Direct-acting antiviral therapy for chronic hepatitis C

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Citation for published version (APA):
de Bruijne, J. (2012). Direct-acting antiviral therapy for chronic hepatitis C

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Hepatitis C virus (HCV) infection was discovered in the late 1980s. Since then, tremendous progress has been made in understanding the pathophysiology of HCV infection together with the development of improved therapies for patients with chronic hepatitis C. Our increased understanding of the natural course of HCV infection, including viral and host factors involved in replication of the virus, enabled the development of various antiviral therapies. The first application of interferon monotherapy for the treatment of non-A non-B hepatitis in the early nineties resulted in success rates not exceeding ten percent. The subsequent treatment with pegylated interferon-alfa (peg-IFN) and ribavirin resulted in an overall sustained viral response rate (SVR) of approximately 60%. Although the treatment of chronic hepatitis C already resembles a success story, the largest progression has yet to come with the ongoing development of HCV specific direct-acting antivirals (DAAs). The promising phase 1 study results of the first HCV specific NS3 protease inhibitor were published in 2003. Currently, many DAAs with different modes of action are being developed which have brought the treatment of chronic hepatitis C into a new era. The first two protease inhibitors (boceprevir and telaprevir) have been registered and are reimbursed by health insurance companies. Triple therapy with boceprevir or telaprevir and peg-IFN plus ribavirin is currently the new standard of care for treatment of chronic hepatitis C.

I. DRUGS INTERACTING WITH THE HEPATITIS C VIRUS LIFE CYCLE

Many HCV-infected patients do not achieve sustained viral clearance after treatment with peg-IFN and ribavirin due to non-responsiveness to IFN-based therapy, poor tolerability or toxicity. As a result, a difficult-to-treat patient population awaits new treatment options. Moreover, patients with extensive liver fibrosis or Child-Pugh A cirrhosis respond poorly to the current standard of care. Therefore, improved treatment strategies to stop disease progression and (disease free) survival are desperately needed. Improvements of antiviral therapy are focused on antiviral drugs directed towards viral and host factors (Chapter 2, 3, 4, 5 and 6) together with alternatives for peg-IFN (IFN-lambda and albumin-IFN) or Toll-like receptor agonists (Chapter 12) and ribavirin analogues (taribavirin) as well as to optimize current therapeutic strategies. Since peg-IFN and ribavirin will remain the cornerstone of DAA-based therapy in the near future, a better understanding of the mechanism of action of IFN and ribavirin will still be necessary.

The HCV life cycle is a complex multistep process. The primary step, viral entry into the hepatocyte, is a potential target for antiviral therapy within the HCV life cycle. The study presented in Chapter 2 is the first to describe the in vitro development of an HCV infection inhibitor and its clinical evaluation in patients with chronic hepatitis C. The in vitro antiviral properties of JTK-652 against HCV pseudotype virus were studied using HepG2 cells, human primary hepatocytes and Huh7 cells as target cells. JTK-652 showed inhibition of infection of HepG2 cells and human primary hepatocytes. However, JTK-652 showed no inhibition of infection of Huh7 cells with HCV genotype 1a pseudotype virus. A possible explanation for the lack of inhibitory effect in these cells could be because of the amount of E1 and E2 expressed on the pseudo particles or because of differences of glycosylation as described before. The in vitro model used for this study is potentially not suitable for testing HCV entry inhibitors. Using other pre-clinical approaches to study HCV infection inhibition, such as lentiviral particles bearing HCV glycoproteins, HCV produced in cell culture or eventually small animal models, could potentially help to bridge the gap between in vitro models and effective HCV entry inhibitors in vivo. Subsequently, a placebo controlled phase 1 study evaluated JTK-652 in 10 patients with chronic hepatitis C to assess safety, tolerability, pharmacokinetics and antiviral activity. Results from this phase 1 study indicated that JTK-652 was safe and well tolerated. Unfortunately, no significant changes in HCV RNA levels compared to baseline were observed in individual patients.

The safety, pharmacokinetics, tolerability and antiviral activity of NS3/4A protease inhibitors narlaprevir, IDX320 and PHX1766 are described in Chapter 3, 4 and 5, respectively. Chapter 3 describes a rapid and persistent decline in plasma HCV RNA levels in both treatment-experienced and naive patients in a randomized, placebo-controlled, two period, blinded study after narlaprevir dosing. This study was the first to investigate boosting of a NS3/4A protease inhibitor by ritonavir for the treatment of chronic hepatitis C. The enhanced trough levels observed when narlaprevir was administered with ritonavir and the associated robust antiviral activity observed in this study provided a proof of principle for the use of pharmacokinetic
enhancement in HCV therapy, following the example of HIV antiretroviral therapy. Moreover, this study demonstrated that administration of narlaprevir for two weeks (with or without ritonavir) plus peg-IFN followed by standard of care resulted in 81% and 38% SVR in treatment-naive and experienced patients, respectively. It is uncertain if ritonavir boosting will be useful in future hepatitis C treatment regimens that potentially include three or four drugs. Coadministration of a metabolic enhancer for one compound will require extra attention to possible interactions with other medications metabolized by CYP3A4. Other (macrocyclic) protease inhibitors such as TMC435 demonstrate potent antiviral activity with once daily dosing without ritonavir boosting. Nevertheless, knowledge about the coadministration of HCV protease inhibitors with ritonavir will be particularly relevant in the large HIV-coinfected subpopulation of patients.

