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

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Chapter 3

Prediction of virologic response in difficult-to-treat chronic hepatitis C patients during high dose interferon induction therapy

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Submitted
Abstract

**Aims & Methods** We treated 100 “difficult-to-treat” chronic hepatitis C patients (46 previous non-responders/relapsers [any genotype], 54 treatment-naive genotype 1 and 4) with triple antiviral induction therapy: Amantadine hydrochloride and ribavirin, combined with 6 weeks interferon alfa-2b induction (week 1-2: 18 MU/day, week 3-4: 9 MU/day, week 5-6: 6 MU/day), thereafter combined with weekly peginterferon alfa-2b. Fast-responders (≥ 3 log$_{10}$ HCV RNA decline at week 4) were randomized to 24 or 48 weeks. Slow-responders (< 3 log$_{10}$ HCV RNA decline at week 4) were treated for 48 weeks. Patients with detectable HCV RNA at week 24 stopped treatment.

**Results** Thirty-six patients achieved a sustained virologic response (SVR): 28 of 60 fast-responders (47%) vs 8 of 32 slow-responders (25%, P<0.05). Relapse rates among fast-responders treated for 24 or 48 weeks were 27% and 20%, respectively (P=ns). SVR in fast-responders was independent of baseline HCV RNA ≥ or < 600,000 IU/mL. All treatment-naive patients with HCV RNA < 5 IU/mL at week 1 or 2 achieved SVR; all treatment-naive patients with HCV RNA ≥ 5 IU/mL at week 16 became non-SVR. In previous non-responders/relapsers, predictive value for SVR was 83% if HCV RNA was < 5 IU/mL at week 2; all previous non-responders/relapsers with HCV RNA ≥ 5 IU/mL at week 8 became non-SVR.

**Conclusion** With high-dose interferon induction SVR and non-SVR can be predicted reliably within 16 weeks. Fast-responders may be treated for 24 weeks, and SVR is independent of baseline viral load in fast-responders.
Introduction

Chronic hepatitis C virus (HCV) infection is a major cause of cirrhosis and hepatocellular carcinoma [1]. Depending on the HCV genotype, 40 to 80% of patients respond to the current standard of care, which consists of administration of peginterferon alfa and ribavirin for 12-48 weeks [2-6]. Patients who do not achieve a sustained virologic response (SVR) after their first course of antiviral therapy, and treatment naive genotype 1 and 4 patients—with only a 40-50% a priori chance of achieving SVR—are considered difficult-to-treat. These patients may benefit from alternative treatment regimens.

The initial decline in HCV RNA levels during antiviral therapy depends on the dose of interferon alfa [7, 8] and the HCV genotype [9, 10]. Daily administration of a high dose of interferon alfa during the first weeks of antiviral therapy (induction) accelerates the initial decline in HCV RNA [7, 8, 11-14]. Interferon induction increased the SVR rate in some, but not all, studies [13-16].

The measurement of HCV RNA levels has become an important tool to (1) predict the outcome, and (2) determine the duration of antiviral therapy. Patients who achieve a ≥ 3 log₁₀ decline in HCV RNA at week 4 [17], and patients who are HCV RNA negative by PCR at week 4 (rapid virologic response, RVR) [2, 6] have a greater chance of achieving SVR compared to patients with a slower response. Shorter treatment may be sufficient for patients with RVR [2, 6]. On the other hand, the chance of achieving SVR is 0% in patients who do not achieve a 2 log₁₀ decline in HCV RNA at week 12 (early virologic response, EVR), and treatment can be stopped earlier than week 24 in these patients [18-20].

We treated 100 difficult-to-treat HCV patients with a high dose interferon induction scheme during the first 6 weeks of antiviral therapy. HCV RNA levels were monitored and fast-responders (≥ 3 log₁₀ decline in HCV RNA at week 4) were randomized (1:1) to shorter treatment for 24 weeks or standard treatment for 48 weeks. The aim of our study was to determine (i) if early viral kinetics or other markers during this modified treatment regimen could predict treatment outcome, and (ii) if fast-responders (≥ 3 log₁₀ decline in HCV RNA at week 4) could be treated for 24 weeks, without compromising SVR rate.

