Artículo:

New approaches and therapeutic modalities for the treatment of patients with chronic hepatitis C

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Concise Review

New approaches and therapeutic modalities for the treatment of patients with chronic hepatitis C

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Abstract

A major challenge in the field of hepatology is the fight against hepatitis C that affects more than 150 million people world-wide. Despite the enormous improvement that has been achieved in the therapy of chronic hepatitis C over the last decade, there is an urgent medical need for new therapeutic approaches. This review focuses on the optimization of the current standard therapy of hepatitis C and future treatment directions beyond pegylated interferon alpha and ribavirin.

Key words: Hepatitis C, Interferon alpha, ribavirin, pegylated interferon alpha, therapy.

Current therapy and challenges

Chronic hepatitis C virus infection is one the major challenges world-wide. More than 150 million people are infected and we are still waiting for the peak of HCV related complications. In the next future we will expect an increase in number of patients with liver cirrhosis, hepatocellular carcinoma, and the need for a liver transplantation due to hepatitis C.[1] Thus, there is an urgent need for effective therapies to prevent and stop HCV related complications.

The current standard therapy is the combination of pegylated interferon alpha (PEG-IFN) and ribavirin. There are two PEG-IFNs available; PEG-IFN alpha-2a (PEGASYS™, Hofmann La-Roche) and PEG-IFN alpha-2b (PEG-Intron™, Schering-Plough). Pegylation of the interferon alfa allows the once weekly administration and improved the overall sustained virological response (HCV-RNA negative more than 6 months after the end of therapy) rates to 54-63%.[2-4] There seems to be no big difference between the pegylated interferons concerning sustained virological response rates.[5] However, both interferons have different pharmacokinetics due to their different polyethyl-

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ene glycol moieties. PEG-IFN alpha-2a is covalently attached to a 40 kDa branched chain polyethylene glycol moiety, whereas PEG-IFN alpha-2b is bound to a single linear 12 kDa polyethylene glycol molecule. The distinct sizes of the PEG-IFNs influence the volume of distribution which impacts the dosing. The larger PEG-IFN alpha-2a has a restricted volume of distribution (predominantly intravascular) and can be given in a fixed dose of 180 μg once weekly, whereas PEG-IFN alpha-2b should be adjusted for body weight (1.5 μg/kg once weekly) due to a large volume of distribution (intravascular and extravascular) [reviewed in[6,7])]. Ribavirin is also dosed by weight, but is not as simple as it used to be (1.000 mg if < 75 kg or 1.200 mg if > 75 kg). A retrospective analysis of the PEG-IFN alpha-2b/ribavirin registration trial revealed that the optimal ribavirin dose is at least 10.6 mg/kg.[2] Therefore ribavirin (Rebetol™, Schering-Plough) is recommended to be given at a concentration of approximately 11 mg/kg in combination with PEG-IFN alfa-2b. Ribavirin (Copegus™, Hofmann La-Roche) is recommended as it used to be when combined with PEG-IFN alpha-2a. However, the PEG-IFN alpha-2a/ribavirin registration trial by Hadziyannis and colleagues showed that 800 mg ribavirin is sufficient for patients with HCV-genotypes 2 and 3 and a benefit of higher ribavirin doses has not been observed. This study also confirmed that 24 weeks of therapy are enough for HCV-genotype 2/3 patients whereas patients with HCV-genotype 1 require 48 weeks therapy.[4]

The main challenge for the future is to improve the success rates for patients with the difficult to treat HCV-genotype 1. While patients with HCV-genotypes 2 and 3 can be cured in more than 75% of cases, the 40-50% sustained virological response rates for patients with HCV-genotype 1 are still unsatisfactory. The following part of this manuscript gives insight how to optimize the current therapies and what we can expect from the future.

