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Concise Review

Current and future treatment options for HCV

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Abstract

Aim of antiviral therapy of patients with chronic hepatitis C is the sustained elimination of the hepatitis C virus (HCV). The standard of care (SOC) is peginterferon alfa-2a/-2b with ribavirin for 48 weeks or 24 weeks in patients infected with HCV genotype 1 or 2/3, respectively. Overall, approximately half of the patients can be cured by SOC. Based on baseline viral load and the speed of virologic response during treatment, individualization of treatment duration is possible. However, this approach is not sufficient to substantially improve the sustained virologic response (SVR) rates. This goal can be achieved with new HCV specific inhibitors against the NS3/4A polymerase and the NS5B polymerase. Recent trials reported SVR rates in the order of 67-69% and 67-75% for the combination of SOC with the protease inhibitors telaprevir and boceprevir, respectively, in patients with HCV genotype 1 infection. Several new HCV specific inhibitors such as protease inhibitors, nucleoside and non-nucleoside polymerase inhibitors as well as non HCV specific compounds with anti-HCV activity such as cyclophilin inhibitors, silibinin, and nitazoxanide are currently in clinical evaluation. The review describes recent developments and discusses limitations posed by resistance development and drug toxicity.

Key words: Hepatitis C, STAT-C, peginterferon, ribavirin, protease inhibitors, polymerase inhibitors.

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Current treatment options

The standard of care (SOC) for patients with chronic hepatitis C is pegylated interferon alfa in combination with ribavirin. Two pegylated interferons, alfa-2a (40 kD) and -2b (12 kD), are approved. The aim of antiviral therapy is the sustained elimination of the hepatitis C virus.

The HCV genotype is the most important predictive factor for treatment response of patients with chronic hepatitis C and has become an important decision criterion for treatment duration and ribavirin dosage.[1] The SOC treatment duration is 48 weeks and 24 weeks for patients infected with HCV genotype 1 and 2/3, respectively. The SOC ribavirin dosage is 1,000-1,200 mg and 800 mg for patients infected with HCV genotype 1 and 2/3, respectively.

The initial virologic response of patients with chronic hepatitis C shows large individual variation and can be classified into rapid virologic response (HCV undetectable after 4 weeks of therapy), complete early virologic response (HCV undetectable after 12 weeks of therapy), partial early virologic response ($\geq 2 \log_{10}$ decline of HCV RNA after 12 weeks of treatment but still HCV RNA positive), slow virologic response ($\geq 2 \log_{10}$ decline of HCV RNA after 12 weeks of treatment but still HCV RNA positive followed by undetectable HCV RNA by week 24) and non response (detectable HCV RNA 24 weeks after start of antiviral treatment). The faster a patient develops undetectable HCV RNA, the higher is his/her probability to achieve a sustained virologic response. Based on the rapid virologic response criterion, individualization of treatment duration is possible without reduction of the overall sustained virologic response rate. Patients infected with HCV genotype 1 who start with a low baseline viral load (< 600.000 IU/mL) and who achieve a rapid virologic response were shown to have favorable sustained virologic response rates after 24 weeks of antiviral treatment indicating that a shorter treatment duration can be considered in this group of patients.[2]

The possibility of shorter treatment duration was also investigated in patients with HCV genotype 2/3 infection. Smaller trials showed that a shorter treatment duration of 12-14 weeks is equally effective as the standard treatment duration in patients infected with HCV genotype 2/3 who achieve a rapid virologic response after 4 weeks of therapy.[3,4] However, the large ACCELERATE

trial comparing 16 versus 24 weeks of treatment in patients with HCV genotype 2/3 infection showed that a shorter treatment duration of 16 weeks results in lower sustained virologic response rates compared with the standard treatment duration.[5] In the ACCELERATE trial, a shorter course of therapy over 16 weeks has been shown to be as effective as a 24 week course in those patients with genotype 2/3 infection who have a baseline viral load ≤ 400.000 IU/mL and rapid virologic response.[5] In patients with genotype (2 and) 3 infection without a rapid virologic response (< 50 IU/mL) at week 4, a longer than 24 weeks treatment duration may be necessary to optimize sustained virologic response rates.[6]