Patients and Methods

Study design

The results reported here are from 2 open labelled pilot studies in 2 groups of difficult-to-treat chronic hepatitis C patients (treatment naive patients with HCV genotype 1 or 4, and patients [all genotypes] with previous non-response, relapse or breakthrough). The treatment regimens of the 2 studies were identical. These studies were designed to assess the influence of daily high dose interferon induction on early HCV RNA kinetics and other markers in relation to treatment outcome. Both studies were approved by the institutional review board. Written informed consent was obtained from each patient.
Chapter 3

Treatment regimen

Patients were treated with amantadine hydrochloride 200 mg/d in 2 divided doses (Symmetrel®, Novartis, Basel, Switzerland), ribavirin (Rebetol®; Schering-Plough, Kenilworth, NJ, USA) 1000 or 1200 mg/d in 2 divided doses based on body weight (patients weighing > 75 kg received 1200 mg/d) combined with a high induction dose interferon-alfa2b (IntronA®, Schering-Plough) during the first 6 weeks, thereafter combined with weekly peginterferon alfa-2b (Pegintron®; Schering-Plough) 1.5 μg/kg for a total of 24 or 48 weeks. The induction scheme during the first 6 weeks was as follows: weeks 1 and 2: 18 MU/d in 3 divided doses; weeks 3 and 4: 9 MU/d in 3 divided doses; weeks 5 and 6: 6 MU/d in 2 divided doses.

Patients with a ≥ 3 log_{10} decline in HCV RNA from baseline to week 4 (fast-responders) that were TMA negative at week 24 were randomized by computer (1:1, permuted block randomization) to stop treatment early at 24 weeks (Figure 1a) or continue to 48 weeks (Figure 1b). Patients with a < 3 log_{10} decline in HCV RNA from baseline to week 4 (slow-responders) were treated for 48 weeks (Figure 1b). Treatment was stopped in all patients who were positive for HCV RNA at week 24 (Figure 1a). All patients were followed for 24 weeks after cessation of treatment.

Figure 1. Study Design

Patients with a decline in HCV RNA ≥ 3 log_{10} at week 4 (and TMA negative at week 24) were randomized to stop treatment at 24 weeks (Figure 1a) or continue to 48 weeks (Figure 1b). Patients with a decline in HCV RNA < 3 log_{10} at week 4 were treated for 48 weeks (Figure 1b). Treatment was stopped in all patients who were positive for HCV RNA at week 24 (Figure 1a). All patients were followed for 24 weeks after completion of therapy.

Patient selection

Patients were eligible for inclusion when they fulfilled the following criteria: Age between 18 and 75 years, HCV RNA > 615 IU/mL, HCV genotype 1 or 4 (treatment naive patients), or patients (any genotype) with non-response or breakthrough during, or relapse after previous treatment with a treatment-free interval of at least 24 weeks after previous antiviral therapy. Standard exclusion criteria for interferon alfa and ribavirin were applied, except that well controlled (insulin dependent) diabetes mellitus, a history of depression or other psychiatric
disease, daily cannabis use and/or methadone maintenance therapy were no exclusion criteria in these studies. Patients with a history of psychiatric disease were only included after approval by a psychiatrist and if indicated treatment with an SSRI was started before antiviral therapy. Pre-treatment diagnostic work-up included a chest X-ray, assessment of random blood glucose level, and determination of autoantibodies (ANF, AMA, ASMA, LMA, anti-TPO) and thyroid-stimulating hormone (TSH).

**Patient monitoring and specimen collection**

Patients were examined at least 2 weeks before the treatment started. The first dose of interferon was administered by the treating physician. Patients visited the outpatient clinic at days 0, 1, 2, weeks 1, 2, 3, 4, 6, 8 and then every 4 weeks until end-of-treatment. After cessation of treatment, patients were examined at weeks 4, 12 and 24. At each visit, a medical history, a physical examination (except day 2), routine blood tests (except days 1 and 2) and HCV RNA tests (except week 3) were performed. EDTA plasma samples for assessment of HCV RNA were processed and frozen at -80°C within 24 h of collection. TSH was assessed every 12 weeks during treatment and at end-of-follow-up.