Optimizing current treatment

Adherence to therapy

Patients who do not take their medication on a regular basis do not respond as well as patient with full adherence to therapy. It is very obvious to come to this conclusion and this is off course the case for almost all medical ther-

apies. However, this concept has been systematically studied and now we have confirmation how adherence to therapy influences the response to therapy.[8] The definition of adherence used here is the 80/80 rule. Patients who received more than 80% of the medication and were treated for more than 80% of the planned duration of treatment are considered adherent. One of the first studies investigating the effect of adherence demonstrated that patients who fulfilled the 80/80 rule had 63% sustained response compared to 52% of those with less than 80% adherence.[8] Therefore, it is important to reduce side effects and motivate the patients. One major problem in the management of chronic hepatitis C patients under IFN-based therapies is depression. Psychiatric care and the use of antidepressants may help to reduce IFN induced depression and consequently improve the response rates.[9] Prospective placebo-controlled trials are under way to confirm these preliminary findings.

Treatment Duration

There are two different concepts to optimize the treatment duration. While some patients with HCV-genotype 1 may need longer treatment to improve the response, patients with HCV-genotypes 2 and 3 may be treated for a shorter period of time to reduce costs and side-effects.

Many studies are investigating the concept to reduce the treatment duration for HCV-genotypes 2 and 3 to 16, 14, or even 12 weeks. The first reported results are very promising, but it turns out that we have to consider individual factors when treating patients for less than 24 weeks. The early virological response (EVR) after 4 weeks of therapy (HCV-RNA negative at TW4) is one of the critical factors that are associated with the success of a shorter therapy. Only the patients who showed an EVR at week 4 had high sustained virological response rates after 16 weeks,[10] 14 weeks,[11] or even after 12 weeks of therapy,[12] whereas those without an EVR had low response rates, nevertheless with the 24-week schedule. However, 12 weeks seem to be the limit for some patients, since the relapse rates after 12 weeks were higher compared to the standard 24 week schedule.[12] In addition to the EVR further factors are associated with the response in patients with HCV-genotypes 2 and 3 as these were the HCV-genotype and the baseline viral load. Patients with HCV-genotypes 2 and 3 should be analyzed separately because patients with HCV-genotype 2 respond much better to PEG-IFN and ribavirin therapy than those infected with HCV-genotype 3.[12,13] Furthermore, the shorter treatment schedules revealed that HCV-genotype 3 patients with low baseline viremia (< 800.000 IU/mL) had a much better chance to respond than those with high viral load.[10,11] In conclusion, patients with HCV-genotype 2 and patients with HCV-genotype 3 and low viral load who have an EVR after 4 weeks of therapy can be treated for less than 24 weeks and patients without an EVR (especially HCV-

genotype 3 and high viral load) may be treated even for more than 24 weeks. Tailoring treatment individually for patients with HCV-genotype 2 and 3 will reduce costs, side effects and further optimize the response rates.

We face the opposite problem in patients with HCV-genotype 1 who are still difficult to treat. Extending the treatment duration beyond 48 weeks is one strategy that may improve response rates in these difficult-to-treat patients. The rationale is to extend the time of HCV-RNA negativity, especially in patients with a slow viral decline (first time HCV-RNA negative between TW12 and TW24) to reduce the relapse rate in these patients. Several studies investigated the efficacy and safety of 48 weeks versus 72 weeks of treatment with PEG-IFN plus ribavirin in patients with chronic hepatitis C. Sanchez-Tapias and colleagues reported a benefit of an extended therapy in patients who were HCV-RNA positive at treatment week 4. The relapse rate after 72 weeks of therapy was significantly reduced in these patients.[14] However, a treatment duration beyond one year may lead to more drop out rates, which results in lower intent-to-treat responses.[14,15] Multivariate analyses of these studies will hopefully reveal factors such as viral kinetics that will help to identify the patients who will benefit from an extended therapy. In conclusion, extension of therapy to 72 weeks may improve response rates for patients with a slow viral response but high motivation and compliance of the patient is mandatory.

Adjuvants

A strategy to further enhance the sustained response rates especially in patients with HCV-genotype 1 is the addition of other drugs to the combination therapy. Triple therapies regimes that are currently investigated include the addition of amantadine or thymosin alpha-1 to the standard PEG-IFN alpha and ribavirin therapy.

