

Review article: HCV – STAT-C era of therapy

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Review article: HCV – STAT-C era of therapy

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hepatitis C (STAT-C), non-structural (NS) protein.	

Summary

Background: Novel, directly acting antiviral agents, also named "specifically targeted antiviral therapy for hepatitis C" (STAT-C) compounds, are currently under development in order to improve treatment opportunities of chronic hepatitis C virus (HCV) infection.

Aim: To review the potential of STAT-C agents which are currently under clinical development with a focus on agents that target HCV proteins.

Methods: Studies evaluating STAT-C compounds were identified by systematic literature search using PubMed as well as databases of abstracts presented in English at recent liver and gastroenterology congresses.

Results: Numerous directly acting antiviral agents are currently under clinical phase I-III evaluation. Final 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 pegylated interferon-alfa and ribavirin strongly improves the chance to achieve a SVR in treatment-naive HCV genotype 1 patient as well as in prior non-responders and relapsers to standard therapy. Monotherapy with directly acting antivirals is not suitable since it frequently results in the selection of resistant quasispecies and viral breakthrough. NS5B polymerase inhibitors in general have a lower antiviral efficacy than protease inhibitors and their potency to improve SVR rates remains to be established.

Conclusion: STAT-C compounds in addition to pegylated interferon-alfa and ribavirin are capable to improve SVR rates at least in HCV genotype 1 patients and will therefore be included in future treatment recommendations and guidelines. Future research needs to evaluate whether a SVR can be achieved by combination therapies of STAT-C compounds in interferon-free regimens.

Introduction

With the current standard of care, a combination therapy of pegylated interferon-alfa plus weight based ribavirin for 24 to 72 weeks, only half of all patients with chronic hepatitis C can be cured ¹⁻⁴. The chance to achieve a sustained virologic reponse (SVR) by such regimens differs significantly between HCV genotypes with SVR rates of 40-50% in patients infected with genotype 1, contrasted by SVR rates of approximately 80% in those infected with genotypes 2 or 3^{1-5} . In addition, treatment with pegylated interferon- α and ribavirin is long (up to 72 weeks) and associated with numerous side effects like anemia, flu-like symptoms or depression. In view of these facts there is an urgent need for improved treatment strategies. The exploding knowledge of the HCV life cycle and of structural features of the HCV proteins has supported the development of many promising directly acting antiviral agents, also named "specifically targeted antiviral therapy for hepatitis C" (STAT-C) compounds⁶⁻¹³. Figure 1 summarizes the HCV life cycle and potential targets for STAT-C^{11, 12}. Many of these direct antivirals 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 which have been shown to significantly enhance SVR rates in HCV genotype 1 patients, when applied in addition to pegylated interferon-alfa and ribavirin ¹⁴⁻¹⁶. These and other STAT-C compounds will be described in this review with a focus on agents that were already evaluated in clinical trials (Table 1). Antivirals targeting host proteins which are mandatory for HCV replication (e.g. nitazoxanide, celgosivir or DEBIO-025) are reviewed elsewhere¹⁷⁻²¹.

Compounds targeting HCV polyprotein procession

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^{6, 7}. Nevertheless, many NS3/4A protease inhibitors are under development, which in general provide a high antiviral efficacy but a low genetic barrier to resistance. Protease inhibitors can be divided into two chemical classes, macrocyclic inhibitors and linear tetra-peptide α -ketoamid derivatives. NS3/4A protease inhibitors of both classes strongly inhibit HCV replication during monotherapy, but also frequently cause the selection of resistant mutants which may be followed by viral breakthrough^{13, 22}. However, it was shown that the frequency of resistance development against protease inhibitors can be vastly reduced by the additional administration of pegylated interferon and ribavirin. The most advanced compounds are telaprevir and boceprevir, which are currently under phase III evaluation and are expected to be approved in 2011/2012.

Ciluprevir (BILN 2061)

The first NS3/4A inhibitor applied in clinical studies was ciluprevir (BILN 2061), an orally bioavailable, peptidomimetic, macrocyclic drug binding non-covalently to the active center of the enzyme²³. Ciluprevir monotherapy was evaluated in a double-blind, placebo-controlled pilot study in treatment-naïve genotype 1 patients with compensated liver disease²⁴. In this study, ciluprevir, administered twice daily for two 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. Another study with equivalent design assessed the influence of the HCV genotype on treatment with this protease inhibitor. Compared to genotype 1 patients, the antiviral activity of ciluprevir was less pronounced and more variable in patients infected with genotypes 2 or 3²⁵. 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.