Patients infected with HCV genotype 1 and slow virologic response have a high risk to relapse after 48 weeks of treatment with peginterferon and ribavirin. An approach to reduce the relapse rates is treatment extension to 72 weeks. In a German multicenter study, patients infected with HCV genotype 1 were randomized for treatment with peginterferon alfa-2a/ribavirin 800 mg for 48 weeks and 72 weeks, respectively. In this study, the overall sustained virologic response rate was not superior in patients infected with genotype 1 treated for 72 weeks with peginterferon alfa-2a/ribavirin 800 mg compared with patients treated for 48 weeks.[7] However, the subgroup analysis of patients infected with HCV genotype 1 and a slow virologic response showed a significantly lower relapse rate in patients treated for 72 weeks compared with patients treated for 48 weeks indicating that a subgroup of patients may benefit from extended treatment duration. The SUCCESS study was the first prospective study to compare 48 weeks of treatment with 72 weeks of treatment in slow responders. In this trial slow responders were randomized at week 36 of treatment to receive peginterferon alfa-2b (1.5 μg/kg/week) plus weight-based dosed ribavirin (800-1400 mg/day) for a total of 48 or 72 weeks. Of the 1,427 patients included in the trial 157 (11%) were slow responders. In the intent-to-treat analysis, sustained virologic rates were not different between the two groups (43% and 48% in the 48 and the 72 week treatment arms, respectively). Patients, however, who were 80/80/80 compliant had sustained virologic response rates of 44% and 57% in the 48 weeks and 72 weeks treatment arms, respectively. Overall, these studies indicate that extended treatment duration can be considered in slow responders, however, adherence to treatment is highly crucial important.[8]

The treatment options for patients with chronic hepatitis C and non-response to antiviral treatment are sparse. It was hypothesized that long term maintenance therapy with interferon may reduce progression to liver cirrhosis and its complications. In the HALT-C trial 1,050 patients with prior non response to peginterferon alfa/ribavirin and advanced fibrosis/cirrhosis were randomized for treatment with low dose peginterferon alfa-2a 90 μg/week or no treatment for 3.5 years.[9] The primary end point was progression of liver disease defined as liver related death, hepatocellular carcinoma, hepatic decompensation or increase of the ISHAK fibrosis score of 2 or more points. The level of aminotransferases, HCV-RNA and necroinflammatory scores decreased significantly, however, there was no difference between the groups in the rate of any primary outcome. Similar results were observed in the CoPilot[10] and the EPIC3[11] trials investigating long term peginterferon alfa-2b 0.5 mg/kg *vs* colchicine for 4 years or peginterferon alfa-2b 0.5 mg/kg *vs* no treatment in patients for 5 years with prior non-response to peginterferon/ribavirin, respectively. In agreement with the HALT-C trial, peginterferon alfa-2b maintenance therapy was not superior to colchicine or no treatment, respectively, in preventing the occurrence of clinical events. Secondary analyses of the CoPilot and the EPIC3 trial showed a benefit of peginterferon-maintenance compared with the control arms in patients with cirrhosis and portal hypertension. Overall, these trials indicate that long term maintenance therapy with peginterferon alfa does not reduce disease progression in patients with advanced fibrosis/cirrhosis and prior non response to peginterferon/ribavirin treatment. Whether, long-term maintenance therapy is really effective in the subgroup of patients with portal hypertension, has to be confirmed in prospective trials.

Future treatment options

Advances have been made in the development of new interferons, specifically targeted therapy against hepatitis C (STAT-C) and host cell targets inhibiting HCV replication. This review focuses on recent clinical trials in that field. Other potential treatment approaches like toll like receptor agonists and therapeutic vaccines are beyond the scope of the review.

New interferons

Albinterferon is a genetic fusion polypeptide of albumin and interferon alfa-2b with a longer half life than pegylated interferons. The phase 2 study comparing different doses of albinterferon alfa-2b and ribavirin with peginterferon alfa-2a and ribavirin indicated similar sustained virologic response rates with a better tolerability of albinterferon alfa-2b based treatment. Based on the encouraging findings from the phase 2 study, the efficacy and safety of albinterferon alfa-2b administered every two weeks in combination with ribavirin for 48 weeks and 24 weeks in patients infected with HCV genotype 1 and 2/3, respectively, was investigated in two phase 3, randomized, active controlled, multi-center studies.[12,13] Both studies ACHIEVE-1 and ACHIEVE-2 were designed to demonstrate non-inferiority of the albinterferon alfa-2b regimes compared with peginterferon alfa-2a. Both studies achieved the primary objective. In the in-

tention-to-treat population, the sustained virologic response rates in the peginterferon alfa-2a, albinterferon alfa-2b 900 μg and albinterferon alfa-2b 1,200 μg groups were 51.0%, 48.2%, and 47.3% in patients infected with HCV genotype 1 and 84.8%, 79.8%, and 80.0% in patients infected with HCV genotype 2,3 respectively. The overall incidence of adverse events, serious or severe adverse events in the phase 3 studies was similar between the two treatments indicating that albinterferon alfa-2b is not better tolerated than peginterferon alfa-2a.