Amantadine is an antiviral agent that is used to treat influenza A infection. In 1997 JP Smith published results that amantadine treatment could improve both biochemical and virological markers in patients with hepatitis C who had previously not responded to treatment with interferon alpha.[16] The effect of an amantadine monotherapy could not be confirmed in other studies. However, these data led to numerous studies analyzing the efficacy of amantadine in combination with interferon alpha or interferon alpha/ribavirin. Brillanti and colleagues were among the first who reported very promising sustained response rates with the triple therapy (IFN alpha/ribavirin/amantadine) in prior IFN nonresponders. The sustained response rates were 48% in contrast to 5% with the IFN alpha/ribavirin standard therapy.[17] The dilemma of all these small studies was that the results varied from study to study. While some studies could confirm the good results, others demonstrated no additional benefit of amantadine in combination with IFN or IFN/ribavirin.
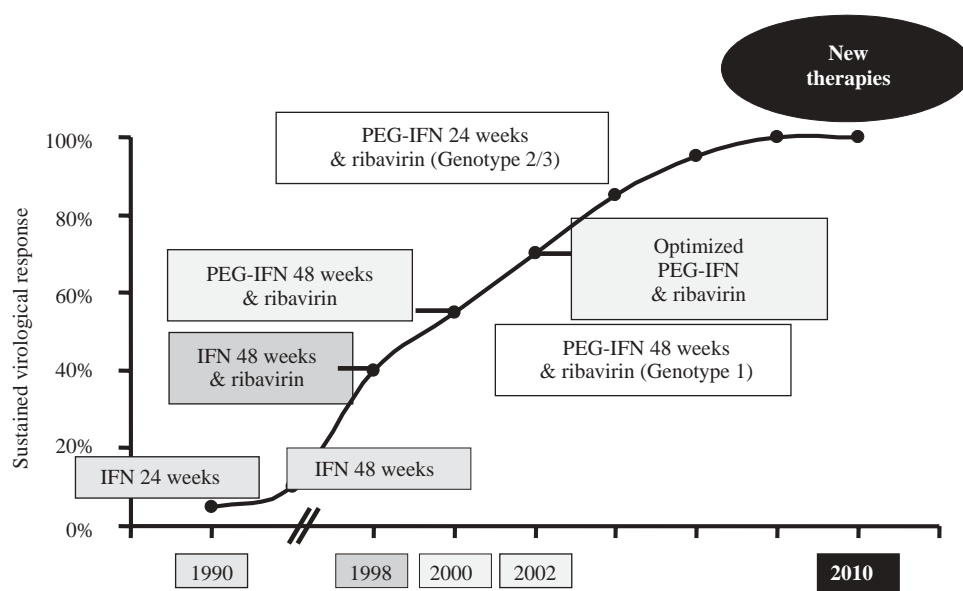
Figure 1. The development of the therapy of chronic hepatitis C is a story of success. The sustained virological response rates have been improved from 5% with an interferon monotherapy in the 90s to now 60% with the current standard therapy of PEG-IFN alpha and ribavirin. We are looking ahead and expect further advances in the therapy of chronic hepatitis C.
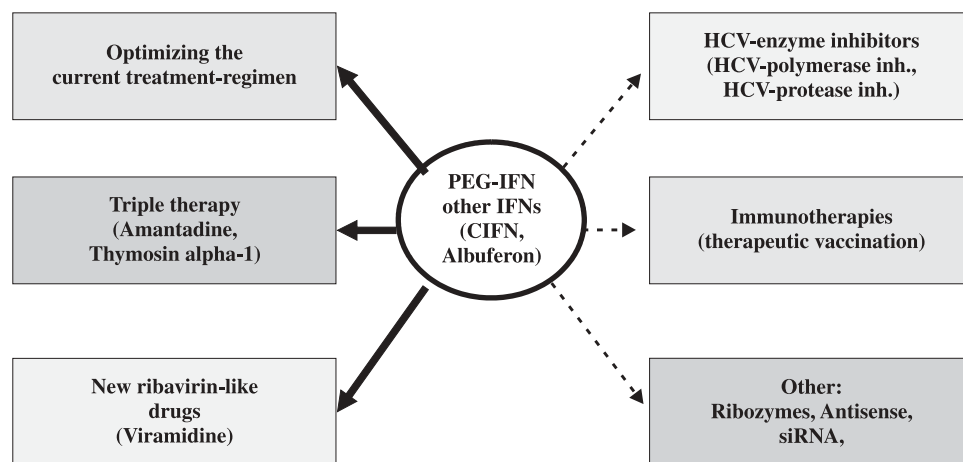
Figure 2. The new approaches in the therapy of chronic hepatitis C include the optimization of the current PEG-IFN-based therapy and the development of new interferons, which may remain indispensable even with the future therapeutics. Nevertheless, we anticipate to abandon the use of interferon alpha in the future (dashed lines) when we have the new therapeutic tools (enzyme inhibitors, immunotherapy, etc) in our hands.