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Viral resistance to ciluprevir

Because of the high replication rate of HCV and the poor fidelity of its RNA-dependent RNA polymerase, numerous variants (quasispecies) are continuously produced during HCV replication. Among them, variants carrying mutations altering the conformation of the binding sites of STAT-C compounds can develop. During treatment with specific antivirals, these preexisting drug-resistant variants have a fitness advantage and can be selected to become the dominant viral quasispecies. Many of these resistant mutants exhibit an attenuated replication fitness with the consequence that, after termination of exposure to specific antivirals, the wild-type may again replace the resistant variants^{22, 26}. Nevertheless, HCV quasispecies resistant to NS3/4A protease inhibitors or non-nucleoside polymerase inhibitors can be detected at very low levels in some patients who were never treated with specific antivirals before²⁷⁻²⁹. The clinical relevance of these pre-existing mutants is not completely understood, although there is evidence that they may reduce the chance to achieve a SVR by therapies based on HCV protease or non-nucleoside polymerase inhibitors.

Exposure of genotype 1 replicon cells to ciluprevir and subsequent sequence analyses of the NS3 region has led to the identification of several mutations conferring ciluprevir-resistance: A156T, R155Q and D168V/A. These mutations result in a 357-fold, 24-fold and 144-fold reduced susceptibility to ciluprevir, respectively, compared to wild-type³⁰⁻³². The A156T mutant confers varying levels of cross-resistance to ciluprevir, telaprevir and boceprevir. The A156T mutation causes a significantly reduced enzymatic function attenuating the HCV life cycle, which, however, can be overcome by additional mutations at P89L, Q86R or G162R³⁰⁻³². No data are available on clinically selected resistance mutations after administration of ciluprevir in patients with chronic hepatitis C.

Telaprevir (VX-950)

Telaprevir is an orally bioavailable NS3 protease inhibitor which belongs to the α -ketoamids and binds the enzyme covalently but reversibly, with a half-life of 58 minutes of the enzyme-inhibitor complex. Currently, telaprevir is under phase III evaluation (ADVANCE- and ILLUMINATE-Study for treatment-naïve patients, REALIZE study for non-responders).

Phase I studies

Telaprevir monotherapy study

A double-blind, randomized placebo-controlled phase Ib clinical trial evaluating telaprevir monotherapy over 14 days was performed in patients with chronic HCV genotype 1 infection³³. In this study, antiviral activity, safety, optimal dosage, and pharmacokinetics were assessed in treatment-naïve patients, relapsers or non-responders to standard treatment. Doses of telaprevir were 450 mg or 750 mg every 8 hours or 1250 mg every 12 hours. Telaprevir was well tolerated and led to a rapid decline of HCV RNA serum levels in all groups. The best results were obtained in the 750 mg telaprevir q8h dose group with a median reduction of HCV RNA of 4.4 log₁₀ after 14 days of treatment, which is the basis for telaprevir-dosage in most of the following clinical trials. However, viral rebound due to selected mutants occurred in all patients after treatment completion and in some patients even during therapy. The selection of resistant mutants was more frequent in patients who received suboptimal doses²².

Telaprevir / pegylated (peg) interferon α -2a / ribavirin combination studies

A second phase I study investigated the safety, viral kinetics and the development of telaprevirresistant mutants of telaprevir monotherapy and in combination with pegIFN α -2a in treatmentnaïve genotype 1 patients³⁴. Telaprevir dosage was 750 mg every 8 hours after an initial loading dose of 1250 mg and it was administered either alone or in combination with pegIFN α -2a in comparison to pegIFN α -2a monotherapy. Treatment was given for 14 days and caused a median reduction of HCV RNA of 1.09 log₁₀ in the pegIFN α -2a/placebo group, of 3.99 log₁₀ in

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the telaprevir/placebo group and of 5.49 \log_{10} in the telaprevir/pegIFN α -2a group at the end of therapy. As observed before, selection of telaprevir-resistant mutants occurred during telaprevir monotherapy. However, their frequency was significantly lower during combination therapy with pegIFN α -2a and no viral breakthrough was seen during the combination therapy within 14 days³⁴.

A parallel study evaluated the safety and efficacy of telaprevir (750 mg every 8 hours) in combination with pegIFN α -2a and weight-based ribavirin in treatment-naive genotype 1 patients for 28 days³⁵. At the end of the 28-day treatment period, all patients had undetectable HCV RNA serum levels.

Phase II studies

Telaprevir and peg interferon with and without ribavirin

Studies in treatment naïve patients (PROVE 1 and 2, C208, C209, C210)

Larger phase II clinical trials (PROVE 1 and 2) in treatment naïve genotype 1 patients assessed whether with additional telaprevir to pegIFN α -2a and ribavirin overall treatment duration can be reduced and / or SVR rates be improved (Figure 2 and 3). PROVE 1 was conducted in the USA whereas PROVE 2 was conducted in Europe. In addition, a study comparing two versus three times daily administration of telaprevir in combination with either pegylated interferon alfa 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 to standard treatment. SVR rates were 35%, 61% and 67%, respectively, compared to 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. Most common adverse events were skin rash, anemia and gastrointestinal disorders¹⁵.