The enormous antiviral potential of macrocyclic protease inhibitors such as IDX320 is presented in Chapter 4. The pharmacological profile of IDX320 offered the opportunity for once daily dosing. In contrast, registered linear protease inhibitors boceprevir and telaprevir require three times daily administration and are associated with additional side effects, such as anemia or rash. Frequent dosing and side effects can negatively affect compliance. Once daily administration of IDX320 for 3 days was safe and well tolerated and resulted in a rapid HCV RNA decline in all patients. The results of this study demonstrated the clinical potential of IDX320. However, further development of IDX320 was stopped when 3 patients developed elevated serum liver tests during a drug-drug interaction study of IDX320 with the nucleotide HCV polymerase inhibitor IDX184. A hepatotoxic effect was not observed during a phase 1 study in which IDX320 was administered as monotherapy. Drug-drug interactions could cause serious side effects in future treatment regimens when three or four DAs will be combined. More drug-drug interaction studies are needed to prevent serious side effects, especially in coinfected patients (HBV, HIV) or patients with impaired liver function.

A novel overlapping single- and multiple-dose escalating study design, presented in Chapter 5, was highly informative in a short period of time. Moreover this study design was the most cost-effective approach without compromising the safety of the participants. This accelerated trial design was used to investigate the safety, tolerability, and pharmacokinetics of PHX1766, a potent, tight-binding, reversible, and highly selective NS3/4A protease inhibitor, in healthy volunteers and patients with chronic hepatitis C. There was a clear discrepancy between the results of the clinical trials and the earlier described preclinical results concerning the pharmacokinetic profile and antiviral effect. In contrast to the potent in vitro replication results of PHX1766 (EC50 8 nM), PHX1766 dosing resulted in only a modest HCV RNA decrease in HCV-infected patients. The highest dose group (800 mg twice daily) produced a trough-level 120 hours after the first dose of only three times the EC50 which might explain the disappointing viral decline.

The overlapping design of the single-dose and multiple-dose protocols has proven to be a time-efficient and safe way to investigate PHX1766. Due to this design fewer patients were exposed to the experimental protease inhibitor, which is of relevance to minimize possible health risks. Secondly, less exposure reduces the risk to generate mutations and, subsequently, resistance, because this phenomenon is inevitable with the administration of a protease inhibitor as monotherapy. We therefore suggest that this design should be considered in future phase 1 studies, especially when DAA monotherapy is investigated.

The objectives of the first-in-human study described in Chapter 6 were to evaluate the safety, tolerability, and pharmacokinetics of IDX375 in healthy male subjects and HCV infected genotype 1 patients following single ascending-doses. Antiviral activity was assessed in patients with HCV genotype-1 infection following a single-day dose. IDX375 proved to be a potent and selective palm-binding non-nucleoside inhibitor (NNI) of the RNA-dependent RNA polymerase (NS5B). NNIs bind to allosteric sites on the surface of NS5B. Here, a high number of variants with multiple amino acid exchanges can occur without functionally altering the NS5B polymerase activity. It is therefore challenging to design a NS5B inhibitor with a high genetic barrier to resistance. Variants that are resistant to most non-nucleoside inhibitors were selected within a few days of treatment during phase 1 monotherapy trials. Moreover, variants with reduced sensitivity to non-nucleoside NS5B inhibitors may be present at high frequencies already before initiation of DAA therapy. In concordance with other NNIs in early clinical development, IDX375 was well tolerated without dose-limiting toxicity and showed moderate antiviral activity (0.5-1.1 log10 HCV RNA IU/mL decline). The modest antiviral activity could be due to the small number of doses (n=3) and the short dosing period (single day) with a suboptimal dose of IDX375. The antiviral activity of IDX375 was restricted to genotype 1a/b in the replicon model and therefore unsuitable for non-genotype 1 infected patients. Despite the modest antiviral activity, genotype restrictions and low genetic barrier to resistance, NNIs could be used in future DAA combination regimens due to the favourable toxicity profile and lack of cross-resistance with NS3 protease inhibitors, NS5A inhibitors and nucleoside analogs.

A new standard of care

The use of protease inhibitors in combination with peg-IFN and ribavirin is generally claimed to yield higher SVR rates with shorter treatment duration in treatment-naïve patients. Patients who did not achieve SVR with IFN and ribavirin therapy may also benefit from triple therapy. The data are especially encouraging for patients who experienced partial response, breakthrough or relapse with their prior course of IFN-based therapy. The optimism about the improved SVR rates with this new standard of care is accompanied with many questions, uncertainties and even new problems arise. The most urgent issues involve side effects, adherence, toxicity, viral resistance, high costs, activity against non-genotype 1 infected patients and difficult-to-treat patients (previous non-responders, HBV/HIV co-infected patients, blacks and patients with extended fibrosis or cirrhosis).

