335+BI207127).

Conclusions

Numerous DAA agents are currently under clinical phase I–III evaluation. Monotherapy with most of these agents will be not suitable since it frequently results in the selection of resistant variants, which may cause viral breakthrough. R155 is an overlapping mutation conferring resistance to all clinically evaluated protease inhibitors. However, results of phase II clinical trials evaluating the most advanced compounds, telaprevir and boceprevir, indicate that the addition of these NS3/4A protease inhibitors to pegIFN-α and ribavirin substantially improves the chance of achieving an SVR in treatment-naïve HCV genotype 1 patients and in prior non-responders and relapsers to standard therapy. In addition, at least during treatment with telaprevir-based regimens, overall treatment durations can be shortened significantly. Additional findings of the milestone studies PROVE 1 and 2 were that ribavirin is still necessary in HCV protease-inhibitor-based therapy to achieve high SVR rates, and RVR during triple therapy is an important predictor of treatment success and can be applied to define individualised treatment durations. As DAA agents are burdened with additional side effects such as anaemia, rash or gastrointestinal disorders, there will likely be a debate as to which HCV genotype 1 patients require a triple-therapy approach. In this context, the impact of recently discovered polymorphisms near the *IL28B* gene on SVR rates during triple therapy as well as of other classic predictors of virological response, such as baseline viral load, needs to be characterised in future studies.[62–64] Compared with NS3/4A protease inhibitors, most HCV polymerase inhibitors display a lower antiviral activity during monotherapy, and SVR data of triple therapies containing NS5B inhibitors are awaited. However, some polymerase inhibitors are equally effective against different HCV genotypes, whereas it was shown that protease inhibitors such as telaprevir are less potent in genotypes other than HCV genotype 1. In addition, NS5B inhibitors, at least of the nucleoside analogue family, display a high genetic barrier to resistance. Combination of different DAA agents may be sufficient to achieve an SVR or to suppress HCV during long-term application in IFN-free regimens, which has to be proved in future studies. An additional task for future research will be the identification of DAA agents efficient for genotypes other than HCV genotype 1. ■

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