The study design of PROVE 2 was similar to 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% for patients treated with telaprevir plus pegIFN alone for 12 weeks (n=78), telaprevir and pegIFN and ribavirin for 12 weeks (n=82), and with telaprevir, pegIFN and ribavirin for 12 weeks of pegIFN plus ribavirin alone (n=81), respectively. The SVR rate achieved by standard treatment was 46%. However, the rate of relapse in the groups treated for 12 weeks was relatively high with 30% and 48% of all patients who were treated with and without ribavirin, respectively. Two patients who discontinued treatment at day 60 and 65 experienced a late relapse 36 and 48 weeks after the end of treatment, respectively¹⁴.

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 to 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 to the standard of care. The RVR during triple therapy is an important predictor for treatment success and can be applied for defining individualized treatment durations.

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

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C208 was a small study (n=161) comparing three times daily 750mg with two times daily 1125mg telaprevir combined with pegylated interferon alfa 2a or 2b, respectively, and ribavirin³⁶. In all 4 treatment arms comparable 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 centers 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 48 weeks therapy. Between 80% - 83% of all patients treated with pegIFN alfa 2a, and 67% - 69% of all patients treated with pegIFN alfa 2b achieved an RVR and could therefore be treated for 24 weeks.

Because 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 genotypes. Like ciluprevir, telaprevir alone or in combination with pegIFN and ribavirin was less effective in treatment-naïve patients infected with other genotypes 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 log₁₀ 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 log₁₀ decline)^{37, 38}.

Studies in non-responders and relapsers (PROVE 3)

The PROVE 3 trial was conducted to determine SVR rates of treatment with telaprevir in combination with pegIFN-alfa and ribavirin in treatment-experienced patients (Figure 4). Telaprevir was administered in combination with pegIFN α -2a with and without ribavirin for 12 to 24 weeks followed by pegIFN α -2a and ribavirin alone for up to 24 weeks. Retreatment of previous non-responders with 12 weeks of triple therapy followed by 12 weeks of standard

treatment led to a SVR rate of 51% (69% Relapser, 39% Non-Responder), which is significantly higher compared to SVR rates achieved with the standard of care (14%). Retreatment of nonresponders with 24 weeks of triple therapy followed by 24 weeks of standard treatment led to a SVR rate of 53% (76% Relapser, 38% Non-Responder) and retreatment 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 1b³⁹.

Phase III studies

Design of phase III clinical trials: telaprevir with pegylated interferon-alfa and ribavirin

Phase III clinical trials evaluating telaprevir in combination with pegIFN-alfa and ribavirin have been initiated. The ADVANCE trial enrolled more than 1000 treatment-naïve HCV genotype 1 patients to evaluate 24 weeks of telaprevir-based therapy. Telaprevir was dosed at 750 mg every 8 hours and given for 8 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 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 REALIZE study enrolled more than 650 patients with prior failure to standard treatment. PegIFN- α -2a and ribavirin was given for 48 weeks including 12 weeks of telaprevir at a dose of 750 mg every eight hours. In one treatment arm, telaprevir treatment was initiated after a 4 week lead-in phase of pegIFN- α -2a and ribavirin alone. SVR data of the ADVANCE, ILLUMINATE and REALIZE study are expected to be published in 2010.

Viral resistance to telaprevir

To date, mutations conferring telaprevir-resistance have been identified at four positions, V36A/M/L, T54A, R155K/M/S/T and A156S//T^{22, 30, 31, 40}, see Table 2 and 3. 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. A detailed kinetic analysis of telaprevir-resistant variants was performed in genotype 1 patients during 14 days of telaprevir monotherapy and combination therapy with peqIFN α-2a⁴¹. Telaprevir monotherapy initially led to a rapid HCV RNA decline in all patients due to a strong reduction in wild-type virus. In patients who developed a viral rebound during telaprevir monotherapy, mainly the single mutation variants R155K/T and A156/T were uncovered by wild-type reduction and became dominant after day 8. These single mutation variants were selected from preexisting quasispecies. During the viral rebound phase these variants typically were replaced by highly resistant double-mutation variants (e.g., V36M/A +R155K/T). The combination of telaprevir and pegIFN α -2a was sufficient to inhibit the breakthrough of resistant mutations in a 14-day study. It is important to note that after up to 3 years after telaprevir treatment low to medium levels of V36 and R155 variants were still observed in single patients⁴². Another study modeling the dynamics of wild type HCV genotype 1 in patients treated with telaprevir with and without pegylated interferon-alfa and ribavirin showed a first and second phase reduction in virus decline which was up to 10-fold stronger than reported for the standard of care⁴³.