In most trials conducted to date, combining an oral DAA with peg-IFN and ribavirin has increased the likelihood of side effects and treatment discontinuation. Additional side effects together with a three times daily dosing schedule, will prove challenging in daily clinical practice. Adherence will therefore be of key importance during administration of DAs, as poor adherence will lead to earlier selection of resistant variants, their rapid acquisition of fitness (ie, a selective replicative advantage in vivo), and thus a higher likelihood of treatment failure.
Adherence to peg-IFN and ribavirin remains pivotal as well, since antiviral pressure supplied by these agents must be maintained for the full duration of treatment. Second generation macrocyclic inhibitors (such as TMC435) currently in phase 3 development were equally effective compared to linear protease inhibitors. The favourable side effect profile and once daily administration of macrocyclic protease inhibitors could potentially become the first-choice treatment for patients with chronic hepatitis C genotype 1 upon registration. The approval of potent drug combinations yielding high cure rates will be tempting for physicians to start treatment in patients likely to develop severe liver disease with life threatening complications, e.g. patients with compensated and decompensated cirrhosis, HIV/HCV coinfection and liver transplant recipients. However, there is little experience with DAAs in these patients. Therefore, DAAs should only be used in these patient populations in the setting of clinical trials. Such trials are currently ongoing in HIV/HCV co-infected patients.

The increased financial burden of DAA-based therapy is another important, non-medical, item that needs attention. peg-IFN and ribavirin treatment is expensive and is only reimbursed in developed countries. The addition of a protease inhibitor to the current standard of care will cause a considerable increase of medical costs in the short run. In the long run, protease inhibitors might reduce medical costs since enhanced SVR rates will lead to fewer patients who need re-treatment, a reduction of patients who develop HCCs or decompensated liver disease and consequently less patients in need of liver transplantation. The benefit of DAA-based therapy with increased SVR rates will be restricted to a certain patient population since reimbursement will most likely only happen in developed countries. In that light it would be helpful if pharmaceutical companies are willing to lower treatment costs in low-income countries, following the example of HIV treatment. This might help to restrict further spread of HCV-infection and reduce mortality as a result of liver damage due to chronic hepatitis C.

In the recent Dutch, EASL and AASLD guidelines for the treatment of chronic hepatitis C there are no strict indications when to start antiviral therapy. In theory, all patients with chronic hepatitis C without decompensated liver disease or specific contra-indications are potential candidates to undergo antiviral therapy. However, natural history studies indicate that approximately 20% of chronically HCV-infected individuals develop cirrhosis over periods of approximately 10 to 30 years. Persons with HCV-related cirrhosis are at risk for developing end-stage liver disease as well as HCC (a risk of approximately 1-4% per year). More-than-portal fibrosis on liver biopsy (Metavir ≥2 or Ishak ≥3) is an important predictor of future progression of liver disease and these patients should definitively be treated. The ongoing increase of antiviral efficacy with DAA-based therapy might lower the barrier to initiate antiviral treatment for patients, even if liver damage is not apparent. On the other hand, to reduce costs and avoid unnecessarily exposure to peg-IFN, ribavirin and a direct-acting antiviral, guidelines could advise to start treatment only if liver biopsy shows septal or bridging fibrosis or at least moderate inflammation.

Individualized Antiviral Therapy

Response-guided therapy

Treatment duration can be tailored using the on-treatment virological response. In reality, the potential to shorten the duration of therapy with peg-IFN/ribavirin to less than 48 weeks without impairing the chance of an SVR in patients with HCV genotype 1 infection who have not previously received treatment, is currently limited to the small number of patients with a low viral load (<800,000 IU/mL) who have undetectable HCV RNA at week 4. In contrast, more than half of the patients who received telaprevir with peg-IFN/ribavirin had undetectable HCV RNA levels at weeks 4 and 12, indicating an extended rapid virologic response (eRVR). Relapse occurred infrequently in these patients after 24 weeks of treatment, suggesting that total treatment duration of 24 weeks is sufficient for patients with an eRVR. The role of a 4-week lead-in phase with peg-IFN plus ribavirin before the addition of telaprevir was investigated in a smaller phase 2 study with boceprevir. It seemed that lowering the viral load with pretreatment with peg-IFN plus ribavirin may have reduced the emergence of protease-resistant variants, lowered the rate of virologic breakthrough during treatment, and improved the SVR rate. However, no significant differences in SVR rate between the concurrent and delayed initiation of telaprevir or boceprevir with peg-IFN plus ribavirin were observed. Thus, the additional value of a lead-in phase with regard to increasing the SVR rate is not demonstrated in phase 3 trials and it appears questionable whether a lead-in phase should be applied in daily clinical practice.

