Towards optimal treatment for chronic hepatitis C infection
Gelderblom, H.C.

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Chapter 1

General Introduction
Introduction

Chronic hepatitis C virus (HCV) infection is a major cause of liver disease [1, 2]. HCV is endemic in most parts of the world, and it is estimated that at least 170 million people are or have been infected with HCV worldwide [3]. HCV is transmitted mainly via the percutaneous route. The majority of infected people develop chronic HCV infection, which frequently results in progressive liver disease. HCV related end-stage liver disease is now the main indication for liver transplantation in the USA and Western Europe. There is no vaccine or immunoglobulin to prevent HCV infection. Reliable in vitro culture systems have only recently become available. Current treatment regimens, based on administration of interferon alfa and ribavirin for 24-48 weeks, result in sustained remission of viremia in ~50% of patients. New classes of antiviral drugs that are expected to improve treatment significantly are currently being tested in phase I and phase II clinical trials.

Epidemiology

HCV is an important infection in both developed and developing countries [3, 4] (Figure 1). Compared to other viral infectious diseases such as chronic hepatitis B virus infection (HBV, 350 million people with chronic infection worldwide) and infection with human immunodeficiency virus type 1 (HIV-1, 40 million people infected worldwide), HCV is particular in the sense that it also is quite prevalent in developed countries (estimated seroprevalence 0.5% in the Netherlands, 1.1% in France, 2.2% in Italy and 1.8% in the USA, Table 1). The estimate of 170 million people that have been infected worldwide is based on studies that reported seroprevalence data of antibodies to HCV, but did not assess presence

Figure 1. Worldwide anti-HCV seroprevalence in 2001, source WHO, 2002 [242].
Chapter 1

of HCV RNA [3]. Given the fact that about 75% of those infected do not clear the virus and develop chronic infection, the actual number of people with chronic HCV infection is roughly around 130 million worldwide. The data are from selected populations and countries, and data obtained from population based studies are scarce [3, 4]. Therefore, the true number of HCV infected people may be underestimated. About 4.5 million people worldwide may be co-infected with HCV and HIV-1 [5]. About 30% of HIV-1 infected people in the USA and Europe have been co-infected with HCV, the rate of co-infection differs per region and depends on the main mode of transmission with the highest co-infection rates in hemophiliacs and intravenous drug users (IVDU).

The worldwide HCV epidemic is a result of the increased use of syringes, the therapeutic use of blood and blood products and other invasive and/or parenteral medical procedures in the 20th century [4, 6]. Re-use of inadequately sterilized syringes and needles during mass treatment and/or vaccination campaigns has caused the spread of HCV in countries around the world [7-9]. In the 1970s and 1980s, post-transfusion non-A non-B (NANB) hepatitis was the most frequent infectious disease transmitted by blood transfusion, with an incidence of 2-20% for Europe and 10-12% for the USA. An estimated 80-90% of post-transfusion hepatitis cases were classified as NANB [10]. After the discovery of HCV in 1989 [11] and the development of a serologic assay for the detection of HCV-antibodies [12], HCV turned out to be the main cause of NANB hepatitis [13-16]. Blood transfusion prior to the start of donation testing in 1991 appears to account for between 6 and 12% of all infections with HCV in the United Kingdom. Approximately 24,000 recipients were estimated to have been infected with HCV, and by the end of 1995, 9100 of these were estimated to be alive [17].

### Transmission / Risk factors

The main route of HCV transmission is parenteral via contaminated blood [18]. However, in up to 50% of cases no recognizable risk factor can be identified. Persons at considerable risk for HCV infection include recipients of unscreened blood, blood products and organs (before 1991/1992 in developed countries); and anyone who injects drugs or has ever injected drugs, even if only once [19]. Other (minor) risk factors include haemodialysis, occupational exposure to blood, intranasal cocaine use, sexual promiscuity, beauty treatments, professional pedicure and manicure, ear piercing, incarceration, contact sports, barber shop shaving, sharing of injecting paraphernalia other than syringes or needles among injecting

<table>
<thead>
<tr>
<th>region</th>
<th>No. of people with chronic HCV</th>
<th>Prevalence of chronic HCV</th>
<th>Mortality due to HCV in 2002</th>
<th>No. of people with HIV-AIDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>total</td>
<td>128,000,000</td>
<td>2%</td>
<td>366,000</td>
<td>40,000,000</td>
</tr>
<tr>
<td>Western Europe</td>
<td>7,000,000</td>
<td>1%</td>
<td>17,000</td>
<td>720,000</td>
</tr>
<tr>
<td>North America</td>
<td>4,000,000</td>
<td>1.3%</td>
<td>10,000</td>
<td>1,300,000</td>
</tr>
<tr>
<td>Sub-Saharan Africa</td>
<td>18,000,000</td>
<td>3%*</td>
<td>45,000</td>
<td>25,000,000</td>
</tr>
</tbody>
</table>