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 to genotype 1b. This difference was shown to result from nucleotide differences at

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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 1 nucleotide change in HCV subtype 1a and 2 nucleotide changes in subtype 1b isolates⁴⁴.

Boceprevir (SCH 503034)

Boceprevir is another novel peptidomimetic orally bioavailable α -ketoamid HCV protease inhibitor that forms a covalent but reversible complex with the NS3 protein⁴⁵. Like telaprevir, boceprevir is currently in phase III evaluation.

Phase I studies

Boceprevir monotherapy study

An initial phase I trial evaluated safety, tolerability and antiviral efficacy of boceprevir monotherapy (100 to 400 mg daily) in HCV genotype 1 patients with prior failure to standard therapy⁴⁶. After the 14-day treatment period, a mean log₁₀ reduction in HCV RNA load of 2.06 was achieved in patients treated with 400 mg boceprevir daily. Boceprevir was well tolerated at all doses without significant adverse effects. However, viral breakthrough with selection of resistant variants occurred in some patients with a frequency depending on boceprevir dosage⁴⁷.

Boceprevir / peg interferon α -2b combination study

A subsequent phase lb study evaluated the combination of boceprevir and pegIFN α -2b in HCV genotype 1-infected non-responders to standard therapy⁴⁸. In this randomized, double-blind crossover study, boceprevir was administered at doses of 200 or 400 mg every eight hours either alone for seven days or in combination with pegIFN α -2b for 14 days in comparison to 14 days of pegIFN α -2b monotherapy. Because HCV genotype 1 non-responders to standard

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treatment are heterogeneous, the study design intended each patient to receive boceprevir alone, in combination with pegIFN α -2b and pegIFN α -2b alone with washout-periods in between in a randomized crossover sequence. Mean maximum reductions in HCV RNA load were 2.45 and 2.88 log₁₀ for boceprevir 200 mg and 400 mg plus pegIFN α 2b, 1.08 and 1.61 log₁₀ for boceprevir monotherapy and 1.08 and 1.26 log₁₀ for pegIFN α 2b monotherapy. Boceprevir was well-tolerated alone and in combination with pegIFN α 2b. Viral breakthrough due to selection of preexisting resistant mutants was observed in some patients, in particular during boceprevir monotherapy⁴⁹.

Phase II studies

Boceprevir and peg interferon with and without ribavirin

Treatment naïve phase II study (SPRINT-1)

The aim of the SPRINT 1 trial was to investigate safety, tolerability and antiviral efficacy of boceprevir (800 mg three times a day) in combination with pegIFN α 2b and ribavirin in treatment-naïve HCV genotype 1 patients¹⁶. Treatment with boceprevir in combination with pegIFN α 2b and ribavirin was either performed continuously for 28 or 48 weeks or for 24 or 44 weeks after a previous 4-week lead-in phase of pegIFN α 2b and ribavirin alone. The lead-in design was chosen to determine a potential benefit of pretreatment 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 4 weeks of pretreatment lead in with pegIFN α 2 and ribavirin (Figure 5). SVR rates after 48 weeks of triple treatment were 67% and 75% after 44 weeks with an additional 4 weeks of pretreatment were 67% and 75% after 44 weeks triple therapy with boceprevir, pegIFN and ribavirin 38% of patients achieved an RVR. The most common side-effects related to boceprevir were anemia, nausea, vomiting and dysgeusia. In

general, SPRINT-1 has proven a higher antiviral efficacy of combination therapy with boceprevir in comparison to the standard of care with slightly better results after a 4 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, lead to an RVR rate of approximately 70%.

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 to standard treatment (14% versus 2%)⁵⁰.

Design of phase III studies

A phase III clinical trial (SPRINT-2) evaluating boceprevir in treatment-naïve patients was initiated recently and has enrolled more than 1000 patients. Equivalent to the SPRINT-1 study design, patients receive 800 mg boceprevir three times daily in combination with pegIFN- α 2b and weight based ribavirin for 28 or 48 weeks. RESPOND-2 evaluates boceprevir in combination with pegIFN- α 2b and ribavirin at the same doses but for 36 and 48 weeks in relapsers and non-responders. In all investigational arms a lead-in strategy with pegIFN- α 2b and ribavirin is followed.

Viral resistance to boceprevir

In the replicon system, mutations at three positions conferring boceprevir resistance were discovered (Table 3). T54A, A156S and V170A confer low level resistance to boceprevir whereas A156T, which also confers telaprevir and ciluprevir resistance, exhibited greater levels of resistance²⁶. In patients with chronic hepatitis C three additional mutations were detected during boceprevir monotherapy (V36G/M/A, V55A, R155K)⁴⁷. In a number of these patients one

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year and in single patients even 4 years after the end of boceprevir treatment still resistant variants were detected in the HCV quasispecies by clonal sequence analysis⁵¹. However, an additional study revealed that the antiviral activity of boceprevir was not impaired in patients who were treated with boceprevir with and without pegIFN-alfa before⁴⁹.