Response-guided therapy could reduce treatment duration, decrease peg-IFN or ribavirin related side effects and save costs while likely improving adherence. This will only succeed if physicians are familiar with the treatment guidelines and accompanying definitions (RVR, eRVR, EVR, DVR, SVR) and have access to regular HCV RNA testing with a sensitive quantitative assays enabling measurements between 100-1000 IU/mL necessary to comply with stopping rules during treatment.

Prediction of treatment outcome

A reliable prediction of achieving an SVR or non-response early during the treatment course can help to prevent unnecessary toxicity in a considerable number of patients by withholding further treatment. Several pre-treatment clinical and virological factors have been identified that enable prediction of SVR, with viral genotype, baseline viral load, race, fibrosis stage and metabolic clinical signs being most widely appreciated. Unfortunately, none of these markers turns out to be 100% predictive for treatment success or failure. The favourable C/C single nucleotide polymorphisms (SNP, rs12979860) upstream of the interleukine 28B gene, encoding for IFN-λ-3, showed a high correlation with SVR in chronic hepatitis C patients. In patients with acute hepatitis C this SNP predicts progression to chronic hepatitis C or spontaneous clearance. The specific mechanisms of how variations in IL28B SNPs affect HCV suppression remain unknown. Although IL28B genotyping is highly predictive of SVR at the population level in HCV genotype 1 infected patients, its predictive power at the individual patient level is not an absolute prerequisite for treatment-induced HCV suppression.
Interferon-gamma inducible protein (IP-10 or CXCL10) is a chemotactic chemokine. High systemic levels of IP-10 were found in a large proportion of patients with chronic hepatitis C, and baseline levels of IP-10 were elevated in patients infected with HCV genotypes 1 or 4 who did not achieve an SVR after completion of therapy. Furthermore, associations between higher baseline IP-10 levels and high viral load, high body mass index, and the presence of bridging fibrosis or cirrhosis have been reported. Patients infected with HCV genotype 1, cut-off levels of 150 and 600 pg/mL have yielded positive and negative predictive values for SVR of 71% and 100%, respectively. There is little doubt that parameters such as genotype, baseline viral load, race, fibrosis stage, IL28B genotype and the serum IP-10 level are important predictors of peg-IFN/ribavirin responsiveness. It is unlikely, however, that any of these parameters alone will have sufficient predictive value to guide treatment decisions. Therefore, predictive algorithms combining several baseline parameters need to be developed and prospectively validated for practical clinical decision-making.

II. VIRAL PHYLOGENY, KINETICS AND RESISTANCE

Epidemiological changes of HCV infection and implications for antiviral therapy

HCV infection has become a common infectious disease with a worldwide prevalence. After the discovery of HCV, all blood donations were screened for HCV and plasma derivatives adequately sterilised in developed countries. This was accompanied with a decreased popularity and, in some countries, an effective harm reduction strategy of intravenous drug use (IDU). In low-income countries, the prevalence and spread of HCV is largely unknown, but it is likely that transmission is maintained due to unsafe blood transfusions and inadequately sterilised (medical) instruments. In Europe, North America, Asia and Australia, most HCV-infected patients (>80%) are infected with genotype 1, 2, or 3. Genotype 4 is the most common genotype in the Middle East and in northern and central Africa, accounting for more than 20% of all chronic HCV-infections worldwide. Genotype 4 is considered difficult to treat and has an SVR rate of approximately 60%, where the SVR rates are 40 to 50% for genotype 1 and 80 to 90% for genotypes 2 and 3. The genetic diversity and evolutionary origin of HCV-4 infection in The Netherlands are described in Chapter 7. This study demonstrated that HCV-4 is effectively spreading corresponding to three separate epidemiological profiles: import of HCV-4a mainly by immigrants from areas in Africa and the Middle East (mainly Egypt) where HCV is endemic, the local spread of HCV-4d in individuals reporting intravenous drug use, and its spillover into HIV-positive men who have sex with men (MSM). The sharing of drug use equipment, and more recently high-risk sexual behaviour among MSM, will presumably lead to further spread of HCV-4 within The Netherlands. These results could have implications for the development of DAA-based therapies. Most clinical trials investigating DAAs are focused on patients infected with genotype 1, despite the high worldwide prevalence of genotype 4 infections. The current registered protease inhibitors telaprevir and boceprevir have minimal or no antiviral efficacy against genotype 4 strains [ref]. The addition of NS3 protease inhibitors to the current standard of care increases the SVR rate of genotype 1 infected patients to approximately 70% (phase 3 refs). Consequently, genotype 4 could become the hardest to treat genotype in the near future. The ongoing spread and increased prevalence of genotype 4 infection together with the poor treatment results of peg-IFN and ribavirin combination therapy warrant the development of panenzotypic DAAs. Unfortunately, few protease inhibitors and non-nucleoside polymerase inhibitors, active against non-genotype 1 HCV-infections, are currently being developed. In our opinion, clinical research and drug development programs must be stimulated to further deter the spread of HCV-4 infection worldwide.

