Towards optimal treatment for chronic hepatitis C infection
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Summary

This thesis deals with approaches to improve the treatment of chronic hepatitis C virus (HCV) infection.

Chapter 1. Chronic HCV infection is an important liver disease. An estimated 130 million people are infected. This global epidemic has been created in part by modern medicine. The main mode of transmission is via contaminated blood (unsafe injections, blood transfusion, intravenous drug use and abuse). HCV is a positive strand RNA virus that is classified within the Flaviviridae family. Chronic HCV infection can ultimately lead to cirrhosis and hepatocellular carcinoma. Current treatment regimes are based on subcutaneous administration of interferon alfa (a cytokine) and oral ribavirin (a nucleoside analogue) for 12-48 weeks. This treatment is successful in only 50% of patients and causes significant side effects. Clinicians need tools to identify those patients that need treatment, and predict treatment outcome as early as possible. Currently many clinical studies are aimed at optimising the current treatment regimens (i.e., treating patients with a rapid response or no response to treatment as short as possible, and those with a slower response as long as necessary). Treatment duration depends on the HCV subtype (genotype) and the speed of the HCV RNA decline during treatment. Other studies focus on the identification of new antiviral compounds, and predictors of response and non-response.

In chapter 2 the performance of a new real-time PCR assay for detection and quantification of HCV RNA is described. Decisions on treatment duration are nowadays based on HCV RNA levels at baseline, week 4, week 12 and week 24. When used for guidance of treatment duration, reliable and accurate detection and quantification of HCV RNA are mandatory. However, we demonstrated that the assay was not as sensitive as the manufacturer claimed, and the HCV RNA quantification for non-1 HCV genotypes—a common pitfall—was poor. We were not the only ones to find these results. The assay, that had already received FDA and CE approval, had to be retracted from the market.

In chapter 3 we describe 2 clinical trials in difficult-to-treat chronic HCV patients treated with high doses of interferon alfa during the first 6 weeks, followed by weekly peginterferon alfa, ribavirin and amantadine hydrochloride for a total of 24 or 48 weeks. We aimed to shorten treatment duration in patients with a fast-response (≥ 3 log_{10} HCV RNA decline at week 4), and to predict treatment outcome as early as possible through frequent assessment of HCV RNA. We found that fast-responders can be treated for 24 weeks, without compromising sustained virologic response rates. We could predict positive and negative treatment outcome using HCV RNA levels within the first 6 weeks of treatment in the majority of patients. In some patients we were able to predict an unfavourable outcome after just 1 day of treatment.

Chapter 4 deals with a new phenomenon that we observed in the studies described in chapter 3. We monitored treatment at regular intervals with an assay for detection of HCV RNA that
is 10 times more sensitive than PCR. We found reappearance of low levels of HCV RNA after 16 or 20 weeks of antiviral therapy in patients with subsequent breakthrough or relapse, but not in patients who achieved SVR. Thus, reappearance of low levels of HCV RNA during treatment proved to be an early sign of impending treatment failure.

Chronic HCV infection is essentially an immunologic disease, as patients with chronic HCV are those that have failed to achieve immunologic control during the acute infection. It is unclear if successful treatment with interferon alfa and ribavirin leads to an improvement in the HCV specific T-cell response. Chapter 5 deals with the function of HCV specific T-cells during antiviral therapy in 31 HCV genotype 1 patients from one of the 2 studies described in chapter 3. Chronic HCV infection is characterised by a loss in IL-2 secreting HCV specific T-cells. We hypothesized that successful treatment would lead to a restoration of IL-2 secreting HCV specific T-cells. However, we observed that T-cell function in general declined during interferon alfa treatment, irrespective of treatment outcome. Our results suggest that successful treatment outcome in the setting of high dose interferon induction treatment is due to the direct antiviral effects of interferon alfa.

In chapter 6, we describe development of diabetes mellitus in 9 patients with chronic HCV infection treated with standard peginterferon alfa and ribavirin therapy or with the high dose regimen described in chapter 3. Patients with chronic HCV infection frequently suffer from concomitant autoimmune disease, and treatment with interferon alfa can trigger autoimmune disease. Prompted by 2 chronic HCV patients who developed diabetes mellitus during treatment with interferon alfa, we started monitoring glucose levels in all chronic HCV patients before, during and after treatment. We found that the incidence of diabetes mellitus, type 1 diabetes mellitus in particular, was much higher than previously reported.

Chapter 7 deals with assessment of neopterin and ALT as markers of inflammation in chronic hepatitis C patients during administration of the new HCV NS3•4A protease inhibitor telaprevir (VX-950) and/or peginterferon alfa 2a, in 2 phase 1b trials. Administration of telaprevir not only resulted in a rapid decrease in HCV RNA, but also in a rapid decrease in inflammation.