Data from [3, 19, 240], [241]*, WHO and UNAIDS
drug users, and medical or dental procedures with inadequately sterilized instruments or shared equipment [14, 15, 20-29]. Transmission through sexual exposure or household exposure occurs but seems uncommon [30, 31]. An inoculum of 20 copies may be sufficient to transmit infection [32-35], and HCV remains viable in the environment for more than 16 hours [36], this may explain why in some patients no risk factors can be identified. The extent of household transmission may also depend on the prevalence of HCV in the population and on behavioural factors [30]. HCV RNA has been demonstrated in semen [37], saliva [38], bile [39], feces [40], cervical smears [41], cornea [42], tear fluid [43], toothbrushes [38], and ticks [44]. Recent reports on recently acquired HCV infection indicate that sexual transmission may occur more frequent than previously assumed [24, 45]. Perinatal infection occurs mainly when the mother is co-infected with HIV [46].

Prospective studies of health care workers who have experienced accidental percutaneous exposure indicate that HCV is less transmissible than HBV, and more than ten times more transmissible than HIV [47] (Figure 2).

<table>
<thead>
<tr>
<th>Prevalence of HIV-AIDS</th>
<th>Mortality due to HIV in 2005</th>
<th>No. of people with chronic HBV</th>
<th>Prevalence of chronic HBV</th>
<th>Mortality due to HBV in 2002</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.67%</td>
<td>2,800,000</td>
<td>350,000,000</td>
<td>5.8%</td>
<td>563,000</td>
</tr>
<tr>
<td>0.1%</td>
<td>18,000</td>
<td>1,400,000-3,500,000</td>
<td>0.2-0.5%</td>
<td>2200-5600</td>
</tr>
<tr>
<td>&lt;0.5%</td>
<td>12,000</td>
<td>600,000-1,500,000</td>
<td>0.2-0.5%</td>
<td>1000-2400</td>
</tr>
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<td>3.8%</td>
<td>2,000,000</td>
<td>52,000,000-130,000,000</td>
<td>8.20%</td>
<td>84,000-210,000</td>
</tr>
</tbody>
</table>

In developed countries, blood donations are now screened using third and fourth generation tests for detection of HCV antibodies in combination with tests based on selective amplification of HCV specific RNA (Nucleic Acid Testing, NAT). The estimated risk of transfusion transmission of HCV is now 1 in approximately 2,000,000 to 30,000,000 transfusions [33, 48-50]. In comparison, the chance of death after a liver biopsy is 1 in 10,000-
12,000 [51], and the lifetime odds for dying in a transport accident in the USA are 1 in 78 [52]. Of the 38 deaths related to blood transfusion in the UK between 1996 and 2001, from a total of 15,900,000 components transfused, none was related to viral infection [53]; the 38 deaths were associated with other transfusion hazards, such as transfusion-associated graft versus host disease (n=13, risk 1:1,220,000), bacterial and parasitic infections (n=7, risk 1:2,860,000), transfusion-related acute lung injury (n=6, risk 1:2,650,000), the wrong blood transfused to the wrong patient (n=5, risk 1:3,180,000), and acute and delayed transfusion reactions [54]. However, one should not forget that many people who now suffer from the consequences of chronic HCV infection acquired the disease through blood transfusion before 1991.

**Continuing spread of HCV in the developing world through unsafe injections and unscreened blood**

In contrast to the situation in the West, the risk factors in developing countries are similar to those in developed countries prior to 1992. The use of unscreened blood or blood products is still widespread. Parenteral exposure to blood occurs through use of inadequately sterilized instruments and needles during procedures such as tattooing, piercing, acupuncture, scarification, circumcision, other forms of traditional medicine, or inadequate disposal or even handing out to children of used syringes and needles by local dispensers or primary care workers [47, 55-57].

Hauri et al. estimated that in 2000, unsafe injections caused approximately 2,000,000 HCV infections, 21,000,000 HBV infections and 260,000 HIV-1 infections, accounting for 40%, 32% and 5%, respectively of new infections [53]. The number of unsafe injections (defined as the reuse of syringe or needle between patients without sterilization) in developing countries is still substantial and model based estimates suggest that 2.3-4.7 million HCV infections may result every year from unsafe injections (Figure 3) [47, 55]. Blood transfusions are not included in these estimates.
The most dramatic example of unsafe injection practices is probably the transmission of HCV through the campaigns for parenteral treatment of schistosomiasis in Egypt, which represents the largest ever iatrogenic transmission of blood borne pathogens [58].