HCV resistance to direct-acting antiviral therapy

Viral resistance in vivo is influenced by four major factors: the genetic barrier, viral fitness, drug exposure and the fidelity of the HCV RNA-dependent RNA polymerase (RdRp). The genetic barrier to resistance is defined as the number of nucleotide substitutions needed for a viral variant to acquire resistance to the drug in question. The genetic barrier can be different between viral genotypes. Several resistance mutations (V36M, R155K/T) are more often seen in genotype 1a than 1b infections, as a single nucleotide exchange may suffice to change a resistance-associated amino acid codon in the former, while 2 nucleotide switches are needed in the latter. In daily clinical practice when protease inhibitors are added to the regimen it might therefore be necessary to determine the viral subtype since resistance, and consequently therapy failure, is more likely to occur in genotype 1a infected patients. The in vivo fitness of the viral variant population is defined as its ability to survive and grow in the replicative environment. DAA exposure profoundly inhibits replication of the dominant, wild type drug-sensitive viral population, and the resistant variants could gradually occupy the vacant replication space. Resistant variants have a greater viral fitness compared to wild type virus in the presence of a DAA. This advantage disappears in the absence of DAA treatment and it is expected that wild type virus regains its dominance. Previously it was shown that the wild type viral population grows only slowly after DAA treatment discontinuation and generally takes several weeks or months to regain its dominance. The aim of the study presented in Chapter 8 was to analyse the selection and long-term persistence of resistant variants of the NS3 protease domain of HCV genotype 1-infected patients treated with narlaprevir. This study demonstrated that narlaprevir rapidly selected (high level) resistant variants in genotype 1a infected patients leading to therapy failure. The R155K variant was present in all 5 patients and additional resistant variants V36M, T54S or A156S together with R155K/T were observed in the same 5 patients. The ongoing replication of a viral population with the R155K mutation during treatment with a protease inhibitor might allow selection of additional resistant variants. Clonal sequencing of narlaprevir treated patients demonstrated that most viral variants that emerged during narlaprevir re-exposure were no longer detected over time, while mutation R155K persisted in 2 patients. The swift
return of wild type virus suggests an inverse correlation between replicative viral fitness and
narlaprevir resistant variants. The increased presence of these resistant variants could result
in a reduced viral decline and viral breakthrough upon retreatment with a cross-resistant
protease inhibitor due to rapid reduction of wild type virus and selection of R155K variants.
This hypothesis should be investigated in future retreatment studies with cross-resistant DAA
and sensitive techniques to detect viral resistance.
It is likely that resistant variants remain present at undetectable levels as a result of continuous
de novo generation of resistant variants due to the high replication rate and the error-prone
NS5B polymerase. These drug-resistant variants pre-exist as minor populations within the
patient’s quasispecies, as a result of the error-prone activity of the RdRp, the large viral
populations and the short half-life of the virus in peripheral blood. Preexisting drug-resistant
variants are rarely detected with population-based sequencing prior to therapy, because the
amino acid substitution(s) that confer resistance also generally reduce replicative capacity
in the absence of the drug. Sensitive techniques, such as clonal sequencing or ultra-deep
pyrosequencing (UDPS), could be used to identify such resistant variants prior to (re)treatment.
We performed 2 studies (Chapter 9 and 10) to investigate the presence of resistant variants
prior to and after treatment with a NS3/4A protease inhibitor using UDPS. The aim of the study
described in Chapter 9 was to assess the prevalence of resistant variants in patients before
telaprevir dosing and after a prolonged follow-up period by using clonal sequencing and UDPS.
Variants at previously well-characterized NS3 protease positions V36, T54, R155 and A156
were assessed at baseline and after a follow-up of 4±1.2 years by UDPS in 15 patients who
received telaprevir monotherapy for 14 days. The prevalence of resistant variants at follow-up
was compared to baseline. In general, prevalence of resistance mutations at follow-up was
not increased compared to baseline. Only one patient had a small, but statistically significant,
increase in the number of V36M and T54S variants 4 years after telaprevir dosing. Phylogenetic
analysis suggested that these mutations arose at a later timepoint and not during telaprevir
treatment. Limitations of UDPS are the possibility of oversampling and the intrinsic error rate
of this technique. Oversampling was minimized since all samples exceeded 10^6 IU/mL, with a
viral RNA input >10^6 virus copies per test, demonstrating that redundancy or oversampling was
not a problem in the UDPS test set up. Viral variability that was observed may have been caused
by sequencing errors. A cut off of 0.1% - 0.5% is often used for reliable detection of mutants
based on plasmid controls. Sequencing errors in our system set up seem to occur at a much
lower level than 0.5%, illustrated by the observation that the residues of the catalytic triad
(H57-D81-S139) showed none or very little (<0.01%) variation. This study demonstrated that
frequencies of resistant NS3 variants measured by an extremely sensitive sequence analysis
technique are comparable to baseline in most patients 4 years after telaprevir monotherapy.
Since HCV is not known to be archived, patients could potentially be retreated in the future
with more expanded combination therapy regimens that still contain telaprevir or other cross-
resistant protease inhibitors.
We subsequently investigated the antiviral activity, safety, and tolerability of a TMC435 (a
macrocyclic NS3/4A protease inhibitor)-containing treatment regimen in patients who had
previously received TMC435 monotherapy (Chapter 10). Four weeks of treatment with
TMC435 in combination with peg-IFN/RBV resulted in SVR in 3 of 5 HCV genotype 1-infected
patients who had previously failed IFN-based therapy and had been subsequently exposed to
5 days of TMC435 monotherapy during which resistant variants emerged. Detailed virologic
analyses suggest that in some patients, the frequency of emerging resistant viral variants might
decrease over time in the absence of selective pressure imposed by the DAA, to a level that
does not negatively impact clinical outcome upon subsequent retreatment with a regimen
containing the same DAA. However, in other patients, resistant variants might persist at low
or undetectable levels that can still subsequently impact the efficacy of a second course of a
DAA-containing regimen, although this may also be influenced by a poor intrinsic peg-IFN/RBV
response. This study showed that successful retreatment after prior exposure to TMC435 with
emergence of resistance variants is possible. Whether the selection of resistant variants during
DAA-based treatment impairs future treatment choices needs to be further explored in larger
prospective DAA re-treatment studies.
Knowledge of the hepatitis C treatment history (naïve, relapser, breakthrough, partial- or null-
responder) is of great importance to predict treatment success and this may also help to decide
whether or not to start antiviral therapy. Moreover, strict application of stopping rules may
help to avoid the selection and long-term persistence of HCV resistance variants. In 58% of
patients who had variants with reduced sensitivity to telaprevir, such variants were no longer
detectable by population sequencing 6 months after treatment discontinuation.9 Undoubtedly,
the treatment of chronic hepatitis C has become more complex and one could argue that
treatment should only be initiated in selected certified medical centres by experienced
physicians and nurses. Moreover a molecular laboratory should be present with an acceptable
turn around time for determining qualitative and quantitative HCV RNA levels. Currently, this is
not the case in the Netherlands.
The potential complexity of DAA-based treatment strategies in patients who previously failed
IFN-based therapy is illustrated by the case report described in Chapter 11. This patient failed
to respond to IFN-based treatment twice before triple therapy with telaprevir, peg-IFN, and
ribavirin was initiated. Therapy could not be completed due to grade 3 laboratory side effects.
This therapy resulted in a viral relapse with a viral population dominated by V36A resistant
variants. Subsequently, this patient had a transient disappearance of HCV RNA for more than
1 year in the absence of antiviral therapy. Thereafter, HCV RNA reappeared again with a viral
population consisting of only wild type virus. No other cases of a temporary viral clearance
after treatment failure in a DAA containing regimen have been reported so far. We propose that
this genetic bottlenecking may occur whenever a treatment regimen produces an extremely
significant reduction in viral load. We hypothesize that the reduction in viral heterogeneity
potentially led to a reduced viral capacity to adapt to a host immune response leading to a
transient loss of detectable HCV RNA. Long-term follow-up of patients who fail to respond to a
combination regimen including a direct-acting antiviral agent is needed to further study these
phenomena.
Clearing chronic hepatitis C without interferon and/or ribavirin