Locteron is a controlled-release interferon alfa-2b which is injected every 2 weeks. In a short term study controlled release interferon alfa-2b showed less flu like symptoms than peginterferon alfa-2b injected every week indicating that the controlled-release formulation may have a better tolerability. Larger trials powered to examine adverse event profiles and antiviral activity are being initiated.[14]

Peginterferon-λ is a pegylated type III interferon that binds to a unique receptor with more limited distribution than the type I interferon receptor. In a phase 1 healthy volunteer study, peginterferon-λ was pharmacologically active without flu-like symptoms or hematologic side-effects. In a phase 1b study the mean decline of HCV-RNA in patients with relapsed HCV genotype 1 infection was 1.9-3.6 $\log_{10}$ IU/mL after 4 weeks of re-treatment with peginterferon-λ. Peginterferon-λ is currently investigated in combination with ribavirin.[15]

Overall, the new interferons may improve convenience and tolerability of interferon-based therapy. However, the current results on viral efficacy indicate that response rates will not be improved by the new interferons.

Specifically targeted therapy against hepatitis C virus (STAT-C)

The structure identification of the NS3/4A protease and the HCV NS5B polymerase and the development of a (sub) genomic replicon system have enabled the development and testing of HCV specific compounds. Further attractive targets within the HCV genome for antiviral therapy are the envelope proteins which are involved in HCV entry and NS5A which is involved in replication and in interferon alfa resistance. The clinical development of NS3/4A protease inhibitors is currently most advanced.

Protease inhibitors

The NS3/4A protease has key functions in the hepatitis C virus replication cycle. NS3/NS4A cleaves at four downstream sites in the polyprotein to generate the N-termini of the NS4A, NS4B, NS5A, and NS5B proteins. The NS3/4A serine protease has also been shown to cleave and inactivate the host proteins Trif and Cardif. Both proteins have important roles in the interferon response mediated by TLR3 and RIG-I, respectively.[16,17] Furthermore, it has been shown that NS3 is not only a protease but also an integral part of the viral RNA replication complex, functions as a RNA helicase and a nucleotide triphosphatase (NTPase). Due to the multiple functions, NS3 is an attractive target for anti-HCV therapy. Several protease inhibitors were investigated in clinical trials. Monotherapy with protease inhibitors ciluprevir, telaprevir and boceprevir was shown to be effective in lowering the viral load. The development of ciluprevir was stopped due to cardiotoxicity in animal studies. Clinical evaluation of telaprevir and boceprevir is most advanced. Both protease inhibitors showed a rapid occurrence of drug resistant HCV strains within 2 weeks of therapy indicating that protease monotherapy is not sufficient for treatment of patients with chronic hepatitis C. Because peginterferon alfa/ribavirin has a completely different mode of action and resistance profile than protease inhibitors the current protease inhibitors are investigated in combination with peginterferon with and without ribavirin.

Telaprevir

The peptidomimetic inhibitor of the NS3/4A serine protease telaprevir showed a 3 $\log_{10}$ IU/mL decline of HCV RNA during the first 2 days of monotherapy in patients infected with HCV genotype 1 and previous non response to interferon based antiviral treatment. However, during 14 days of monotherapy, a continuous decline of HCV RNA was noted in only 7 of 28 patients (25%). Using a highly sensitive sequencing method several mutations associated with resistance to telaprevir were identified. Mutations associated with resistance occurred in the NS3 catalytic domain either as single mutation (V36A/M, T54A, R155K/T, A156S/T/V) or as double mutation (at positions 36+155 or 36+156). Low level resistance mutations (V36A/M, T54A, R155K/T, and A156S) and high level resistance mutations (A156V/T, 36+155, 36+156) can be distinguished.

Combination therapy of telaprevir with peginterferon alfa-2a and ribavirin was effective in preventing the rapid occurrence of resistance. The combination therapy of peginterferon alfa-2a/ribavirin/telaprevir was investigated in the PROVE1 and 2 studies.[18,19] Both studies are completed and telaprevir is one of the first STAT-C compound for which sustained virologic response rates have been reported for the combination therapy with peginterferon alfa-2a and ribavirin. In both trials triple therapy was given for 12 weeks. The sustained virologic response rates in PROVE1 and PROVE2 were 67% and 69% in patients treated with peginterferon alfa-2a/ribavirin/telaprevir for 12 weeks followed by peginterferon/ribavirin for 36 or 12 weeks, respectively. The sustained virologic response rates in these telaprevir arms were significantly higher compared with the sustained virologic

response rates in the standard of care control arms (41% and 46% in PROVE1 and PROVE2 respectively). Overall, the PROVE-studies confirm that protease inhibitors are able to increase sustained virologic response rates in patients with HCV genotype 1 infection. Furthermore, the PROVE2 study indicates that by addition of telaprevir to SOC higher sustained virologic response rates can be achieved with shorter treatment duration.