**HCV RNA assessments**

We used 1 quantitative and 2 qualitative assays to assess HCV RNA: quantitative bDNA (VERSANT® HCV 3.0 assay; Bayer Diagnostics, Berkeley, CA, USA; linear dynamic range 6.15 x 10^2 to 7.7 x 10^6 IU/mL [21]), qualitative PCR (COBAS® Amplicor HCV Test v2.0; Roche Molecular Systems, Branchburg, NJ, USA; Lower limit of detection (LLD) 50 IU/mL [22]), and qualitative TMA (Transcription-Mediated Amplification; VERSANT® HCV qualitative assay; Bayer Diagnostics; LLD 5 IU/mL [23]).

**Definition of treatment outcome**

- **SVR:** undetectable HCV RNA (TMA negative) at end-of-follow-up (24 weeks after end-of-treatment).
- **Non-SVR:** all patients who did not achieve SVR (i.e., non-response, breakthrough, relapse, and including dropouts in intention to treat analyses).
- **Non-response:** detectable HCV RNA (TMA positive) at all timepoints during treatment and at end-of-treatment.
- **Relapse:** undetectable HCV RNA (TMA negative) at end-of-treatment but detectable HCV RNA at end-of-follow-up.
- **Breakthrough:** undetectable HCV RNA (TMA negative) during treatment but detectable HCV RNA at end-of-treatment.
- **Drop out:** any patient who stopped treatment prematurely between day 0 and week 24.

**Prediction of treatment outcome using 1st and 2nd phase HCV RNA kinetics**

First and 2nd phase HCV RNA kinetics were calculated according to the model described by Neumann et al. [7, 24], with minor modifications. The effect of antiviral therapy at day
1 was calculated as decline in \( \log_{10} \) HCV RNA from baseline, and as efficacy \( e_1 \) \( [(\text{HCV RNA baseline} - \text{HCV RNA day 1}) / \text{HCV RNA baseline}] \). The slope of the second phase decline (\( \partial \)) was determined by linear regression of \( \log_{10} \) HCV RNA at day 2, week 1 and week 2.

**Prediction of treatment outcome using TMA**

Prediction of treatment response was based on HCV RNA levels during treatment. Predictive value (PV) for SVR was defined as the percentage of patients who were TMA negative with subsequent SVR. PV for non-SVR was defined as the percentage of patients who were TMA positive with subsequent non-SVR.

**Genotyping**

HCV genotypes were determined using the TruGene \(^\circledR\) HCV genotyping assay and the OpenGene\(^\circledR\) automated DNA sequencing system (Bayer Diagnostics).

**Liver biopsy**

Liver biopsies were obtained < 2 years before treatment from 88 patients. For this study, the biopsies were classified as cirrhotic (Child-Pugh A) or non-cirrhotic. Of the 12 patients where a liver biopsy was not obtained, 11 were classified as having no cirrhosis based on clinical criteria, and 1 patient with thrombocytopenia and oesophageal varices was classified as having cirrhosis.

**Statistical analysis**

Graphical representation and statistical analysis were performed using GraphPad Prism version 4.0b for Macintosh (GraphPad Software, San Diego, CA, USA), and SPSS version 12.0.2 for Windows (SPSS Inc., Chicago, IL, USA). Data were analysed on intention to treat (ITT, defined as all patients who received 1 or more doses of treatment) and/or per protocol basis. We used Student’s t test, Mann-Whitney U test, and Fisher’s exact test for the identification of possible factors related to treatment outcome. A P value < 0.05 was considered significant. Confidence intervals (95%) of PVs were calculated using the modified Wald method [25].