Resistance to antiviral drugs is classically prevented by combining several drugs with potent antiviral activity and no cross-resistance. Several anecdotal cases of HCV eradication (one chimpanzee and two patients) with DAAs and without interferon were previously reported, providing evidence that chronic HCV infection could be cleared without exogenous IFN administration. The Inform-1 study provided a proof of concept for an oral approach to the treatment of chronic hepatitis C, in which a combination of DAAs was safely co-administered without peg-IFN and ribavirin. This phase 1 study examined the safety and antiviral efficacy of a combination treatment of danoprevir (protease inhibitor) and RG7128 (nucleoside inhibitor) during 2 weeks. Oral treatment was administered to 73 genotype 1 infected patients and resulted in a median viral load reduction of 5.1 log_{10} IU/mL after 13 days. No viral breakthrough occurred during the two week course, owing to the high barrier to resistance of this combination therapy. Other dual DAA combination treatment studies were subsequently initiated to enhance SVR rates but were hindered by rapid selection of resistance associated variants in the majority of patients. Triple combinations with ribavirin prevented most of these breakthroughs, but still selected for single mutations in a minority of patients within 4 weeks. Next, quadruple therapy for 24 weeks, combining a NSSA inhibitor (BMS-790052) with a protease inhibitor (BMS-650032) with or without peg-IFN and ribavirin was studied in previous null-responders. Quadruple therapy resulted in an impressive 90% SVR rate (n=9/10) in this difficult-to-treat population. Combination therapy with BMS-790052 and BMS-650032, but without peg-IFN and ribavirin, resulted in a 36% SVR rate (n=4/11). This study does provide new hope for a growing number of HCV infected patients who cannot be effectively treated with current treatment regimens. It also clearly demonstrated that chronic HCV infection can be cured without the addition of peg-IFN and ribavirin.