Therefore meta-analysis tried to shed light into this field as one meta-analysis found a significant benefit from IFN/amantadine therapy compared to IFN alone.[18] Another meta-analysis revealed that triple therapy had a significant effect on sustained response rates, but this effect was restricted to prior IFN nonresponder patients and naïve patients had no benefit.[19] However, meta-analyses hamper from the quality of the studies. Many studies had inadequate sample size, were not randomized or placebo controlled and the patient populations were often very heterogeneous. Prospective, placebo-controlled trials are the only way to answer the question if amantadine has a positive effect on hepatitis C therapy. A large German placebo-controlled multicenter study treated 400 naïve patients with IFN/ribavirin/placebo or with IFN/ribavirin/amantadine. Triple therapy could increase the sustained response rates by 8% in HCV-genotype 1 patients. This was not statistically significant.[20] Again, we have a trend but no prove. A study of more than 700 patients is need-

ed. We hope that the placebo-controlled study in cooperation with the German network of competence for viral hepatitis (Kompetenznetz Hepatitis) testing the addition of amantadine to PEG-IFN alpha-2a/ribavirin therapy will give the answer. Until that result, we can conclude that amantadine seems to have some effect - at least a strong trend - and we know that it can reduce IFN induced side effects such as fatigue and depression.[21] Amantadine is inexpensive and due to that knowledge it is a useful tool for the treatment of difficult to treat patients with chronic hepatitis C.

Another promising adjuvant is thymosin alpha-1 (TA1, Zadaxin™, SciClone Pharmaceuticals) a synthetic 28-amino acid peptide that acts as an immune response stimulator. Already in 1998, Sherman and colleagues performed a randomized, double-blind, placebo-controlled trial to compare the efficacy of IFN alpha plus TA1 with IFN alone in 109 patients with chronic hepatitis C. The results suggested a positive effect of TA1.[22] Currently,

phase-III studies are ongoing to test the triple therapy PEG-IFN/ribavirin/TA1 in difficult-to-treat patients.[23]

New strategies

New interferons

Another type-1 interferon, however not so new anymore, is the interferon alphacon-1 or consensus interferon. Consensus interferon (CIFN) is a bio-engineered "consensus" molecule, composed of the most frequently observed aminoacid at each position of the type-1 interferons. CIFN shares an 89% and 30% homology with IFN-alpha and IFN-beta, respectively. CIFN has a 10-fold increased affinity to the type-1 IFN-receptor compared to IFN alpha-2a or IFN alpha-2b. In comparison to the mass base, CIFN displays 5-10 times greater biological activity than other type-1 interferons.[24,25] Despite this advantage in-vitro, the head to head study comparing CIFN and standard IFN alpha monotherapy revealed only minor differences. The results suggested that patients with HCV-genotype 1 may have a small advantage with CIFN.[26] A recent study reported better sustained response rates in naïve patients with chronic hepatitis when they were treated with CIFN and ribavirin compared to standard IFN alpha and ribavirin.[27] S. Kaiser from Germany presented data from several studies investigating the effect of high and daily dosing of CIFN in combination with ribavirin in naïve as well as in nonreponder patients. The overall sustained response rates were very promising. Patients who were nonresponders to PEG-IFN alpha and ribavirin had a sustained virological response of 27-31% with CIFN and ribavirin depending on the CIFN dose.[28] We have also some experience with daily dosing of CIFN in combination with ribavirin in prior IFN and IFN/ribavirin nonresponder patients. The response rates were 30% with better responses in the group of IFN nonresponders compared to combination nonresponders (Cornberg et al., manuscript in preparation). We think that the use of daily CIFN in combination with ribavirin might be a promising alternative to treat prior nonresponder patients. However, the daily dosing requires high motivated and compliant patients since adherence to therapy is especially here one of the most important factors that influences the treatment outcome.