**Results**

**Treatment outcome**

The breakdown of the study is depicted in Figure 2. Baseline characteristics of the patients are summarized in Table 1. A total of 106 patients were entered into the study, 6 patients did not take any study medication and were excluded. A 100 patients received at least 1 dose of study medication (intention to treat, ITT). Of these, 36 patients achieved SVR, 9 patients broke through during therapy, 15 patients relapsed after cessation of therapy, 27 patients were non-responders (i.e., they remained HCV RNA positive during 24 weeks of treatment),
13 patients dropped out during the first 24 weeks. The SVR rate was 44% in treatment naive patients, and 26% in previous non-responders/relapsers.

**Baseline characteristics**

We did not identify any baseline characteristics associated with SVR. However, patients with a non-response compared to patients with SVR, relapse and breakthrough were significantly older and had significantly higher Gamma GT, AST, and APRI (AST to platelet ratio index [26]) (Figure 3). Baseline HCV RNA ≥ 600,000 IU/mL, cirrhosis, a history of depression or

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**Figure 2.** Study flowchart.

Study flowchart. 13 patients dropped out: 8 during the first 4 weeks, 5 between week 4 and 24, 3 dropouts achieved a ≥ 3 log10 decline in HCV RNA at week 4 (fast-responders). All patients were followed for 24 weeks after cessation of treatment.

*SVR rate in the fast-responders treated for 48 weeks was calculated as 9 of 15 (14 patients who completed 48 weeks of treatment and 1 patient who stopped early at week 32 with virologic breakthrough, 2 patients who stopped at weeks 28 and 32 and relapsed** were excluded from the virologic response analysis in this arm)

TMA, transcription-mediated amplification; LLD, 5 HCV RNA IU/mL; SVR, sustained virologic response; BT, breakthrough; NR, non-response; REL, relapse
other psychiatric disease, methadone maintenance therapy, or previous non-response/relapse were not associated with non-SVR (Fisher’s exact test).

<table>
<thead>
<tr>
<th>Table 1. Baseline characteristics of patients who received at least one dose of treatment (intention to treat, ITT).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment naive</td>
</tr>
<tr>
<td>-------------------------------</td>
</tr>
<tr>
<td>No of patients (intention to treat)</td>
</tr>
<tr>
<td>male/female</td>
</tr>
<tr>
<td>mean age (years, range)</td>
</tr>
<tr>
<td>Genotype</td>
</tr>
<tr>
<td>HCV 1</td>
</tr>
<tr>
<td>HCV 2</td>
</tr>
<tr>
<td>HCV 3</td>
</tr>
<tr>
<td>HCV 4</td>
</tr>
<tr>
<td>HCV 5</td>
</tr>
<tr>
<td>Previous high dose IFN</td>
</tr>
<tr>
<td>HCV RNA (log10 IU/mL, mean ± SD)</td>
</tr>
<tr>
<td>HCV RNA &lt; 600,000 IU/mL (5.78 log10)</td>
</tr>
<tr>
<td>ALT (U/L, mean ± SD)</td>
</tr>
<tr>
<td>normal ALT (ULN 45 U/L)</td>
</tr>
<tr>
<td>AST (U/L, ULN 40 U/L, mean ± SD)</td>
</tr>
<tr>
<td>GammaGT (U/L, ULN 60 U/L, mean ± SD)</td>
</tr>
<tr>
<td>thrombocytes 10E9/L</td>
</tr>
<tr>
<td>APRI†</td>
</tr>
<tr>
<td>cirrhosis (Child-Pugh A)</td>
</tr>
<tr>
<td>psychiatric comorbidity</td>
</tr>
<tr>
<td>methadone maintenance</td>
</tr>
</tbody>
</table>

* P = 0.04
ULN, upper limit of normal
APRI, AST Platelet Ratio Index: [(AST/ULN)/thrombocytes*100]