**Virology**

HCV was discovered in 1989 [11]. HCV is a hepatotropic virus that is the sole member of the genus hepacivirus in the Flaviviridae family [59]. The virus is ~50 nm in diameter. Within an envelope derived from host membranes is a nucleocapsid containing a positive-sense, single-strand RNA genome of ~9600 nucleotides [60]. The virus does not integrate in the host genome. A polyprotein from just over 3000 amino acids is translated from a single open reading frame. The viral genome is flanked by highly conserved 5’ and 3’ termini that contain signals for translation and replication. The polyprotein is processed by viral and cellular proteases into 10 structural and non-structural proteins [61] (Figures 4 and 5). Reliable in vitro culture systems that produce infectious virus have recently become available [62]. There are 6 major genotypes [63, 64], differing as much as 30 percent in their nucleotide sequences. HCV replication occurs at a high rate (10^{12} virions/day) and is error-prone, therefore HCV exists within the host as a quasispecies [65-67]. Patients can be co-infected with multiple HCV strains [68-72], but recombination is rare [73-76]. HCV also infects PBMCs and other non-hepatic cells, but the relevance of extrahepatic replication is unknown [77-84].

![Figure 4. HCV genome organization (top) and polyprotein processing (bottom). The central 9.6-kb open reading frame codes for a polyprotein of just over 3000 aa depending on the HCV genotype. The polyprotein processing and the location of the 10 HCV proteins relative to the ER membrane are schematically represented. Straight black arrows indicate ER signal peptidase cleavage sites; dashed arrows indicate autocatalytic cleavage of the NS2/NS3 junction, and the NS3/NS4A proteinase complex cleavage sites. Abbreviations: UTR, untranslated region; C, core; E, envelope, NS, non-structural.](image-url)
Natural history

Chronic HCV infection is not a new disease, but—given the slow pathogenesis and the recent spread of the epidemic—the period since the discovery of the virus 17 years ago is relatively short. Several retrospective studies but only few prospective studies on the natural history of HCV infection exist [85-87].

Transmission of HCV leads to an infection that is subclinical, and therefore undiagnosed, in most patients. Less than 20% of new infections lead to symptomatic acute hepatitis [88-90], fulminant hepatitis is rare [91]. HCV RNA is detectable 7-10 days after infection [92, 93], HCV specific antibodies are detectable after 49-70 days [94, 95]. In general about 75% of HCV infected persons develop chronic infection, and 25% resolve the infection [22, 49, 96, 97]. Recent studies have shown higher spontaneous clearance rates of 53%—after acute infection—among blood donors in Ghana [98], and 1% per year—after establishment of chronic infection—in Alaska Natives [99]. These high rates of spontaneous clearance probably depend on both host and viral factors.

Chronic HCV infection is defined as persistence of HCV RNA > 6 months after infection. The spectrum of the disease ranges from minimal chronic hepatitis, to chronic active hepatitis (activity may be intermittent), to progressive hepatitis with piecemeal necrosis, 10-20% of patients will develop cirrhosis after 20-30 years of chronic infection. Progression of the disease and risk of developing cirrhosis is slower in females and those who are young at the time of infection, but faster in persons with high disease activity, alcohol consumption, and
General Introduction

HIV co-infection [100]. In cirrhotics, 1-5% per year develop hepatocellular carcinoma (HCC). Death is caused by decompensated cirrhosis or HCC [1, 2, 85, 87, 101]. End-stage liver disease due to HCV infection is now the major indication for liver transplantation in the western world [102]. The incidence of HCC and the number of HCV associated deaths due to cirrhosis or HCC–now estimated at 10,000 per year–will increase in the USA over the coming decades [19, 103]. Although not all infected persons develop an active hepatitis, and many infected persons seem unaffected by the disease, it is not clear to which extent these people are at risk of progressing to active hepatitis and the late sequelae of HCV infection. Caution should be taken though as symptoms may be aspecific, and many patients suffer from extrahepatic diseases that are HCV related such as cryoglobulinemia, lichen planus, Sjögren’s syndrome, or others [104, 105]. All patients with newly diagnosed chronic HCV should therefore be evaluated thoroughly.

It is unknown whether the mode of acquisition affects the course of the disease. Young IV drug users with chronic HCV are more likely to die from IVDU related complications rather than from liver disease due to HCV [106]. About 50% of transfusion recipients die of causes other than HCV in the first decade after transfusion, and about 50% of transfusion recipients are alive >10 years after transfusion [97]. However, survival of transfusion recipients is similar to non-transfused people in those who are alive > 10 years after transfusion [97], and HCV related morbidity is expected in the period > 20 years after infection. This suggests that the number of patients with cirrhosis due to blood transfusion acquired HCV infection will increase in the coming decades [86].

Thus, chronic HCV is a slowly progressive disease and only a minority of all HCV infected persons will die from HCV related liver disease. However, due to size of the epidemic HCV now causes approximately 10,000 deaths per year in the USA (Table 1), and this number may increase in the years to come.