The high antiviral efficacy of telaprevir in combination with interferon alfa raises the question whether ribavirin is still necessary in the era of protease inhibitors and if double combination with peginterferon and a protease inhibitor is sufficient for a sustained virologic response. In PROVE2 the sustained virologic response rate in patients treated with telaprevir/peginterferon alfa-2a without ribavirin for 12 weeks was lower than in patients treated with telaprevir/peginterferon alfa-2a plus ribavirin for 12 weeks (36% vs. 60%). The lower rate of sustained virologic response in the group without ribavirin was due to a higher relapse rate compared to the groups with ribavirin (48% *vs* 14-29%). The results of the PROVE2-trial provide evidence that ribavirin has additive antiviral activity to telaprevir and peginterferon alfa-2a and that triple therapy is required for optimal sustained virologic response rates.

Telaprevir in combination with peginterferon alfa-2a and ribavirin was also investigated in patients with prior non response to standard of care. The PROVE3 trial was a randomized, placebo-controlled phase 2 study assessing safety and efficacy of telaprevir plus peginterferon alfa-2a ± ribavirin in HCV genotype 1 patients who previously failed peginterferon/ribavirin treatment.[20] The overall sustained virologic response rates were significantly higher in the telaprevir arms (peginterferon alfa-2a/ribavirin/telaprevir for 12 or 24 weeks followed by peginterferon alfa-2a/ribavirin for 12 and 24 weeks, respectively) compared with the control arm (51%, 52% *vs* 14%). Remarkably, the subgroup analysis of previous peginterferon/ribavirin non responders showed superior sustained virologic response rates in the triple therapy arms compared with the SOC control arm or the peginterferon/telaprevir arm without ribavirin (38-39% *vs* 9-10%). Overall, the study provides evidence that protease inhibitors in combination with standard of care will be a treatment option for patients who failed previous antiviral therapy.

Boceprevir

Boceprevir, another NS3/4A serine protease inhibitor, binds reversibly to the NS3 protease active site and has potent activity in the replicon system alone[21] and in combination with interferon alfa-2b.[21] As for telaprevir several mutations associated with resistance were identified during boceprevir monotherapy (V36, T54, R155, A156, V170, V55A) (22). Boceprevir shows an overlapping resistance profile with telaprevir indicating that a combination therapy of both protease inhibitors is not promising.

Recently, the final results of the HCV SPRINT-1 study assessing safety and efficacy of boceprevir in combination with peginterferon alfa-2b (1.5 μg/kg/week) and ribavirin in treatment naïve patients with chronic hepatitis C genotype 1 infection were presented.[23] The triple combination arms with a total treatment duration of 48 weeks with or without a 4 weeks peginterferon-alfa2b/ribavirin lead-in were associated with significantly higher sustained virologic response rates than the low dose ribavirin arm and the standard of care control arm (75% and 67% *vs* 36% and 38%, respectively). The results from the PROVE and the SPRINT trials confirm the concept that specific protease inhibitors are able to improve the cure rates of patients with chronic hepatitis C. Furthermore, both trials indicate that ribavirin is still highly necessary for achieving a sustained virologic response.

New protease inhibitors

ITNM-191, SCH 900518, TMC435, BI201335 and MK-7009 are novel NS3/4A protease inhibitors currently in clinical trials. Sustained virologic response rates are not available so far. ITMN-191 is a potent HCV NS3/4A protease inhibitor that achieves high liver concentrations following oral administration.[24] ITNM-191 in combination with peginterferon alfa-2a/ribavirin showed a stronger decline of HCV RNA compared with peginterferon alfa-2a/ribavirin standard of care after two weeks of treatment (4.7-5.7 $\log_{10}$ IU/mL *vs* 2.0 $\log_{10}$ IU/mL). After 2 weeks, 13-57% of patients in the triple therapy arm while no patient in the standard of care arm showed undetectable HCV RNA. SCH 900518 with and without ritonavir boostering showed robust reductions in HCV RNA levels in both treatment-experienced and naïve HCV genotype 1-infected patients (4.01 $\log_{10}$ IU/mL and 4.5 $\log_{10}$ IU/mL *vs* 0.09-0.19 $\log_{10}$ IU/mL after 8 days in patients treated with SCH 900518 400 mg twice/day plus peginterferon alfa-2a/ribavirin plus ritonavir 100 mg/d and SCH 900518 800 mg thrice/day plus peginterferon alfa-2a/ribavirin, respectively, vs. patients receiving peginterferon alfa-2a/ribavirin alone).[25] TMC435 administered for 4 weeks in combination with peginterferon-alfa2a/ribavirin was well tolerated and demonstrated potent antiviral activity in HCV genotype 1 infected treatment-experienced patients (4.3-5.3 $\log_{10}$ IU/mL in the TMC435 arms *vs* 1.5 $\log_{10}$ IU/mL in the control arms).[26] BI 201335 was investigated as monotherapy for 14 days and in combination with peginterferon alfa-2a/ribavirin for 28 days in experienced patients and showed a median HCV RNA decline of 3-4.2 $\log_{10}$ IU/mL in monotherapy and 4.8-5.3 $\log_{10}$ IU/ml in combination therapy.[27-29] MK-7009 is a noncovalent competitive inhibitor of HCV NS3/4A protease. In treatment naïve patients MK-7009 was adminis-