Remaining key questions for combining several DAAs include the number of drugs that should be used together, how they should be combined to achieve a high genetic barrier to resistance, and their ability to trigger a sustained second slope of viral decline that will eventually lead to viral eradication, especially in patients in whom a triple combination of peg-IFN, ribavirin and a protease inhibitor failed to eradicate HCV. Mathematical modelling suggested that at least three drugs should be used, but the final number will depend on drug-drug interactions, their modes of action and the likelihood that HCV variants bearing substitutions in different regions of the genome conferring resistance to the different classes of drugs are present in the same strain. Recent data suggest that, even in the absence of peg-IFN, ribavirin accelerates the second slope of viral decline, prevents relapses and may eventually increase SVR rates, as it does when combined with peg-IFN, through molecular mechanisms that remain to be identified. Although many experts anticipated that ribavirin would become redundant with the rise of DAAs, it seems that a pivotal role in preventing viral resistance is awaiting ribavirin. Immune control induced by peg-IFN administration was thought to be crucial for clearance of chronic HCV-infection. Our perspective on the necessity of peg-IFN immune modulation to cure chronic hepatitis C should be altered in the context of successful DAA combination studies. Interferon, the first drug that was registered for the treatment of hepatitis C, might potentially be the first to become superfluous.

Figure 1. Potential therapy outcome with direct-acting antiviral combination therapy.

Sustained virologic response (SVR) rates in HCV genotype 1 infected patients increase from 40% with pegylated interferon (peg-IFN) and ribavirin (RBV) to approximately 65% or more with the addition of a NS3/4A protease inhibitor (PI). Relapse rates are expected to decline, although rates of discontinuation may well increase because of the increased risk of adverse effects with triple therapy. In the more distant future, higher response rates will hopefully be achieved by combining multiple drug regimens that limit discontinuation rates, enhance efficacy and tolerability, and with some possible small enhancement in relapse and non-responder rates. peg-IFN, pegylated interferon; RBV, ribavirin; PI, protease inhibitor; (N)NI, (non-)nucleoside inhibitor; NSSA, inhibitor of NSSA; Cyclophilin, inhibitor of cyclophilin.

III. IMMUNE MODULATING THERAPY

Treatment with peg-IFN and Ribavirin

IFN has potent antiviral activity but does not act directly on the virus or replication complex. Rather, it acts by inducing IFN-stimulated genes (ISGs), which establish a non-virus-specific antiviral state within the cell and IFN has important interactions with the adaptive and innate immune responses. In Chapter 12 we investigated the phenotypic and functional characteristics of the total CD8+ T-cell population of Cytomegalovirus (CMV)-seropositive individuals with and without chronic
hepatitis B or C to determine the effect of eventual clearance (HCV) or suppression (HBV) of the virus upon IFN-based therapy. Other studies showed that chronic systemic ‘latent’ viral infections such as CMV infection are known to leave a fingerprint in the total T-cell population, identified by an increased percentage of highly differentiated effector-memory type T-cells. We studied whether chronic viral infections with a ‘persistent’ viremia, such as chronic hepatitis B or chronic hepatitis C, that are characterized by local organ-specific inflammation, also impact the total peripheral T-cell population or other virus specific T-cells that do not target hepatitis viruses. The results showed no phenotypic or functional differences between CD8+ T cells in HBV or HCV infected patients and healthy controls. However, expression of the chemokine receptor CXCR3 was significantly higher on total peripheral CD8+ T-cells (p<0.005) in patients with chronic hepatitis B or C compared to healthy controls. Chemokine receptors such as CXCR3 are important for T-cell recruitment to the liver and chemokine ligands specific for CXCR3 have been found to be upregulated in chronic hepatitis. The higher expression of CXCR3 on T cells may reflect the pervasive influence of a persistent viral infection, even when restricted to the liver, on total peripheral blood CD8+ T cells. Modulating chemokine (receptor) expression could be a potential target for future therapy optimizing the anti-viral immunologic environment in the liver.

Recently, it was shown that non-responders have an upregulated and largely ineffective IFN response, so that administration of exogenous IFN adds little.52 Another study provided unequivocal evidence that patients who responded poorly to therapy show pre-activation of their IFN system, and that the pre-activation is confined to the liver and was not evident in PBMCs.53 In patients with low initial ISG expression, representing future responders to therapy, expression of ISGs in response to peg-IFN did not exceed that seen in non-responders, either before or after therapy. This could suggest that patients with the initial pre-activation of the IFN system, future non-responders, have some defects at steps downstream of ISG expression. These findings in human patients are in accordance with observations in chimpanzees chronically infected with HCV.54 These results suggest that downstream inhibitors, either viral or host-related, are active, rendering both endogenous and exogenous IFN ineffective. In this group of IFN non-responders (oral) treatment regimens consisting of DAAs only could prevent ineffective exogenous peg-IFN administration.

