Direct-acting antiviral therapy for chronic hepatitis C

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SUMMARY

The main focus of this thesis is to improve our understanding of direct-acting antiviral therapies for chronic hepatitis C. This thesis comprises the in vitro and in vivo development of several direct-acting antivirals. In addition, we studied the emergence and persistence of viral resistance upon (re-)treatment with NS3/4A protease inhibitors. The different chapters will be briefly summarized.

One of the mechanisms to combat HCV infection is to prevent HCV entry into the hepatocyte. In chapter 2 we presented the safety and antiviral activity of JTK-652, a novel HCV infection inhibitor. JTK-652 showed potent inhibitory activity against HCV genotype 1a and 1b pseudotyped viruses bearing HCV E1E2 envelope proteins in HepG2 cells and human primary hepatocytes. Subsequently, a phase 1 study was initiated in which 10 HCV genotype 1 infected patients received an oral dose of JTK-652 or placebo for 4 weeks. Eight patients completed the study. Premature discontinuation occurred in 2 patients (1 active, 1 placebo) due to an almost generalized rash of mild intensity. This rash disappeared within days after stopping JTK-652 administration and additional treatment was not necessary. No significant clinical laboratory, vital signs, electrocardiogram or physical examination abnormalities were observed. At end of treatment, no significant changes in HCV RNA levels compared to baseline were observed in the individual patients. Results from this phase 1 study indicated that, beside a mild rash in 1 patient, JTK-652 was safe and well tolerated. However, plasma HCV RNA levels did not decline during 28 days of dosing and further development of this compound was stopped.

Safety and antiviral activity of narlaprevir, a NS3/4A protease inhibitor, administered as monotherapy or in combination with ritonavir and peg-IFN in chronic hepatitis C patients is described in chapter 3. This was a randomized, placebo-controlled, two period, blinded study in 40 HCV genotype-1 infected patients (naïve and treatment-experienced). Narlaprevir (with or without ritonavir) alone or in combination with peg-IFN was found to be safe and well tolerated. Pharmacokinetic studies showed that the trough plasma concentrations of narlaprevir were greater than the 90% inhibitory concentration as determined by the HCV replicon assay. A rapid and persistent decline in plasma HCV RNA levels was observed in both treatment-experienced and naïve patients. Population sequencing showed that 5 narlaprevir dosed patients had at least one of the following resistance associated variants: R155K, A156T/S, V36M/L. This study demonstrates that administration of narlaprevir for two weeks (with or without ritonavir) plus peg-IFN followed by standard of care for 24 weeks or 48 weeks resulted in 81% and 38% SVR in treatment-naïve and experienced patients, respectively. 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.

Chapter 4 demonstrates the great anti-viral potential of macrocyclic protease inhibitors such as IDX320. The pharmacological profile of macrocyclic protease inhibitors offers the opportunity for once daily dosing. In contrast, registered linear protease inhibitors boceprevir and telaprevir should be taken three times daily which could hamper the compliance and cause additional side-effects, such as rash and anemia. This randomized, double-blind, placebo-controlled, multiple dose, phase 1 study assessed the safety, tolerability, antiviral activity and pharmacokinetics of IDX320 in treatment-naïve patients with chronic HCV genotype 1 infection. Single and multiple doses of IDX320 were safe and well tolerated. Oral, once daily, administration of IDX320 for 3 days resulted in a rapid HCV RNA decline in all patients. Although the results of this study demonstrated the clinical potential of IDX320, further development of IDX320 was stopped when 3 serious adverse events of elevated liver enzymes occurred during a drug-drug interaction study of IDX320 and IDX184 (nucleotide HCV polymerase inhibitor).