Figure 6. Generalized course of spontaneously resolving HCV infection (a) and chronic HCV infection (b). HCV RNA (black squares) is detectable in plasma within 1 to 2 weeks after infection. Plasma alanine aminotransferase (ALT) levels indicative of hepatocellular injury rise 2 to 8 weeks after infection. Symptoms are present in a minority of patients. Anti-HCV becomes detectable after a few weeks. After acute resolving infection anti-HCV persist in most patients. HCV RNA and ALT levels fluctuate considerably during the first 24 weeks. Chronic HCV infection is defined as persistence of HCV RNA in plasma > 24 weeks after infection.
Chapter 1

Immunology

Most patients exhibit strong HCV-specific CD8\(^+\)-T-cell and CD4\(^+\)-T-cell responses during the acute phase of infection and the outcome of acute HCV infection appears to be related to certain HLA class I and II alleles [107, 108], underlying the importance of T cell responses in attaining viral control.

Patients who spontaneously clear HCV infection maintain this strong and broad HCV-specific CD8\(^+\)-T-cell and CD4\(^+\)-T-cell response for years after resolution of the acute infection [109-112]. In patients who develop chronic infection the CD4\(^+\)-T-cell and CD8\(^+\)-T-cell responses are not sustained [113-115]. In chronic HCV infection the HCV-specific CD8\(^+\)-T-cell and CD4\(^+\)-T-cell response is generally weak and narrowly focused [109, 110]. Possible reasons for this include T cell exhaustion, and viral mutation and escape [116, 117]. However, T cell responses can be identified in patients with chronic HCV in the absence of viral mutation so that other explanations are also required.

Chronic HCV infection is associated with a significant loss of IL-2 secreting HCV specific CD4\(^+\)-T-cells compared to IFN-\(\gamma\) secreting cells, but in spontaneously resolved infection IL-2 secretion is preserved [118]. There is evidence that HCV infects B cells [77-83], CD4\(^+\)-T-cells [77, 79, 80], CD8\(^+\)-T-cells [77, 80, 82], monocytes [77-79], and dendritic cells [81-83, 119] in vivo, but these findings are controversial and how HCV subverts the functions of infected cells is not clear. Selective impairments in the function of professional antigen presenting cells known as dendritic cells have been described, and as these cells drive the T-cell response, this may be a cause of T-cell failure in chronic HCV infection [119-122]. There is evidence that HCV interferes with intracellular host defense mechanisms [123].

Some exposed but uninfected antibody negative persons do show cellular immune responses, this suggests that the rate of spontaneous clearance may be higher than the current estimate of 25% [98, 124-128].

Studies on the treatment of acute HCV infection have shown response rates of 90-100% with interferon alfa monotherapy, regardless of the HCV genotype [129]. The phenomenon that HCV is easier to treat when the infection is recent (< 6 months duration) suggests that in chronic infection the immune system has more difficulty to clear the virus. Interferon alfa has direct antiviral effects and immunomodulatory properties, ribavirin prevents virological relapse but by which mechanism is unknown [130]. A number of studies have suggested enhancement of HCV specific T-cell responses during successful antiviral therapy [131, 132], especially in patients with a rapid initial decline in HCV RNA [133, 134].

Detection

The diagnosis and management of HCV infection is based on a number of laboratory tests: (1) serologic assays detecting HCV core antigen or specific antibody to HCV (anti-HCV), (2) assays to detect and quantify HCV RNA, (3) assays to determine the HCV genotype [135].

Serologic assays

The presence of anti-HCV does not discriminate between resolved or ongoing HCV infection, 75% of anti-HCV positive individuals are also positive for HCV RNA [49, 96]. Anti-HCV may
disappear after spontaneously resolved infection [112]. Detection and quantitation of HCV core protein is possible using a HCV core antigen quantitative ELISA, but this assay is less sensitive than HCV RNA assays.

HCV RNA assays
Nucleic acid testing (NAT) methods such as RT-PCR and transcription-mediated amplification (TMA) can detect HCV RNA levels as low as 5 IU/mL, and can be used to confirm ongoing HCV infection [136]. Quantitative PCR and branched DNA methods are less sensitive but can be used to monitor HCV RNA load and HCV RNA kinetics during antiviral therapy. The branched DNA (bDNA) method is based on hybridization of HCV specific probes to HCV RNA followed by signal amplification. New quantitative real-time PCR methods with increased sensitivity may bridge the gap between lower limit of quantitation and lower limit of detection, but at present these assays are not always reliable [137-139]. The linear dynamic ranges of the quantitative assays and dynamic ranges and lower limits of detection of the qualitative assays are depicted in Figure 7.