A really new interferon alpha is Albuferon (Alphaferon), which is an 85.7 kilodalton protein consisting of interferon alpha-2b genetically fused to human serum albumin. The fusion with serum albumin extends the half-life of the interferon alpha to approximately 148 hours. Albuferon was detectable for up to four weeks following the second subcutaneous injection. This pharmacokinetic profile allows dosing at intervals of 2-4 weeks compared to one week with the pegylated interferons. Just recently, the results of a phase-II trial testing multiple doses of Albuferon in HCV-genotype 1-patients were presented at the 40th

EASL.[29] Albuferon monotherapy with the optimal doses reduced the HCV viral load by 3.2 $\log_{10}$ after 28 days of therapy. 69% of patients treated with the optimal doses had a > 2 $\log_{10}$ reduction in HCV viral load. These are exciting data that led to the initiation of clinical trials evaluating the efficacy of Albuferon in combination with ribavirin.

New Ribavirin like molecules

The introduction of Ribavirin has reduced the relapse rates after interferon alpha monotherapy and significantly improved the overall sustained response rates.[30,31] Yet, ribavirin associated hemolytic anemia is a major problem of the therapy, since this complication may result in ribavirin dose reduction or even discontinuation, which may significantly affect the overall sustained virologic response rates, especially in patients with HCV-genotype 1.[2] Therefore it is a main task to reduce anemia. Treatment with erythropoietin can effectively reverse ribavirin associated anemia and allow full adherence to ribavirin therapy.[32] This will improve the response rates, but the treatment is expensive and will not be reimbursed in many countries. This problem emphasizes the need for alternative ribavirin-like drugs with less toxicity and/or higher antiviral efficacy. Unfortunately, the mechanism how ribavirin enhances the efficacy of interferon alpha treatment remains unknown. Proposed mechanisms are immunmodulatory effects, inhibition of the inosine monophasphate dehydrogenase (IMPDH) activity and the induction of RNA mutagenesis.[33] More potent IMPDH inhibitors such as mycophenolate mofetil (MMF, Cell Cept) or VX-497 were studied,[34] but with limited effect at least for MMF.[35] Another approach is the development of a ribavirin pro-drug. Viramidine is the amidine version of ribavirin and is converted by the enzyme adenosine deaminase to ribavirin mainly in hepatocytes. Therefore there is less uptake of ribavirin into red blood cells with the prodrug viramidine and consequently less hemolytic anemia.[36] A phase II study investigated the toxicity and effect of viramidine in combination with PEG-interferon alpha-2a. Different doses of viramidine were tested in combination with PEG-IFN alpha-2a and compared to 1.000/1.200 mg ribavirin plus PEG-IFN alpha-2a. Patients who received treatment with 800 mg, 1.200 mg or 1.600 mg of viramidine had anemia in 0%, 2%, and 11%, respectively, whereas ribavirin induced in 27% of the cases anemia. The overall sustained response rates were not statistically different between the treatment groups.[37] A phase III study is under way testing the dose of 1.200 mg viramidine in combination with PEG-IFN alpha-2b.

HCV-enzyme inhibitors

The knowledge about the structure of the hepatitis C proteins allowed the design of new drugs that directly target the sites of the HCV enzymes that are important for

the replication of the virus. The HCV protease and the HCV polymerase are the main targets for these enzyme inhibitors. The first drug that has been tested in patients and demonstrated the proof-of-concept in humans for a HCV protease inhibitor was BILN-2061 (Boehringer-Ingelheim). BILN-2061 given twice daily as monotherapy for 2 days reduced HCV-RNA by 2-3 $\log_{10}$ in most of the patients infected with HCV-genotype 1.[38] Interestingly, the effect was more specific for HCV-genotype 1 as the antiviral efficacy of BILN-2061 was less pronounced and more variable in patients with HCV genotypes 2 and 3, presumably due to a lower affinity of BILN-2061 for the HCV protease of these genotypes.[39] Unfortunately, further clinical trials are on hold due to animal toxicity issues.