In chapter 8 we describe ex vivo generation of monocyte derived dendritic cells from chronic HCV patients and their form and function. Dendritic cells are professional antigen presenting cells that drive the T-cell response. In theory, therapeutic vaccination with autologous dendritic cells could be applied as an adjuvant therapy to boost the HCV specific T-cell response in chronic HCV infection. However, we found that the dendritic cells showed an immature phenotype and aberrant cytokine pattern. Our results suggest that the dendritic cells–as generated with our protocol–would be of limited benefit.
Conclusion

Relevance of this thesis

The studies presented in this thesis represent some steps towards optimal treatment of chronic hepatitis C infection:

1. Assays for detection and quantification of HCV RNA must be reliable. We demonstrated the poor performance of a real time PCR assay that could have lead clinicians into making erroneous treatment decisions (chapter 2).

2. Our results with high dose interferon induction therapy suggest that treatment can be shortened in chronic hepatitis C patients with a rapid decrease in HCV RNA during the first 4 weeks of treatment (chapter 3).

3. Our studies with high dose interferon induction therapy show that frequent assessment of HCV RNA allows earlier prediction of SVR and non-SVR. Thus treatment can also be shortened in patients with predicted non-SVR (chapters 3 and 4).

These are important points. The major reason why week 12 and week 24 have become standard time points for assessment of HCV RNA is because these appeared to be the only time points when HCV RNA was assessed in the pivotal clinical trials for the registration of peginterferon and ribavirin. However, HCV RNA levels were also assessed at week 4 in the 3 clinical trials for the registration of peginterferon and ribavirin performed in 1998-2002 but the data were not shown in the original articles describing the overall results published in 2001, 2002 and 2004. An independent clinical trial published in 2005 showed that patients with HCV genotype 2 or 3 who were HCV RNA negative by PCR at week 4 can be treated for 12 weeks instead of 24 weeks. Subsequently additional data analyses from the 3 clinical trials for the registration of peginterferon and ribavirin describing the predictive value of HCV RNA assessment at week 4 and possibility of shorter treatment were published later in 2005, 2006. Thus additional data from 3 large clinical trials were published 2-4 years after the main articles. It is interesting to note that the 3 pivotal clinical trials for the registration of peginterferon and ribavirin were sponsored by the manufacturers of the drugs. It is clear that duration of treatment is of importance for the pharmaceutical industry. Any delay in


publishing this information could be explained as an action that is commercially driven and not in the interest of our patients.

4. We have shown that the HCV specific adaptive immune response does not improve during successful high dose interferon alfa therapy (chapter 5).

5. Side effects and adverse events during interferon alfa and ribavirin therapy are notorious and dangerous. We have shown that the incidence of diabetes mellitus during and shortly after therapy is much higher than previously reported. We propose routine glucose monitoring as an easy method to identify all patients who develop diabetes mellitus during therapy (chapter 6).

6. Monotherapy with the HCV specific protease inhibitor telaprevir (VX-950) results in a rapid decrease in HCV RNA, but also a rapid decrease in hepatocyte injury and monocyte/macrophage activity (chapter 7). Our results show that HCV related inflammation is multifaceted, and that different antivirals have different pro- and anti-inflammatory effects.

7. There is no risk of reintroduction of virus during therapeutic vaccination with autologous dendritic cells from chronic hepatitis C patients. However, the dendritic cells—as we generated them—will probably not enhance HCV specific immunity (chapter 8).

**Treatment for chronic hepatitis C in the future**

The future of HCV treatment is bright. Direct inhibitors of viral enzymes such as the NS3 serine protease, NS3 helicase, and NS5B RNA-dependent RNA polymerase, monoclonal and polyclonal antibodies, antisense RNA, therapeutic vaccination, Toll-Like Receptor agonists and other immunomodulatory drugs, modifications of interferon and ribavirin, and other compounds that inhibit HCV are currently being tested in phase I to IV clinical trials. Frequent monitoring of HCV RNA during treatment will help tailor treatment regimes (i.e., treating each patient as long as necessary, but not any longer). With HCV specific antivirals, future treatment of chronic HCV infection will hopefully be shorter, and will probably consist of combination therapy with these new antivirals and interferon alfa plus ribavirin. Side effects during new treatment regimens will probably be less.

**The future of hepatitis C**

In summary, HCV infection is a global problem that can be dealt with. This will take time, but it can be done: A combined effort of basic researchers, clinicians, public health officials and other healthcare workers, patient organisations, governments and the pharmaceutical industry should lead to better treatment modalities and treatment strategies, identification of infected persons, prevention of transmission among intravenous drug users and in the developing world, lower prices for the current and new drugs in the developing world, and ultimately a preventive vaccine.