**Figure 7.** Comparison of the linear dynamic ranges of the quantitative assays and dynamic ranges and lower limits of detection of the qualitative assays. HCV RNA levels are displayed as log10 IU/mL. HCV RNA levels are within the gray area in approximately 90% of untreated patients. Samples with HCV RNA above 100,000 IU/mL have to be diluted by a factor 10-100 for use with the Monitor assay. The TaqMan assay is qualitative below 43 IU/mL, the x depicts the lower limit of detection at 15 IU/mL. Both the TaqMan and Abbott are real-time PCR assays that have not been approved nor validated properly.

HCV genotype assays
The duration of interferon alfa and ribavirin combination therapy depends on the HCV genotype. Hence, the HCV genotype should be assessed in all patients. Two assays are routinely used for determination of the HCV genotype, one is based on direct sequencing and comparison of the sequence to a number of genotype reference sequences, the other is based on hybridization of PCR amplicons with genotype-specific oligonucleotide probes [135].

Persistence of HCV
A number of studies have demonstrated presence of low levels of HCV RNA in PBMCs and/or hepatocytes in some patients after spontaneous or treatment-induced resolution of HCV infection [77, 88, 140-153]. These findings may explain the relapses that have been observed in a small number patients [154-159]. Taken together, these findings suggest that spontaneously resolved acute HCV and treatment induced SVR may lead to a state wherein the virus can persist at very low levels, somehow unable to replicate to high levels. Other
viruses such as CMV, EBV, HBV, Herpes Simplex, measles virus, and woodchuck hepatitis virus are also known to persist at very low levels.

**Antibody negative HCV infection and occult HCV infection**
Cases of antibody negative HCV RNA positive infection have been described [50, 160], and prolonged periods of antibody negative HCV infection occur frequently in IVDU [70, 161, 162]. Castillo et al. [163-168] have recently described occult HCV infection in patients with elevated liver enzymes of unknown etiology, undetectable HCV RNA in plasma, and absence of anti-HCV. A number of these patients were positive for HCV RNA by in situ hybridisation and RT-PCR in liver tissue and PBMCs. Apparently, a carrier state of HCV infection can occur.

**Therapy**
Antiviral therapy for chronic HCV infection consists of administration of a modified form of recombinant interferon alfa (a cytokine) and ribavirin (a nucleoside analogue) for 12-48 weeks, leading to a sustained virologic response in ~ 50% of patients [169]. Both drugs were around before HCV was discovered and have been applied successfully as anti-HCV drugs. New classes of antiviral drugs that have been designed specifically for treatment of chronic HCV infection are currently being tested in phase I and phase II clinical trials.

**The past**

**Interferon alfa**
Interferons, discovered in the 1950s, are endogenous proteins with immunomodulatory and antiviral properties [170, 171]. The first study indicating that recombinant interferon alfa-2b might have a beneficial effect in chronic HCV infection (then known as chronic non-A, non-B hepatitis) was published in 1986 [172]. The first randomized clinical trials using interferon alfa-2b were started in 1986 and published just after the discovery of HCV [173-175]. These studies demonstrated normalisation of ALT in 38% and histological improvement in 50% of patients, normalisation of ALT was sustained in 16% of patients; HCV RNA levels could not be assessed at that time. The optimal duration of treatment initially appeared to be 48 weeks [176]. Subsequent trials demonstrated that 24 weeks of treatment was sufficient for patients infected with HCV genotype 2 or 3 [177, 178]. Modified forms of interferon alfa were created by adding a polyethyleneglycol (peg) moiety to the interferon alfa molecule, this resulted in an increased half-life facilitating once weekly administration. Three pivotal trials have demonstrated the superiority of one dose of peginterferon alfa per week compared to standard interferon alfa 3 times a week [179-181]
Ribavirin
Ribavirin is a nucleoside analogue with broad spectrum antiviral activity that was developed in the early 1970s [182]. The mechanism of action of ribavirin is unknown [130]. Ribavirin monotherapy results in minor decreases in HCV RNA [183, 184], but profound decreases in ALT levels [183-186]. The first study suggesting an effect on HCV was published in 1991 [185]. The first randomized controlled trial comparing ribavirin and interferon alfa plus ribavirin was published by Brillanti et al. in 1994 [187], combination therapy with ribavirin plus interferon alfa did not influence the response during treatment, but reduced the relapse rate after cessation of treatment. These findings were confirmed in 3 pivotal trials that were published in 1998 [177, 178, 188], and combination therapy with interferon alfa and ribavirin became the standard treatment for chronic HCV infection.