An accelerated trial design was used to investigate the safety, tolerability, and pharmacokinetics of PHX1766 in healthy volunteers and hepatitis C patients, which is presented in chapter 5. PHX1766 is a potent, tight-binding, reversible, and highly selective NS3/4A protease inhibitor in the HCV replicon assay. Single and multiple doses of PHX1766 were safe and well tolerated. This novel overlapping single- and multiple-dose escalating study design was highly informative in a short period of time and the most cost-effective approach without compromising the safety of the participants. Unfortunately, PHX1766 dosing resulted in only a modest HCV RNA decrease, in contrast to the in vitro replicon results, which indicated a robust viral load decline. Consequently further development of PHX1766 was not pursued. This study illustrates that in vitro activity of an antiviral compound is not always a reliable predictor of antiviral activity in vivo.

Among various classes of direct-acting antivirals (DAAs), several non-nucleoside inhibitors of the HCV NS5B polymerase are being clinically evaluated and have shown robust antiviral activity with a favourable toxicity and adverse event profile. IDX375 is a non-nucleoside polymerase inhibitor that was administered for one day to forty healthy male subjects and three patients chronically infected with HCV genotype-1 (Chapter 6). IDX375 was well-absorbed and well-tolerated in all study participants, without dose-limiting toxicity. A single-day 200 mg twice daily dose resulted in exposure-related anti-HCV activity with maximal 0.5-1.1 log_{10} reductions in plasma HCV RNA. Considering the long half-life, trough concentrations above this threshold can easily be achieved with once or twice daily dosing. These initial observations support further clinical investigations of IDX375 at higher doses with longer duration.

In chapter 7 we investigated the genetic diversity and evolutionary origin of HCV-4 infection. NS5B amplification revealed 9 distinct HCV-4 subtypes; two of which could not be assigned to a previously recognised subtype. The majority of HCV-4 isolates were of subtype 4d (n=70, 56.5%) and 4a (n=45, 36.3%). Three distinct phylogenetic patterns of HCV-4 were observed, which corresponded 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). This study demonstrates that HCV-4 is effectively spreading within The Netherlands. 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. Most clinical trials are focused on patients infected with genotype 1, despite the high worldwide prevalence of HCV genotype 4 infections.
A foreseen consequence of treatment with direct-acting antivirals is the development of resistance. Drug-resistant mutations have been shown to be selected in vitro and in vivo in the presence of numerous HCV-specific antivirals. Three chapters presented in this thesis assessed the presence of viral resistance at baseline, during therapy with protease inhibitors and persistence of resistant variants at (long-term) follow-up.

The longitudinal clonal sequencing results of patients who received narlaprevir is presented in chapter 8. This study demonstrated that narlaprevir rapidly selected (high-level) resistant variants in genotype 1a infected patients leading to therapy failure. The viral population consisted of only wild-type virus at baseline. Narlaprevir monotherapy resulted in minor variants at positions 36, 54, 155 and 156. After narlaprevir re-exposure mutations that confer low-level resistance and high-level resistance to narlaprevir were detected in 5 patients in >95% of the clones. For HCV-infected patients who do develop resistance after therapy with protease inhibitors, minimal data exist on the persistence of viral variants that can emerge. We showed that wild-type virus regained its dominance over time although resistant variants remained present for more then 3 years after narlaprevir dosing in 2 patients. This is important, because persistence of resistance could potentially lead to a quick outgrow of resistant-associated variants upon re-treatment with a cross-resistant antiviral and subsequently result in therapy failure.

Based on anti-retroviral monotherapy trials in HIV-infected patients, there is a concern that patients who have been exposed to a DAA will continue to carry mutated drug-resistant strains, narrowing future treatment options. 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 protease inhibitors from the same class. The aim of this 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 ultra-deep pyrosequencing (UDPS). Both clonal sequencing and UDPS methods showed that resistance mutations are detectable at a low frequency at baseline and that the 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 TVR dosing. This study demonstrates that frequencies of resistant NS3 variants measured by two extensive sequence analysis techniques are comparable at baseline and after 4 years of follow-up in most patients.