tered for 28 days in combination with pegylated interferon-alfa/ribavirin. The rapid virologic rate was higher in patients treated with triple therapy than in patients treated with standard of care (68.8-82.4% *vs* 5.6%).[30] All new compounds were relatively safe and well tolerated in monotherapy as well in combination with standard of care and will be further developed for HCV treatment.

Polymerase inhibitors

Two classes of NS5B polymerase inhibitors, nucleoside and non-nucleoside polymerase inhibitors, have been developed. Nucleoside analogue polymerase inhibitors are converted into triphosphates by cellular kinases and incorporated into the elongating RNA strand as chain terminators. Generally, they show similar efficacy against all HCV genotypes. The mechanisms of action of non-nucleoside polymerase inhibitors are different from that of nucleoside polymerase inhibitors. Therefore, cross resistance between these two classes is unlikely to occur.

Several structurally distinct non-nucleoside inhibitors of the HCV RNA-dependent RNA-polymerase NS5B have been reported to date, including benzimidazole, benzothiadiazine, and disubstituted phenylalanine/thiophene or dihydropyranone derivatives. They target different sites within the polymerase. Different resistance profiles due to distinct target sites can be expected for the class of non-nucleoside inhibitors. As with protease inhibitors a single mutation may already confer resistance to non-nucleoside polymerase inhibitors. In contrast to nucleoside polymerase inhibitors, a restricted spectrum of activity of non-nucleoside polymerase inhibitors against different HCV genotypes and subtypes has been described.

Nucleoside analogues

Valopicitabine was the first nucleoside analogue polymerase inhibitors tested in patients with chronic hepatitis C. Valopicitabine showed antiviral activity in monotherapy (mean HCV-RNA decline 0.15-1.21 $\log_{10}$ IU/mL after 14 days in patients infected with HCV genotype 1 and prior non response to interferon based antiviral treatment) and in combination therapy with interferon alfa (mean HCV-RNA decline 3.75-4.41 $\log_{10}$ IU/mL after 36 weeks in treatment naïve patients infected with HCV genotype 1). The development of valopicitabine was stopped due to gastrointestinal adverse events which were severe in some patients.[31] The nucleoside analogue R1479 (4'-azidocytidine) is a potent inhibitor of NS5B-dependent RNA synthesis and hepatitis C virus replication in cell culture.[32] R1626 is a prodrug of R1479.[33] R1626 was investigated in treatment naïve patients with HCV genotype 1 infection in combination with peginterferon alfa-2a and ribavirin. After 48 weeks (4 weeks R1626 plus peginterferon alfa-2a with or without ribavirin followed by 44 weeks of peginterferon alfa-2a plus ribavirin) the virologic response rates were 52-84% in the R1626 treatment arms and 65% in the control arm with peginterferon alfa-2a/ribavirin. Remarkably, end of treatment response was higher in the ribavirin arm than in the non-ribavirin arm (84% *vs* 52-66%) indicating that ribavirin has additional on treatment antiviral activity to polymerase inhibitors.[34] Despite promising results development of R1626 was stopped due to severe neutropenia.

R7128

R7128 is another nucleoside analogue NS5B polymerase inhibitor. Non responders treated with R7128 1,500 mg twice daily showed a mean viral decline of 2.7 $\log_{10}$ IU/mL after 14 days of therapy. R7128 is currently evaluated in combination with peginterferon alfa-2a and ribavirin in treatment naïve patients with chronic HCV-genotype 1 infection.[35] The week 4 rapid virologic response rates in patients treated with peginterferon alfa-2a, ribavirin plus R7128 500 mg or 1,500 mg twice daily were 30% and 85%, respectively, and 10% in patients treated with peginterferon alfa-2a and ribavirin without R7128.