VX-950 (Vertex Pharmaceuticals) is another oral specific inhibitor of the hepatitis C virus protease. Reesink and colleagues demonstrated that HCV-genotype 1-patients treated with 750 mg of VX-950 every eight hours achieved a median reduction of HCV-RNA of 4.4 $\log_{10}$ at the end of a 14-day treatment regimen.[40] These data are very promising especially for difficult-to-treat patients. However, we have to fear resistance to these drugs as known for HIV drugs. Therefore it is important to have multiple drugs in the pipeline. Combining these drugs with interferon alpha or other enzyme inhibitors may be the concept for the future.

There are also HCV polymerase inhibitors under investigation such as the nucleoside analogue valopicitabine (NM283; Idenix Pharmaceuticals). Preliminary clinical data of the combination of PEG-IFN alpha-2b and valopicitabine showed promising anti-HCV activity.[41] In conclusion, there is an enormous effort to design new highly effective agents that directly inhibit the HCV proteins. The recent developments give hope that we have very powerful drugs in the future to further improve anti-HCV therapy.

Immune therapy

Another approach to fight the hepatitis C virus is the induction of HCV-specific immune responses. We know that the spontaneous recovery after acute HCV infection is associated with a strong and broad immune response, while the development of a chronic hepatitis C is associated with an impaired immune system.[42,43] The aim of a therapeutic vaccination is to stimulate the hepatitis C-specific immune responses to control viral replication. There are many different strategies to induce immune responses; among them is the administration of the stimulating antigen as protein, peptides, or DNA.

The first therapeutic vaccine strategy included a protein vaccine. The glycoprotein HCV-E1 served as antigen and a first clinical trial has already been performed.[44] The E1 vaccine (Innogenetics) was able to induce significant E1-specific T cell responses in the majority of the pa-

tients. However, HCV-RNA levels remained unchanged, but ALT levels showed a trend toward a decrease during treatment. The exciting data of these studies were that the increase in anti-E1 antibody levels correlated with a histological improvement including reduced fibrosis.

Another concept that is currently under investigation is the administration of a peptide vaccine (IC41, Intercell) that is supposed to induce HCV-specific CD4 and CD8 T cell responses.[45] Detailed analyses of this study may reveal some helpful information how a therapeutic vaccine change immune responses to hepatitis C. In conclusion, therapeutic vaccines are no dreams anymore and are already tested in clinical trials. The results will give us hints for the final aim, the development of a protective HCV vaccine. We hope we can make the impossible possible.

Others

Last but not least want to focus on small molecules such as ribozymes, antisense oligonucleotides, and small interfering RNAs (siRNAs) that have been widely used to control viral gene expression.

To cut the HCV genome in a region which is crucial for the virus is an approach which is under investigation for several years. Ribozymes are such molecular scissors that can cleave the HCV internal ribosome entry site (IRES).[46] The efficacy of ribozymes have been demonstrated *in-vitro*.[47,48] and the first drug is now in early clinical trials (Heptazyme TM, Ribozyme Pharmaceuticals).

Another way to block HCV-specific gene expression is the development of molecules that complementary bind to the viral RNA to inhibit the expression of proteins required for replication. ISIS-14803 (Isis Pharmaceuticals) is such a antisense molecule and is currently in early stage clinical trials.[49]

A more recent very promising approach to combat viruses is the interference with the virus genome (RNA). RNA interference (RNAi) is a process of posttranscriptional gene silencing using small interfering RNAs (siRNAs).[50] Inhibition of HCV infection (HCV replicon) has already been demonstrated *in-vitro*.[51]

In conclusion, beside the enzyme inhibitors and the immunological approaches there are many more players in the field to charge the hepatitis C virus. At the moment these are still rookies. We have to wait which of these substances will become valuable players in the future or remain wasted talents.

Concluding remarks

The current standard anti-HCV therapy can be further improved with simple available methods such as adherence to therapy, optimizing treatment duration, and the addition of already available drugs. New interferons and ribavirin-like molecules are around the corner. New antiviral compounds with an improved safety profile and en-

hanced antiviral properties compared to current IFN-based therapies are in the pipeline and the hope for the future. The final goal is a short-term treatment with 100% efficacy and the development of a protective HCV vaccine.

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