Other NS3 protease inhibitors

Other NS3 protease inhibitors are currently in phase 1-2 development (R7227/ITMN191, MK7009, BI201335, TMC435350, SCH900518, BMS-650032, PHX1766, ACH-1625)^{13, 52, 53}. In general, they exhibit a high antiviral activity in HCV genotype 1 patients, comparable to telaprevir and boceprevir (Figure 6). Triple therapy studies for a number of compounds have been initiated and confirm that resistance development is significantly reduced by combination with pegylated interferon and ribavirin. Whereas linear tetrapeptide and macrocyclic inhibitors do not differ in general with respect to 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 which are currently in advanced clinical development¹³.

NS4A inhibitors

ACH-806

NS4A is a crucial cofactor of NS3, mandatory for proper folding of the protease and capable to enhance the enzymatic activity of NS3 manifold. ACH-806 targets NS4A and therefore inhibits the NS3/4A protease by a different mechanism than peptidomimetic NS3 inhibitors. ACH-806 binds to newly synthesized NS4A molecules, which leads to the blockade of their assembly with NS3 proteins. A phase lb trial in HCV genotype 1-infected patients demonstrated that ACH-806 has a significant inhibitory impact on HCV replication⁵⁴. Although the development of ACH-806

was halted due to reversible serum creatinine elevations, the concept of NS4A inhibition was proven. Importantly, no cross-resistance between ACH-806 and peptidomimetic NS3/4A protease inhibitors was observed in vitro^{55, 56}. Novel NS4A inhibitors (e.g. ACH-1095) are currently under preclinical development.

Compounds targeting HCV replication

NS5B polymerase inhibitors

NS5B RNA polymerase inhibitors can be divided into two distinct categories. Nucleoside analog inhibitors (NIs) like valopicitabine (NM283), R7128, R1626, PSI-7851 or 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 NS5B^{29, 57-67}. Because the active centre of NS5B is a highly conserved region of the HCV genome, NIs are potentially effective against 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 resistances to NIs.

In contrast to NIs, 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 formed⁶⁸. To inhibit NS5B allostericaly, a high chemical affinity of the compound to the enzyme is required. 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)-, thiophene-(thumb 2)-, benzothiadiazine-(palm 1)- and benzofuran-(palm 2)-binding site^{68, 69}. Theoretically, NNIs targeting different binding sites can be used in combination or in sequence to manage the development of resistance. Because NNIs bind distantly to the active centre of NS5B, their application results more frequently in resistance development than during treatment with NIs. In addition, mutations at the NNI-binding sites do not necessarily lead to impaired function of the enzyme.

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Nucleoside analogs

Valopicitabine (NM283, 2'-C-methylcytidine/NM107) was the first nucleoside inhibitor investigated in patients with chronic hepatitis C. Antiviral activity of valopicitabine was low⁷⁰. 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 1 study showed a high antiviral activity at high doses of R1626 in patients infected with HCV genotype 1⁶³⁻⁶⁵. No viral breakthrough with selection of resistant variants was reported from monotherapy or combination studies with pegylated interferon ± ribavirin. However, severe lymphopenia and infectious disease adverse events led to the stop of R1626 development.

R7128 is another nucleoside polymerase inhibitor with potent antiviral activity during monotherapy in HCV genotype 1 patients. Currently, R7128 is investigated in phase 2 clinical trials in HCV genotype 1, 2 and 3 infected patients in combination with pegylated interferon and ribavirin⁵⁹. Both during monotherapy and combination therapy with pegylated interferon and ribavirin no resistance development against R7128 was observed.

Other nucleoside analog inhibitors of the NS5B polymerase (PSI-7851 and IDX184) are evaluated in phase 1 clinical trials in patients with chronic hepatitis C and many compounds are under preclinical development¹³. For a summary of antiviral activities of nucleoside polymerase inhibitors see Figure 7.

Non-nucleoside analogs

NNI-site 1 inhibitors (thumb 1 / benzimidazole site)

BILB1941, BI207127 and MK-3281 are NNI-site 1 inhibitors which have been investigated in clinical phase 1 trials and exhibit low to medium antiviral activities^{13, 71, 72}. No selection of resistant variants and viral breakthrough has been observed during 5 days of treatment with BILB1941 or BI207127.

NNI-site 2 inhibitors (thumb 2 / thiophene site)

Filibuvir (PF-00868554) is a NNI-site 2 inhibitor with medium antiviral activity in a phase 1 study. In a subsequent trial viral breakthrough was observed in 5 of 26 patients during combination therapy with pegIFN α -2a and ribavirin for 4 weeks⁷².