**Treatment outcome according to HCV RNA decline at week 4**

Treatment outcome and treatment duration of 24 vs 48 weeks in fast-responders

Sixty patients achieved a ≥ 3 log₁₀ decline in HCV RNA at 4 weeks of treatment (fast-responders, Figure 2). Three of the 60 fast-responders dropped out between week 4 and 24, 14 of the 60 fast-responders were HCV RNA positive at week 24 and stopped treatment, 43 of the 60 fast-responders were HCV RNA negative by TMA at week 24 and were randomized to stop treatment at week 24 (n=20) or continue to 48 weeks (n=23). Six fast-responders refused to continue treatment to 48 weeks, they were included in the analysis of the 24 week treatment group. Nineteen of 26 fast-responders (73%) achieved SVR after 24 weeks of treatment, and 9 of 15 fast-responders (60%) achieved SVR after 48 weeks of treatment (P = ns, Fisher’s exact test). Relapse rates among fast-responders treated for 24 or 48 weeks were 27% and
20% respectively (P = ns, Figure 2a, 2 fast-responders in the 48 week arm who relapsed after stopping treatment early at weeks 28 and 32 were not included in the calculation of relapse and SVR rates in the 48 week arm). In total, 28 of the 60 fast-responders achieved an SVR (PV for SVR 47%): 16 of 30 treatment naive fast-responders (PV for SVR 53%), and 12 of 30 fast-responders who were previous non-responder/relapsers (PV for SVR 40%). Thirty-four of the 60 fast-responders were negative by PCR at week 4 (RVR), 23 of these 34 patients (67%) achieved SVR. Twenty-three of the 60 fast-responders were negative by TMA at week 4, 17 of these 23 patients (74%) achieved SVR.

Differences in baseline variables between different treatment outcomes were assessed in three settings: (i) SVR vs non-SVR (i.e., breakthrough + relapse + non-response), (ii) SVR vs non-response, (iii) SVR + breakthrough + relapse vs non-response, were compared using Student’s t test. Only significant P values (<0.05) are shown.

SVR, sustained virologic response; BT, breakthrough; REL, relapse; NR, non-response. APRI, AST Platelet Ratio Index: [(AST/ULN)/thrombocytes*100].
Chapter 3

Treatment outcome in slow-responders

Thirty-two patients did not achieve a 3 log_{10} decline in HCV RNA at 4 weeks of treatment (slow-responders, Figure 2). Two of the 32 slow-responders dropped out between week 4 and 24, 18 of the 32 slow-responders were HCV RNA positive at week 24 and stopped treatment, 12 of the 32 slow-responders were HCV RNA negative by TMA at week 24 and continued treatment to 48 weeks. In total, 8 of the 32 slow-responders achieved SVR (PV for SVR 25%): 8 of 20 treatment naive slow-responders (PV for SVR 40%), but 0 of 12 slow-responders who were previous non-responder/relapsers (PV for SVR 0%). Compared to fast-responders, slow-response was associated with non-SVR (overall P < 0.05, treatment naive patients P = NS, previous non-responders/relapsers P < 0.01, Fisher’s exact test).

Prediction of treatment outcome using 1st and 2nd phase HCV RNA kinetics

First and 2nd phase HCV RNA kinetics were weak predictors for treatment outcome. However, the combination of decline in log_{10} HCV RNA after 1 day (efficacy of antiviral therapy) and the absolute HCV RNA load at day 1 was a strong predictor of non-SVR in previous non-responders/relapsers: 20 previous non-responders/relapsers with a decline in HCV RNA < 0.7 log_{10} (efficacy < 0.80) and/or HCV RNA > 4.74 log_{10} IU/mL at day 1 became non-SVR (PV for non-SVR 100%, Figure 4).

Figure 4. Prediction of non-SVR using viral kinetics at day 1.

The combination of decline in log_{10} HCV RNA at day 1 (efficacy of antiviral therapy) and absolute log_{10} HCV RNA load at day 1 was a weak predictor of non-SVR in treatment naive patients (data not shown) but a strong predictor of non-SVR in previous non-responders/relapsers: All 20 patients with a decline in HCV RNA < 0.7 log_{10} (efficacy < 0.80) and/or an absolute HCV RNA level > 4.74 log_{10} IU/mL at day 1 became non-SVR (PV for non-SVR 100%). Some patients who achieved SVR had a low efficacy of antiviral therapy and high HCV RNA load at day 1 (marked 1, 2 and 3 in the middle of the graph), making the combination of efficacy of antiviral therapy and HCV RNA load at day 1 a weak predictor of SVR.
Prediction of treatment outcome using TMA

Prediction of SVR

All 24 treatment naive patients who achieved SVR became TMA negative (< 5 IU/mL) within 16 weeks (Table 2, Figure 5a). The PV for SVR was 100% in treatment naive patients who were negative by TMA at week 1 or week 2 (Table 2, Figure 5a).