Amantadine
Amantadine is an old antiviral compound that is registered for treatment of Influenza A infection [189]. In vitro studies in Influenza A infected cells have shown that amantadine prevents virus assembly by blocking a virus specific ion channel [190]. Amantadine entered the HCV field in 1997 when Smith [191] reported a spectacular 18% SVR rate after 6 months of amantadine monotherapy in 22 IFN non-responders. To date these results have not been reproduced, Smith did not even mention SVR in her most recent paper [192]. Brillanti et al. published a small study in 1999 wherein 20 previous non-responders were treated for 24 weeks with interferon alfa plus ribavirin with or without amantadine: in the amantadine group 7 of 10 patients became HCV RNA negative during treatment and 3 of 10 achieved SVR, in the group without amantadine only 1 of 10 patients became HCV RNA negative during therapy and 0 of 10 achieved SVR [193]. The design of Brillanti’s amantadine trial was almost identical to his trial that demonstrated the benefits of ribavirin [187]; therefore, these results were considered very promising and led to dozens of clinical trials investigating the benefits of amantadine. The results from these trials are inconclusive, and a decade after the Smith study the benefits of amantadine are still unclear [194]. However, most of these trials were small, some patients received amantadine sulphate and some amantadine hydrochloride, not all patients were treatment naïve, interferon alfa dosing differed between trials, and patients were not stratified according to HCV genotype in some studies. Taken together, these findings suggest that a small antiviral effect of amantadine is likely, but difficult to prove, especially during combination therapy with the more powerful antivirals interferon alfa and ribavirin. The in vitro evidence is solid, a number of studies have shown that amantadine blocks the function of the HCV P7 ion channel, which is essential for virus assembly [195, 196].
The present

Peginterferon alfa and ribavirin combination therapy
The current standard antiviral therapy for chronic HCV infection consists of administration of peginterferon alfa and ribavirin for 12-24 weeks (genotype 2 and 3) [179-181, 197], 24-48 weeks (genotype 1) [179-181, 198], or 48 weeks (genotype 4) [179] and leads to a sustained virologic response in approximately 50% (genotype 1 and 4) to 80% (genotype 2 and 3) of patients. The duration of interferon alfa and ribavirin therapy is based on HCV genotype, baseline HCV RNA load [198], and the HCV RNA level after 4 weeks [197, 198], 12 weeks [199, 200], or 24 weeks of antiviral therapy [199]. Interferon alfa and ribavirin based therapy is expensive (~ €8000 in medication alone for 24 weeks) and causes a wide range of side effects in the majority of patients (Table 2)[51, 201, 202]. Therefore, HCV RNA should be assessed frequently during antiviral therapy in order to (i) stop treatment in patients predicted to experience treatment failure (e.g. in patients without a 2 log10 decline in HCV RNA at week 12) [199, 200, 203], and to (ii) shorten treatment duration in rapid responders (e.g., patients who are HCV RNA negative by PCR at week 4) [197, 198].

Viral kinetics
The initial decline in HCV RNA levels during antiviral therapy is bi-phasic [65, 67]. The depth of the decline depends on the dose of interferon alfa [65, 204, 205] and the HCV genotype [206, 207]. The greatest decline is usually seen during the first phase, which lasts for 24 to 48 hours in most patients; this phase represents the direct inhibition of virus production by interferon alfa. During the second phase the decline in HCV RNA level is slower, a possible explanation for this decline is a decrease in the production and release of virus from a decreasing number of infected hepatocytes. Some authors interpret the second phase decline as “immune mediated killing of infected cells” [65], but in fact it is unknown if the second phase decline is immune mediated and if infected hepatocytes die or if the virus is cleared from the cell without cell death [208].

First and second phase HCV RNA kinetics can be calculated according a mathematical model of the bi-phasic decline [65]. The efficacy of antiviral therapy at day 1 or 2 (ε) can be calculated as (HCV RNA baseline – HCV RNA day 1 or 2) / HCV RNA baseline. An efficacy of 0.9 corresponds to a 90% or 1log10 reduction in HCV RNA, an efficacy of 0.99 corresponds to a 99% or 2log10 reduction in HCV RNA. The slope of the second phase decline (a) can be calculated from HCV RNA levels at early time points, preferably day 2, week 1 and week 2. Some studies have shown that the first and/or second phase of decline in HCV RNA during antiviral therapy correlate with treatment outcome [205, 206, 209-211]; yet some patients with a slow initial decline in HCV RNA (ε < 0.90) during the first 2 days of therapy do achieve SVR [206, 210, 211].

A strong independent predictor of SVR, independent of HCV genotype, is the time needed to achieve a HCV RNA negative status during antiviral therapy [197, 198, 212-216]. Patients who are HCV RNA negative by PCR at week 4 have an increased chance of achieving SVR per se
Table 2. Side effects of treatment with interferon alfa and ribavirin [51, 169, 202].