Other NNI-site 2 inhibitors which were evaluated in phase 1 trials are VCH-759, VCH-916 and VCH-222, their antiviral efficacy is shown in Figure 8 ^{13, 73}. Like during 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.

NNI-site 3 inhibitors (palm 1 / benzothiadiazine site)

ANA598 is a NNI-site 3 inhibitor which displayed antiviral activity during treatment of HCV genotype 1 infected patients. No viral breakthrough was observed during a short term monotherapy trial⁷⁴.

NNI-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^{75, 76}. GS-9190 displays a low antiviral activity in a clinical study and variants conferring resistance were identified in the beta-hairpin of the polymerase.

An overview of the antiviral activities of non-nucleoside polymerase inhibitors in monotherapy studies is shown in Figure 8.

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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 concentrations⁷⁷. 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 results of multiple dose and combination therapy studies have to be awaited.

NS4B inhibitors

NS4B is a hydrophobic protein mandatory for the formation of the membranous web of the HCV replication complex. Moreover, NS4B displays RNA-binding properties which may be crucial in HCV RNA procession and replication. In vitro, inhibition of NS4B by small molecular compounds has been shown to compromise HCV replication significantly⁷⁸.

Combination therapies of specific antivirals

It is a fundamental question whether SVR can be achieved by combination therapies of different STAT-C compounds without pegIFN-alfa and ribavirin. A first clinical trial (INFORM-1 study) evaluated the combination of a polymerase inhibitor (R7128) and a NS3 inhibitor (R7227/ITMN191). In this proof of principle study, patients were treated with both compounds for up to 2 weeks. HCV RNA concentrations decreased up to 5.2 log10 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 patients⁷⁹. Future clinical trials need to address whether a 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 conducted (R7128+R7227, VX-950+VCH222, BMS790052+BMS650032, BI201335+BI207127).

Conclusions

Numerous directly acting antiviral agents are currently under clinical phase I-III evaluation. 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 pegylated interferonalfa and ribavirin substantially improves the chance to achieve a SVR in treatment-naive HCV genotype 1 patients as well as 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.

Results of the milestone studies PROVE 1 and 2 indicate that 12 weeks of telaprevir-based triple therapy is 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 to 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 to the standard of care. The RVR during triple therapy is an important predictor for treatment success and can be applied for defining individualized treatment durations. Important side effects of telaprevir are, as observed during treatment with other protease inhibitors as well, anemia, rash and gastrointestinal disorders. The SPRINT-1 trial demonstrated that SVR rates in treatment-naïve HCV genotype 1 patients can be enhanced by the addition of boceprevir to standard treatment as well. However, the lower antiviral efficacy of boceprevir compared to telaprevir may require longer durations of boceprevir application.

PROVE 3 has shown that telaprevir is also highly effective in the treatment of prior nonresponders or relapsers infected with HCV genotype 1. In contrast, addition of boceprevir to standard treatment only revealed a minor impact on SVR rates in non-responders, but further trials are awaited. In addition to telaprevir and boceprevir, many NS3/4A inhibitors with promising antiviral activities are currently investigated in phase I and II trials.

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Compared to NS3/4A protease inhibitors, most HCV polymerase inhibitors display a lower antiviral activity during monotherapy. SVR data of triple therapies containing NS5B inhibitors need to be 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 other genotypes than HCV genotype 1. In addition, NS5B inhibitors at least of the nucleoside analog family display a high genetic barrier to resistance.

Though it can be vastly reduced by addition of pegylated interferon-alfa and ribavirin, resistance development to directly acting antiviral agents has to be kept in mind. R155 is the overlapping mutation conferring resistance to all clinically yet evaluated protease inhibitors. Although resistance against polymerase inhibitors needs to be better characterized, it is evident that their resistance profiles differ from those of protease inhibitors. Combination of different classes of STAT-C agents may therefore help to overcome limitations of resistance development. The impact of recently discovered polymorphisms near the IL28B gene on resistance development and SVR rates during STAT-C regimens needs to be characterized in future studies⁸⁰⁻⁸².

A pivotal trial evidenced an additive antiviral efficacy of the polymerase inhibitor R7128 in combination with the protease inhibitor ITMN-191 in an interferon- and ribavirin-free regimen. Whether SVR can be achieved with such interferon-free regimens needs to be addressed in future trials.

In conclusion, STAT-C compounds in addition to pegylated interferon-alfa and ribavirin are capable to improve SVR rates at least in HCV genotype 1 patients and will therefore be included in future treatment recommendations and guidelines.