Initially synthesized as a guanosine analogue in 1970, ribavirin was immediately recognized to possess activity against several RNA and DNA viruses. The addition of ribavirin to IFN therapy led to marked improvements in SVR rate and decreased the relapse rate in HCV-infected patients.55

The addition of ribavirin to IFN is suggested to enhance the second phase-phase viral decline.56

How ribavirin augments the response rate to IFN is not known, but multiple mechanisms have been proposed, each with some experimental support. These include a direct inhibition of viral replication by early chain termination, a competitive inhibition of ionsine monophosphate dehydrogenase resulting in depletion of GTP pools and viral mutagenesis leading to non-viable mutants.57 Induction of IFN-stimulated cytokines also correlated with viral kinetics following ribavirin therapy, suggesting that ribavirin promotes IFN signalling.58 Ribavirin in combination with a HCV specific protease- and polymerase inhibitors showed that ribavirin was essential to prevent viral mutagenesis, viral breakthrough and relapse, suggesting that ribavirin has direct antiviral activity in vivo.59-60

The last decade, interferon-based treatment formed the cornerstone of hepatitis C treatment and Peg-interferon will probably remain the backbone of HCV therapy in the near future. However, many new advances in immune stimulation, interferon drug design and administration systems, are being developed. Novel approaches, include controlled interferon release, stimulation of the immune system with interferon lambda or Toll-like receptor (TLR) agonists and prolonging the half-life by combining interferon with albumin (Alb-interferon). These novel approaches aim to offer improved efficacy, less frequent dosing and enhanced tolerability compared with regular Peg-interferon.

Stimulation of the immune system with interferon lambda or TLR agonists is potentially a promising alternative to regular peg-IFN alpha therapy. Interferon lambda (IL-29) is a member of the type 3-interferon family with functional similarities to type 1-interferons, which include interferon alpha. Interferon alpha and lambda share the same intracellular signal transduction pathways. Although all cell types in the liver express the interferon alpha receptor, the interferon lambda receptor distribution is more limited, with less expression in hematologic cells and in non-hepatocyte liver cells.61 The limited distribution of the interferon lambda receptor suggests the potential for reduced adverse events with interferon lambda-based therapy. Recently, an ongoing Phase II study compared the efficacy and safety of ribavirin with peg-IFN lambda or peg-IFN alpha in treatment-naive HCV-infected patients. A planned interim analysis at week 12 showed a superior virologic response in genotype 1- and 4-infected patients who received peg-IFN lambda. Virologic responses in genotype 2 and 3 patients were comparable between both types of interferon. Moreover, peg-IFN lambda was associated with less dose reductions (peg-IFN lambda at the 180 ug dose, 3.8%; peg-IFN alpha at the same dose, 18.8% dose reduction), less flu-like symptoms (9.9 vs 42.9%) and less bone marrow suppression (0.8 vs 15.2%) in peg-IFN lambda versus peg-IFN alpha therapy, respectively.

TLRs are a family of pathogen-recognition receptors. Binding of TLRs to invading microorganisms stimulates the immune system and initiates a host innate and adaptive immune response. Stimulating TLRs by TLR agonists enhances a virus-specific immunomodulatory response. In a recent clinical proof-of-concept study, a TLR7 agonist, isatiboribine, was shown to have an anti-viral effect in the treatment of chronic HCV infection.62 Chapter 13 describes a clinical study which was conducted to investigate the safety, pharmacodynamics, pharmacokinetics and efficacy of ANA773 in patients chronically infected with HCV. ANA773 is an oral prodrg of a small-molecule TLR7 agonist and was investigated in a double-blind, placebo-controlled study in 24 patients chronically infected with HCV of any genotype. This study demonstrated that oral ANA773 was well tolerated and induced a dose-related interferon dependent response leading to a significant decrease in serum HCV RNA levels. These findings justified further clinical exploration with ANA773 for the treatment of chronic HCV infection as a potential alternative to peg-IFN administration. Potential advantages of TLR agonists relative to weekly subcutaneous injections with peg-IFN include the route of administration (oral versus injection), stronger antiviral activity and an improved safety profile. It will be of particular interest to investigate this drug in combination with DAAs or ribavirin.
CONCLUSION

The quest for a definite cure for chronic hepatitis C is well underway. For the foreseeable future, triple combination of peg-IFN, ribavirin, and a protease inhibitor has become the new standard of care for patients infected with HCV genotype 1. Side effects and viral resistance remain serious problems and continue to present a major challenge for the development of potential novel therapeutic agents. Accurate prediction of triple combination treatment failure and response-guided treatment should help minimize pointless drug exposure and the selection of resistant viruses. As HCV infection is inherently curable, the development of new potent combinations of drugs with a high barrier to resistance will likely lead to effective control of chronic hepatitis C in parts of the world where such therapies are affordable. In countries who cannot bear the high costs of DAA therapy an effective prophylactic vaccine, which is currently not available, will remain the only viable option to prevent HCV infection.

REFERENCES