In chapter 10 a detailed genotypic analysis of resistance-associated variants at baseline and during TMC435 re-treatment was assessed using population-, clonal- and 454 sequencing techniques. TMC435 is a macrocyclic NS3/4A protease inhibitor. Six genotype-1 infected patients received TMC435 monotherapy for 5 days in a previously performed phase 1 study. Approximately 1.5 years later, 5/6 patients were re-treated with TMC435 combined with Peg-IFN and ribavirin. We showed that viral variants that emerged during first exposure to TMC435 were mostly replaced by wild-type virus over time, while some persisted at low frequencies based on deep-sequencing analysis. In 3/5 patients a fast initial virologic response was observed upon TMC435 re-treatment which resulted in undetectable HCV RNA levels at week 4 and subsequently a sustained viral response. This study showed that successful re-treatment after prior exposure to TMC435 with emergence of resistance variants is possible.

Combination therapy with a protease inhibitor, peg-IFN and ribavirin significantly increased the sustained viral response rate in treatment-naïve and treatment-experienced patients infected with HCV genotype 1. The complexity of (future) combination DAA-based therapies, including viral resistance and additional side effects, is illustrated by the case described in chapter 11. This previous non-responder to interferon based therapy was treated for 40 weeks with a telaprevir, peg-IFN, and ribavirin regimen. At week 40, peg-IFN/RBV treatment was discontinued due to grade 3 laboratory side effects. This patient experienced a viral relapse 4 weeks after the end of dosing with a viral population consisting of only V36A 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. This case report showed that significant viral load reductions resulted in a genetic bottleneck leading to a reduction of variability in the hepatitis C viral population. 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.

The mechanism by which interferon activates and stimulates the antiviral-activity of the adapted immune system is largely unknown. The phenotypic and functional characteristics of the CDB8 T-cell population of cytomegalovirus (CMV) seropositive individuals with and without chronic hepatitis B or C are described in chapter 12. We investigated whether chronic HBV or HCV infection can alter the total peripheral CDB8 T-cell population just like has been described for CMV infected patients. No phenotypic or functional differences were found between CDB8 T cells in viral hepatitis or healthy controls. However, expression of the chemokine receptor CXCR3 was significantly higher on total peripheral CDB8 T-cells in patients with chronic hepatitis B or C compared to healthy controls. 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 CDB8 T-cells. In fact 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 patients with chronic hepatitis. Further research is necessary in order to investigate whether modulating chemokine (receptor) expression could be a potential target for future therapy optimizing the anti-viral immunologic environment in the liver.

Toll-like receptors (TLRs) are a family of pathogen-recognition receptors that activate the innate immune response. 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. 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 produg 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. Mild to moderate adverse events were...
reported, with an increase in frequency and intensity with increasing dose, including a few patients reporting flu-like symptoms. There were dose-related increases in various markers of interferon-a response after administration of ANA773. The median maximum change in serum HCV RNA level from baseline was -0.30, -0.22, -0.49 and -0.79 log_{10} HCV RNA in the placebo, 800, 1200 and 1600 mg cohorts, respectively. At the 1600 mg dose, ANA773 significantly (p=0.04) reduced serum HCV RNA levels (range: -0.10 to -2.52 log_{10}), and these declines correlated with induction of markers of IFN-response. This study demonstrated that oral ANA773 was well tolerated and induced a dose-related IFN-dependent response leading to a significant decrease in serum HCV RNA levels at the 1600 mg dose. These findings justified further clinical exploration with ANA773 for the treatment of chronic HCV infection as a potential alternative to peg-IFN administration.

This thesis was aimed at gaining more insight into pathogenesis of chronic hepatitis C virus infection, its epidemiology, development of innovative treatments and upcoming viral resistance during therapy. We hope that the results of this thesis contribute to deeper understanding and further progress in development of novel molecular strategies against chronic hepatitis C virus infection.