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Tables

Drug name	Company	Target / Active drug	Study phase		
NS3/4A protease inhibitors		-			
Ciluprevir (BILN 2061)	Boehringer Ingelheim	Active site / macrocyclic	Stopped		
Telaprevir (VX-950)	Vertex	Active site / linear	Phase 3		
Boceprevir (SCH503034)	Schering-Plough	Active site / linear	Phase 3		
TMC435350	Tibotec / Medavir	Active site / macrocyclic	Phase 2		
R7227 / ITMN-191	InterMune / Roche	Active site / macrocyclic	Phase 2		
MK-7009	Merck	Active site / macrocyclic	Phase 2		
BI201335	Boehringer Ingelheim	Active site / macrocyclic?	Phase 2		
Narlaprevir (SCH900518)	Schering-Plough	Active site / linear	On hold		
BMS-650032	Bristol-Myers Squibb	Active site	Phase 1		
PHX1766	Phenomix	Active site	Phase 1		
ACH-1625	Achillion	Active site / macrocyclic?	Phase 1		
Nucleoside analogue NS5B	polymerase inhibitors				
Valopicitabine (NM283)	Idenix/ Novartis	Active site / NM107	Stopped		
R7128	Roche / Pharmasset	Active site / PSI-6130	Phase 2		
R1626	Roche	Active site / R1479	Stopped		
PSI-7851	Pharmasset	Active site	Phase 1		
IDX184	Idenix	Active site	Phase 1		
Non-nucleoside NS5B polyn	nerase inhibitors (NNI)				
BILB 1941	Boehringer Ingelheim	NNI site 1 / thumb 1	Stopped		
BI207127	Boehringer Ingelheim	NNI site 1 / thumb 1	Phase 2		
MK-3281	Merck	NNI site 1 / thumb 1	Stopped		
Filibuvir (PF-00868554)	Pfizer	NNI site 2 / thumb 2	Phase 2		
VCH759	ViroChem Pharma	NNI site 2 / thumb 2	Phase 1		
VCH916	ViroChem Pharma	NNI site 2 / thumb 2	Phase 1		
VCH222	ViroChem Pharma	NNI site 2 / thumb 2	Phase 1		
ANA598	Anadys	NNI site 3 / palm 1	Phase 1		
HCV-796	ViroPharma / Wyeth	NNI site 4 / palm 2	Stopped		
GS-9190	Gilead	NNI site 4 / palm 2	Phase 2		
ABT-333	Abbott	NNI site 4 / palm 2	Phase 1		
NS5A inhibitor					
BMS-790052	Bristol-Myers Squibb	NS5A domain 1 inhibitor	Phase 2		
Indirect inhibitors / unknow	n mechanism of action				
Debio 025	Debiopharm / Novartis	Cyclophilin inhibitor	Phase 1		
NIM811	Novartis	Cyclophilin inhibitor	Phase 1		
SCY-635	Scynexis	Cyclophilin inhibitor	Phase 1		
Nitazoxanide		PKR induction ?	Phase 2		
Celgosivir	Migenix	Alpha-glucosidase inhibitor Phase			

Table 1. STAT-C compounds in the pipeline.

Table 2: Mutations conferring resistance to telaprevir

Telaprevir-Resistant	Level of Resistance
Variant	(Fold change in HCV replicon IC50)
T54A	low (6)
V36M	low (7)
R155K	low (7)
A156S	low (10)
R155T/I	medium (20)
V36M+R155K	high (>60)
A156V/T	high (>62)

Table 3. Resistance mutations to HCV NS3 protease inhibitors (modified from¹³).

	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)	- <u> </u>				- <u> </u>	- <u></u> -	- <u></u> -		
BILN-2061									
(macrocyclic)									
R7227/ITMN191						*	*		
(macrocyclic)									
MK-7009									
(macrocyclic)									
TMC435350									
(macrocyclic)							A		
BI-201335					· · · · · · · · · · · · · · · · · · ·				
(macrocyclic?)									

* mutations associated with resistance in vitro but not described in patients

Figures legends

Figure 1. The HCV replication complex. After clathrin-mediated endocytosis, fusion of HCV with cellular membranes, and uncoating the viral nucleocapsid, the single-stranded positive-sense RNA genome of the virus of approximately 9600 nucleotides is released into the cytoplasm to serve as a messenger RNA for the HCV polyprotein precursor. The HCV genome contains a single large open reading frame encoding for a polyprotein of about 3100 amino acids. The translated section of the HCV genome is flanked by the strongly conserved HCV 3' and 5' untranslated regions (UTR). The 5' UTR is comprised of four highly structured domains forming the internal ribosome entry site (IRES), which is a virus-specific structure to initiate HCV mRNA translation. From the initially translated polyprotein, the structural HCV protein core (C) and envelope 1 and 2 (E1, E2); p7; and the six non-structural HCV proteins NS2, NS3, NS4A, NS4B, NS5A and NS5B, are processed by both viral and host proteases.