R7128 also showed antiviral activity against HCV genotype 2/3 *in vitro*. The combination of R7128 with peginterferon alfa-2a and ribavirin is currently evaluated in patients infected with genotype 2 and 3 (n = 15) with previous treatment failure to interferon-based therapy. Patients are randomized for treatment with R7128 or placebo with peginterferon alfa-2a/ribavirin for 28 days, followed by peginterferon alfa-2a/ribavirin for a minimum of 20 weeks. Virologic response data are still blinded but safety laboratory assessments revealed neither grade 3-4 changes in hematocrit/hemoglobin, neutrophil counts, or platelets, nor clinically significant changes in other safety laboratory parameters, vital signs, or ECGs.[36]

The S282T mutation was reported to be associated with resistance to nucleoside polymerase inhibitors, the mutant, however, has a very low replication fitness. Population sequence analysis of the NS5B region in patients treated with R7128 revealed no evidence of the S282T resistance mutation after 2 weeks or 4 weeks of therapy confirming clinically the high genetic barrier of this nucleoside polymerase inhibitor in short-term studies.[37]

Nucleotide analogues

IDX184

IDX184 is a liver-targeted nucleotide prodrug designed to enhance formation of its active triphosphate in the liver while minimizing systemic exposure of the parent drug and its nucleoside metabolite.[38] Oral administration of IDX184 to HCV-infected chimpanzees resulted in potent antiviral activity (mean HCV-RNA decline after

4 days 1.4 to 3.8 $\log_{10}$ copies/mL). The antiviral activity was achieved with low systemic levels of the parent drug and its nucleoside metabolite. Clinical data are awaited.

Non nucleoside analogues

HCV-796 is a non-nucleoside polymerase inhibitor that has demonstrated potent antiviral activity in vitro and in patients with chronic hepatitis C. Monotherapy showed a maximum antiviral effect after 4 days of treatment with a mean HCV RNA reduction of 1.4 $\log_{10}$ IU/mL. During monotherapy HCV-796 resistant variants were rapidly selected. HCV-796 resistance was associated with the C316Y amino acid substitution. The combination of HCV-796 and peginterferon alfa-2b produced a mean viral reduction of 3.3-3.5 $\log_{10}$ IU/mL after 14 days of treatment compared to 1.6 $\log_{10}$ IU/mL with peginterferon alfa-2b alone.[39] Due to clinically significant elevations of liver enzymes, HCV-796 clinical development was discontinued in the phase 2 program.

Recently, data from several new non-nucleoside polymerase inhibitors were presented. The development of filibuvir (PF 00868554) is most advanced.[40] Filibuvir showed in monotherapy of patients with chronic HCV genotype 1 infection a dose-dependent inhibition of viral replication, with maximum reductions in HCV RNA ranging from 0.97 to 2.13 $\log_{10}$ IU/mL. During monotherapy, mutations associated with resistance at position 423 rapidly occurred indicating a low resistance barrier of filibuvir. Filibuvir is currently investigated in combination with peginterferon alfa-2a and ribavirin. In treatment naïve patients with HCV genotype 1 infection triple therapy was associated with a rapid virologic response rate of 60-75% while no patient in the placebo arm achieved a rapid virologic response. The most frequently reported adverse events were headache, fatigue, insomnia and nausea.

The non-nucleoside polymerase inhibitors VCH-916, ANA598, BI 207127 and VCH-222 were investigated only in monotherapy so far. VCH-916 showed a maximum HCV-RNA decline ranging between 0.2 and 2.5 $\log_{10}$ IU/mL within 14 days of treatment.[41] Like HCV-796 and filibuvir HCV variants conferring resistance were selected during the course of dosing with VCH-916 over a 14-day period. Sequencing revealed selection of L419S/M, M423T/V/I, I482L and V494A variants during monotherapy indicating that VCH-916 should be used in combination to maintain viral suppression and prevent emergence of resistance.[42] ANA598 showed a decline of HCV-RNA after 3 days of monotherapy ranging between 0.4 and 3.4 $\log_{10}$ IU/mL.[43] ANA598 was combined *in vitro* with interferon alfa, the HCV NS3/4 protease inhibitor telaprevir, the NS5B nucleoside polymerase inhibitor PSI-6130, and the TLR7 agonist ANA773, respectively. The in vitro combination studies demonstrated additive to synergistic antiviral effects of ANA598 in combination with other anti-HCV agents having distinct mechanisms of action and non-overlapping resistance profiles. The study indicates that combination therapy may produce a greater viral load reduction and potentially delay the emergence of drug resistance *in vivo*. Further studies are planned with ANA598 in combination with standard of care.[43] BI 207127 monotherapy showed an HCV-RNA decline after 5 days ranging between 0.6 and 3.1 $\log_{10}$ IU/mL in patients with chronic hepatitis C genotype 1 infection.[27] Similar to ANA598, no increase in HCV RNA levels was observed during short term BI 207127 monotherapy. One patient developed a severe generalized erythema with facial involvement, which resolved within 2 days after discontinuation of BI 207127 and after antihistaminic treatment. All other adverse events were rated "mild" or "moderate" and were apparently not dose-related. Further clinical development of the compound in combination therapy is planned. For VCH-222 only preliminary efficacy results on the first 4 treatment-naïve patients with chronic HCV genotype 1 infection treated for 3 days are available showing a decline of HCV-RNA ranging between 3.2 and 4.2 $\log_{10}$ IU/mL.[44]

MK-3281, ABT-072 and ABT-333 are additional non-nucleoside polymerase inhibitors in development for which no results on antiviral activity in patients with chronic hepatitis C are yet available.[45] ABT-072 and ABT-333 were shown to have oral bioavailability in rats and dogs, in vitro metabolic stability and low potential for drug interactions predicting favorable pharmacokinetics in humans.