<table>
<thead>
<tr>
<th>Frequency of side effect</th>
<th>Interferon alfa</th>
<th>Ribavirin</th>
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<tbody>
<tr>
<td>&gt;30% (very common)</td>
<td>flu-like symptoms</td>
<td>hemolysis</td>
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<tr>
<td></td>
<td>headache</td>
<td>nausea</td>
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<tr>
<td></td>
<td>fatigue</td>
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<td></td>
<td>fever</td>
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<td></td>
<td>rigors</td>
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<tr>
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<td>myalgia</td>
<td></td>
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<tr>
<td></td>
<td>thrombocytopenia</td>
<td></td>
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<tr>
<td></td>
<td>induction of autoantibodies</td>
<td></td>
</tr>
<tr>
<td>1-30% (common)</td>
<td>anorexia</td>
<td>anemia</td>
</tr>
<tr>
<td></td>
<td>erythema at injection site</td>
<td>nasal congestion</td>
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<tr>
<td></td>
<td>insomnia</td>
<td>pruritus</td>
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<tr>
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<td>diarrhea</td>
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<tr>
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<td>lack of motivation</td>
<td>eczema</td>
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<tr>
<td></td>
<td>inability to concentrate</td>
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<tr>
<td></td>
<td>irritability, agitation</td>
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<tr>
<td></td>
<td>emotional lability</td>
<td></td>
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<td></td>
<td>depression</td>
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<tr>
<td></td>
<td>autoimmune disease (thyroiditis, M Sjögren)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>leucocytopenia</td>
<td></td>
</tr>
<tr>
<td></td>
<td>taste perversion</td>
<td></td>
</tr>
<tr>
<td>&lt;1% (rare)</td>
<td>polyneuropathy</td>
<td>gout</td>
</tr>
<tr>
<td></td>
<td>paranoia or suicidal ideation</td>
<td>interstitial pneumonia</td>
</tr>
<tr>
<td></td>
<td>diabetes mellitus</td>
<td></td>
</tr>
<tr>
<td></td>
<td>retinopathy</td>
<td></td>
</tr>
<tr>
<td></td>
<td>neuritis optica</td>
<td></td>
</tr>
<tr>
<td></td>
<td>hearing impairment</td>
<td></td>
</tr>
<tr>
<td></td>
<td>seizures</td>
<td></td>
</tr>
<tr>
<td></td>
<td>loss of libido</td>
<td></td>
</tr>
<tr>
<td></td>
<td>cardiotoxicity</td>
<td></td>
</tr>
</tbody>
</table>

and recent studies have shown that treatment can be shortened in these patients [197, 198, 203, 213-216].

Daily administration of a high dose of interferon alfa (induction) during the first weeks of antiviral therapy increases and accelerates the early decline in HCV RNA [65, 204, 217-220]. Interferon induction increased the SVR rate in some, but not all, studies [219-222].
Chapter 1

Non-responders

Up to 90% of patients did not respond to interferon alfa monotherapy, and 40-50% of patients do not respond to the current standard of care. The reasons of non-response are only partially clear. The only defined viral factor predisposing for non-response is HCV genotype 1 or 4. The number of mutations in the NS5A gene has been implicated as a factor predisposing for non-response in genotype 1b infection [223]. Important host factors predisposing for non-response are older age, male sex, and African-American race [224, 225]. Other factors associated with non-response are: obesity [226], high β-lipoprotein levels [227], high pre-treatment Gamma GT [217, 228], high pre-treatment interferon inducible protein 10 (IP-10) [229, 230], and development of interferon alfa neutralizing antibodies during treatment [231]. Non-responders can be retreated with higher doses of interferon alfa and/or longer combination treatment regimens, but the success of retreatment is generally limited.

Liver transplantation

Liver transplantation is an effective treatment for small HCCs and decompensated cirrhosis. For chronic HCV infection it is only a temporary solution: The graft gets infected with HCV, and cirrhosis of the graft occurs in 10 to 25% of recipients within 5 to 10 years after transplantation [102].

The future

Direct inhibitors of viral enzymes such as the NS3 serine protease, NS3 helicase, NS5B RNA-dependent RNA polymerase, therapeutic vaccination, Toll-Like Receptor agonists and other immunomodulatory drugs, monoclonal and polyclonal antibodies, antisense RNA, modifications of interferon and ribavirin, and other molecules that inhibit HCV are currently being tested in phase I, II, III and IV clinical trials (Table 3) [232-239]. Future treatment of chronic HCV infection will hopefully be shorter, and will probably consist of combination therapy with new antivirals and interferon alfa plus ribavirin.
Table 3. Overview of drugs under evaluation for treatment of chronic hepatitis C infection [232, 238, 239].