The core protein forms the viral nucleocapsid carrying E1 and E2, which are receptors for viral attachment and host cell entry. The tetraspanin protein CD81, claudin-1, occludine, scavenger receptor class B type 1 (SR-B1), the low-density lipoprotein (LDL) receptor, glycosaminoglycans and the dendritic cell- / lymph node-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN/L-SIGN) have been identified as putative ligands for E1 and E2⁸³⁻⁸⁶. The non-structural proteins are mainly enzymes essential for the HCV life cycle. P7 is a small hydrophobic protein that oligomerises into a circular hexamer, most likely serving as an ion channel through the viral lipid membrane^{7, 87-91}.

NS2 and NS3 are viral proteases required for the procession of the HCV polyprotein. NS2 is a metalloproteinase that cleaves itself from the NS2/NS3 protein, leading to its own loss of function and to the release of the NS3 protein^{7, 90, 91}. NS3 provides a serine protease activity and a helicase/NTPase activity. The serine protease domain comprises two β -barrels and four α -helices. The serine protease catalytic triad – histidine 57, asparagine 81 and serine 139 – is located in a small groove between the two β -barrels. NS3 forms a tight, non-covalent complex

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with its obligatory cofactor and enhancer NS4A, which is essential for proper protein folding. The NS3/4A protease cleaves the junctions between NS3/NS4A, NS4A/NS4B, NS4B/NS5A and NS5A/NS5B. Besides its essential role in protein processing, NS3 is integrated into the HCV RNA replication complex, supporting the unwinding of viral RNA by its helicase activity.

NS4B and NS5B are involved in the organization of the HCV replication complex by interactions with lipid membranes, which lead to the formation of the so called membranous web^{11, 12, 69, 92}. The membranous web comprises of rearranged intracellular lipid membranes derived from the endoplasmic reticulum. It provides the basis for the highly structured association of viral proteins and RNA, and of cellular proteins and cofactors within the replication complex. In addition, NS4B and NS5B are involved in transport of viral RNA within the replication complex^{11, 12, 69, 92}.

NS5B is an RNA-dependent RNA-polymerase which catalyzes the synthesis of a complementary negative-strand RNA by using the positive-strand RNA genome as a template^{11, 12, 69}. From this newly synthesized negative-strand RNA, numerous RNA strands of positive polarity are produced by NS5B activity which serve as templates for further replication and polyprotein translation. Because of its poor fidelity leading to a high rate of errors in its RNA sequencing, numerous different isolates are generated during HCV replication in a given patient, termed HCV quasispecies. It is thought that due to the lack of proof-reading of the NS5B polymerase together with the high replication rate of HCV every possible mutation will be generated each day. Thus, NS5B is one key factor in the development of viral resistance during STAT-C therapies.

Figure 2. Results of PROVE 1 (USA). Combination therapy of telaprevir (TVR) and pegIFN-α2a + ribavirin in treatment-naive genotype 1 patients.

Figure 3. Results of PROVE 2 (Europe). Combination therapy of telaprevir (TVR) and pegIFN- $\alpha 2a +/-ribavirin$ in treatment-naive genotype 1 patients.

Figure 4. Results of PROVE 3. Combination therapy of telaprevir (TVR) and pegIFN-α2a +/ribavirin in HCV genotype 1 patients with prior non-response or relapse to standard treatment.

Figure 5. Results of SPRINT-1. Combination therapy of boceprevir and pegIFN α 2b + ribavirin (RBV) in treatment-naive genotype 1 patients.

Figure 6. Antiviral activity of NS3/4A protease inhibitors during monotherapy for 3-14 days (modified from¹³).

Figure 7. Antiviral activity of nucleoside analogue NS5B polymerase inhibitors during monotherapy for 3-14 days (modified from¹³).

Figure 8. Antiviral activity of non-nucleoside analogue NS5B polymerase inhibitors during monotherapy for 3-14 days (modified from¹³).

Figures.

Figure 1.













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2

TVR+pegIFN+RBV TVR+pegIFN+RBV

n=79

SVR Rel

12 weeks

 \rightarrow 12 weeks

pegIFN+RBV

67

6

n=79

12 weeks

→ 36 weeks

pegIFN+RBV

78x37mm (300 x ...

SVR Rel

41

n=75

SVR Rel

Standard 48 weeks

pegIFN+RBV

23

100

80

60

40

20

0

35

n=17

SVR Rel

12 weeks

TVR+pegIFN+RBV

33

SVR / Relapse [%]



57 58



RVR SVR Rel

Rel NR

Rel/NR

Standard

PEG2a+Riba

0





74x42mm (300 x 300 DPI)





64x37mm (300 x 300 DPI)



59x45mm (300 x 300 DPI)





79x41mm (300 x 300 DPI)