Combination therapy

The nucleoside polymerase inhibitor R7128 and the protease inhibitor ITNM-191 showed substantial antiviral activity in patients with chronic hepatitis C. The INFORM-1 trial is the first trial to investigate the combination of a nucleoside polymerase inhibitor and a protease inhibitor in patients with chronic hepatitis C.[46] Both compounds have different resistance profiles and thus are good candidates for combination therapy. After 14 days of combination therapy (with yet lower doses for both compounds), a decline of HCV-RNA ranging between 2.9 and 5.0 to $\log_{10}$ IU/mL was observed. One patient had undetectable HCV-RNA. No viral rebound was observed. Higher doses are currently evaluated. An important question is whether combination therapy is sufficient for achieving a sustained virologic response and what treatment duration would be necessary.

HCV-Entry inhibitors

Chronic hepatitis C is characterized by a high turnover of infected cells and continuous de novo infection of target cells. Due to the vital role of de novo infection in maintenance of HCV infection, blocking of de novo

infection is a potential target for antiviral therapy. A target to block de novo infection is the HCV envelope protein E2. Recently, data for the first potential entry inhibitors were presented. A fully humanized monoclonal antibody to a linear epitope of HCV E2 glycoprotein MBL-HCV1 that neutralizes pseudoviruses from multiple HCV genotypes was developed. The antibody was shown to completely neutralize infectious HCV particles in cell culture. Based on these *in vitro* results, the ability of the monoclonal Anti-E2 antibody was investigated to prevent HCV infection in uninfected chimpanzees.[47] Three chimpanzees received a single dose of the Anti-E2 antibody intravenously before challenge with HCV 1a strain H77. No HCV RNA was detected in the serum of the 250 mg/kg dosed chimpanzees through week 20 while the 0 mg/kg and 50 mg/kg dosed chimpanzees both became infected by day 14. These findings indicate that a human monoclonal antibody directed to a conserved epitope of the HCV E2 glycoprotein has the potential to neutralize infectious, replication competent HCV and may prevent infection. Due to the encouraging results a phase 1 study in humans is planned.

JTK-652 is another HCV entry inhibitor which showed potent inhibitory activity against HCV genotype 1a and 1b pseudotypes *in vitro* and which was well tolerated in healthy subjects. The inhibitor was evaluated in HCV genotype 1 infected patients. However, no significant changes in HCV RNA compared to baseline were observed after 29 days suggesting that the compound has little or no antiviral activity in vivo. Further development of this compound was stopped.[48]

Host cell targeting inhibitors

Cyclophilin-inhibitors

Cyclophilins are ubiquitous proteins in human cells that are involved in protein folding. Moreover, cyclophilins participate in HCV replication. It was shown that cyclophilin B binds to the HCV NS5B polymerase and stimulates its RNA-binding activity. Cyclophilin inhibitors show strong antiviral activity in vitro and in vivo. The cyclophilin inhibitor DEBIO-025 showed dual antiviral activity against HCV and HIV in a phase 1 trial with HCV/HIV co-infected patients.[49] Based on the encouraging results from studies in patients with HCV/HIV coinfection, Debio 025 was investigated in combination with peginterferon alfa-2a and ribavirin in HCV genotype 1 null responders to previous peginterferon/ribavirin combination therapy. Triple combination therapy showed a HCV RNA decline after 29 days of 0.88-2.38 $\log_{10}$ IU/mL in the different dosing arms while no antiviral activity was observed in patients receiving DEBIO-025 monotherapy. DEBIO-025 treatment was well tolerated.[50]

NIM811 is another oral non-immunosuppressive cyclophilin inhibitor which has in vitro activity against HCV. In patients with HCV genotype 1 infection with previous relapse to peginterferon/ribavirin therapy, NIM811 in combination with peginterferon alfa-2a showed a mean HCV RNA decline of 2.78 $\log_{10}$ IU/mL after 14 days compared with a 0.58 $\log_{10}$ decline of HCV-RNA in the peginterferon alfa-2a monotherapy arm.[51] The major safety concern was a decrease in platelets and an increase in serum bilirubin.