<table>
<thead>
<tr>
<th>Drug Name</th>
<th>Drug Category</th>
<th>Pharmaceutical Company</th>
<th>Phase</th>
<th>HCV specific</th>
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<tbody>
<tr>
<td>Consensus interferon (Infergen)</td>
<td>Interferon</td>
<td>Valeant</td>
<td>IV</td>
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<td>Albuferon</td>
<td>Interferon — Long Acting</td>
<td>Human Genome Sciences</td>
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<td>Interferon beta-1a (REBIF)</td>
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<td>Ares-Serono</td>
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<td>Viramidine (Taribavirin)</td>
<td>Nucleoside Analogue</td>
<td>Valeant</td>
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<td>ZADAXIN® (thymalfasin)</td>
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<td>SciClone/Sigma-Tau</td>
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<td>AVI-4065</td>
<td>Antisense Compound</td>
<td>AVI Biopharma</td>
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<td>Civacir</td>
<td>Polyclonal Antibody</td>
<td>NABI</td>
<td>II</td>
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<td>IC41/IC45</td>
<td>Therapeutic Vaccine</td>
<td>Intercell</td>
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<td>yes</td>
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<td>INNO0101 (E1)</td>
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<td>Innogenetics</td>
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<td>NM283 (Valopicitabine)</td>
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<td>Idenix</td>
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<td>Roche</td>
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<td>SCH 503034</td>
<td>Protease Inhibitor</td>
<td>Schering</td>
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<td>VGX-410C</td>
<td>IRES Inhibitor</td>
<td>VGX Pharmaceuticals</td>
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<tr>
<td>VX-9500 (telaprevir)</td>
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<td>Vertex</td>
<td>II</td>
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<td>Therapeutic vaccine</td>
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<td>Sirna-034</td>
<td>RNA interference</td>
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<td>CPG 10101 (Actilon)</td>
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<td>Coley</td>
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<td>DEBIO-025</td>
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<td>EM7202</td>
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<td>G126270</td>
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<td>Jenken Biosciences</td>
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<td>Medusa Interferon</td>
<td>Interferon — Long Acting</td>
<td>Flamel Technologies</td>
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<td>Multiferon</td>
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<td>MX-3253 (Celgosivir)</td>
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<td>Migenix</td>
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<td>Omega Interferon</td>
<td>Interferon</td>
<td>Intarcia</td>
<td>II</td>
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<tr>
<td>PF-03491390 (IDN-6556)</td>
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<td>Pfizer</td>
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<tr>
<td>Suvs (Methylene Blue)</td>
<td>Antiviral</td>
<td>Bioenvision</td>
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<td>UT-231B</td>
<td>Imino Sugar Inhibitor</td>
<td>United Therapeutics</td>
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<td>ANA975 (prodrug of Isatoribine)</td>
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<td>A-831</td>
<td>NS5A Inhibitor</td>
<td>Arrow Therapeutics Ltd</td>
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<td>CGI 5005</td>
<td>Therapeutic Vaccine</td>
<td>GlobelImmune</td>
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<td>Vaccine</td>
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<td>Therapeutic vaccine</td>
<td>Pevion Biotect</td>
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<td>R7128</td>
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<td>Pharmasset/Roche</td>
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<td>XTL-2125</td>
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<td>XTL</td>
<td>I</td>
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<tr>
<td>HepeX-C (AB68 &amp; AB65)</td>
<td>Monclonal Antibody</td>
<td>XTL</td>
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<tr>
<td>Alinia (nitrazoxanide)</td>
<td>Thiadolides</td>
<td>Romark</td>
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<td>Bavituimab (Tarvacin)</td>
<td>Anti-Phospholipid</td>
<td>Peregrine</td>
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<td>Belerofon</td>
<td>Interferon — Long Acting</td>
<td>Nautilus Biotech</td>
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<td>BLX-883 (Locteron)</td>
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<td>Implicit Bioscience</td>
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<tr>
<td>R7025 (MAXY-alpha)</td>
<td>gene shuffled interferon</td>
<td>Maxygen/Roche</td>
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</tbody>
</table>
Chapter 1

Outline of this thesis

This thesis deals with chronic HCV infection and the search for optimal treatment and treatment monitoring:

- In chapter 2 the performance of a new real-time PCR assay for detection and quantification of HCV compared to a validated assay is described.
- In chapter 3 we describe 2 clinical trials in difficult-to-treat chronic HCV patients treated with high doses of interferon alfa (induction) during the first 6 weeks, peginterferon, ribavirin and amantadine hydrochloride for a total of 24 or 48 weeks.
- Chapter 4 deals with a new phenomenon that we observed in the studies described in chapter 3: Reappearance of low levels of HCV RNA after 16 or 20 weeks of antiviral therapy as an early sign of impending treatment failure.
- Chapter 5 deals with 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.
- In chapter 6, we describe an unusually high incidence of diabetes mellitus in patients with chronic HCV infection treated with standard peginterferon-ribavirin therapy or with the high dose regimen described in chapter 3.
- 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.
- In chapter 8 we describe ex vivo generation of dendritic cells from chronic HCV patients and their form and function. In theory, therapeutic vaccination with autologous dendritic cells could be applied as an adjuvant therapy to boost the cellular immune response in chronic HCV infection.

References

Chapter 1


General Introduction


238. HCV Advocate www.hcvadvocate.org/hepatitis/hepC/HCVDrugs.html.