SCY-635 is also a non-immunosuppressive analog of cyclosporine A that exhibits potent suppression of HCV RNA replication *in vitro*.[52] SCY 635 binds to human cyclophilin A at nanomolar concentrations. Different doses of SCY-635 were investigated in patients infected with HCV genotype 1 and viral load above 100,000 IU/mL. Consistent decreases in plasma HCV RNA were observed in the highest dose group (mean nadir values were 2.20 $\log_{10}$ IU/mL). One subject achieved undetectable HCV RNA levels at day 15.

Silibinin

Oral silibinin is widely used for treatment of hepatitis C, but its efficacy is unclear. Intravenous silibinin was investigated in non-responders to prior interferon-based antiviral therapy and showed a significant decline in HCV RNA between 0.55 to 3.02 $\log_{10}$ IU/mL after 7 days and a further decrease after additional 7 days in combination with peginterferon alfa-2a and ribavirin in the range between 1.63 and 4.85 $\log_{10}$ IU/mL. Beside mild gastrointestinal symptoms, intravenous silibinin was well tolerated.[53] Next, intravenous silibinin was investigated as rescue treatment for patients with chronic hepatitis C who were still HCV-RNA positive after 24 weeks of treatment with peginterferon alfa-2a/ribavirin.[54] After 24 weeks of treatment with standard of care the patients received additionally 20 mg/kg/d silibinin intravenously for 15 days. Thereafter peginterferon/ribavirin was continued. After 15 days of intravenous silibinin therapy HCV-RNA decreased in all patients and 7 out of 9 patients achieved undetectable HCV RNA plasma levels. After the end of silibinin administration patients were followed for at least 12 weeks. In one patient HCV-RNA increased to 100 IU/mL, and a second course of intravenous silibinin for 15 days was given. HCV-RNA became negative again and remained negative so far. Final results of this study are pending.

Nitazoxanide

Nitazoxanide is a thiazolide anti-infective with activity against a number of protozoa, bacteria, and viruses. It is FDA approved for treatment of cryptosporidium and giardia. Nitazoxanide inhibits replication of hepatitis C virus, hepatitis B virus, and rotavirus in vitro. Based on its broad antiviral activity, the mechanism of action is likely through cellular processes rather than specific anti-

viral targets. Rossignol et al. recently reported that the use of nitazoxanide in combination with peginterferon alfa-2a with or without ribavirin among treatment-naive hepatitis C patients infected with genotype 4 significantly improved viral response rates compared to the standard of care (peginterferon alfa-2a plus ribavirin).[55] The sustained virologic response rates were 79% and 64% in patients treated with peginterferon alfa-2a/ribavirin/nitazoxanide and peginterferon alfa-2a/nitazoxanide, respectively, versus 45% in patients treated with peginterferon alfa-2a/ribavirin. Nitazoxanide in combination with peginterferon alfa-2a and ribavirin is currently investigated in HCV genotype 1 infected patients.[56] The early virologic response rates (HCV RNA undetectable at week 12) in the nitazoxanide group compared with the placebo group were 80% *vs* 68% and 38% *vs* 25% in treatment naïve patients and patients with prior non response to peginterferon alfa-2a and ribavirin. Overall, the studies suggest that nitazoxanide may have antiviral activity in genotype 1 and 4 infected patients.

Summary and conclusion

The sustained virologic response rates have not substantially been improved since the introduction of peginterferon alfa-2a/-b plus ribavirin. Recent phase 2 studies with the HCV specific protease inhibitors telaprevir and boceprevir convincingly demonstrated that two major goals of antiviral therapy (i) higher sustained virologic response rates and (ii) shorter treatment duration in patients infected with HCV genotype 1 can be achieved when HCV-specific inhibitors are combined with SOC. Data from the pivotal phase 3 trials are awaited before triple therapy with telaprevir or boceprevir with peginterferon/ribavirin can become the new standard of care.

The greatest benefit with the new direct antiviral molecules can be expected in patients with HCV genotype 1 infection who are treatment naïve or who relapsed after peginterferon/ribavirin therapy. The question arises if HCV specific inhibitors in combination with peginterferon/ribavirin will also be a treatment option for non-responders to previous antiviral therapy. The PROVE3 study investigating that issue showed that approximately 40% of previous non-responders achieve a sustained virologic response following triple therapy. These results are encouraging, however, require further improvement.

The ultimate goal of antiviral therapy will be HCV eradication without peginterferon/ribavirin. This may potentially be achieved either by a combination of two or more specific inhibitors with non overlapping resistance profiles such as protease inhibitors with nucleoside and/or non-nucleoside inhibitors or the combination of HCV specific inhibitors with non HCV specific inhibitors such as cyclophilin inhibitors. Preliminary results on the combination of a protease and a polymerase inhibitor presented at the EASL conference 2009 indicate good tolerability and additive antiviral activity in patients with chronic hepatitis C. Whether this dual combination will be sufficient to achieve HCV eradication with e.g. 12-24 weeks combination therapy needs to be studied in the future.

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