All 12 previous non-responders/relapsers who achieved SVR became TMA negative (< 5 IU/mL) within 8 weeks (Table 2, Figure 5a). The PV for SVR was 83% in previous non-responders/relapsers who were TMA negative at week 2 (Table 2, Figure 5a).

Table 2a. PV for SVR for patients who were HCV RNA negative or positive by TMA during treatment.

<table>
<thead>
<tr>
<th>Treatment naive patients TMA negative/positive</th>
<th>PV of TMA negativity for SVR (95% CI)</th>
<th>PV of TMA positivity for non-SVR (95% CI)</th>
<th>Sensitivity PV for SVR</th>
<th>Specificity PV for SVR</th>
</tr>
</thead>
<tbody>
<tr>
<td>day 1</td>
<td>0/53 NA</td>
<td>57% (43-69)</td>
<td>0%</td>
<td>100%</td>
</tr>
<tr>
<td>day 2</td>
<td>0/51 NA</td>
<td>55% (41-68)</td>
<td>0%</td>
<td>100%</td>
</tr>
<tr>
<td>week 1</td>
<td>3/49 100% (38-100)</td>
<td>57% (43-70)</td>
<td>13%</td>
<td>100%</td>
</tr>
<tr>
<td>week 2</td>
<td>6/45 100% (55-100)</td>
<td>60% (45-73)</td>
<td>25%</td>
<td>100%</td>
</tr>
<tr>
<td>week 4</td>
<td>12/36 83% (54-96)</td>
<td>64% (48-78)</td>
<td>43%</td>
<td>92%</td>
</tr>
<tr>
<td>week 6</td>
<td>18/30 94% (72-100)</td>
<td>77% (59-88)</td>
<td>71%</td>
<td>96%</td>
</tr>
<tr>
<td>week 8</td>
<td>27/22 78% (59-90)</td>
<td>86% (66-96)</td>
<td>88%</td>
<td>76%</td>
</tr>
<tr>
<td>week 12</td>
<td>33/14 67% (49-80)</td>
<td>86% (59-97)</td>
<td>92%</td>
<td>52%</td>
</tr>
<tr>
<td>week 16</td>
<td>30/16 80% (62-91)</td>
<td>100% (77-100)</td>
<td>100%</td>
<td>73%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Previous non-responders/relapsers TMA negative/positive</th>
<th>PV of TMA negativity for SVR (95% CI)</th>
<th>PV of TMA positivity for non-SVR (95% CI)</th>
<th>Sensitivity PV for SVR</th>
<th>Specificity PV for SVR</th>
</tr>
</thead>
<tbody>
<tr>
<td>day 1</td>
<td>0/43 NA</td>
<td>74% (60-85)</td>
<td>0%</td>
<td>100%</td>
</tr>
<tr>
<td>day 2</td>
<td>0/42 NA</td>
<td>74% (59-85)</td>
<td>0%</td>
<td>100%</td>
</tr>
<tr>
<td>week 1</td>
<td>0/44 NA</td>
<td>73% (58-84)</td>
<td>0%</td>
<td>100%</td>
</tr>
<tr>
<td>week 2</td>
<td>6/37 83% (42-98)</td>
<td>81% (66-91)</td>
<td>42%</td>
<td>97%</td>
</tr>
<tr>
<td>week 4</td>
<td>11/30 64% (35-85)</td>
<td>83% (66-93)</td>
<td>58%</td>
<td>86%</td>
</tr>
<tr>
<td>week 6</td>
<td>15/26 67% (41-85)</td>
<td>92% (75-99)</td>
<td>83%</td>
<td>83%</td>
</tr>
<tr>
<td>week 8</td>
<td>17/21 65% (41-83)</td>
<td>100% (82-100)</td>
<td>100%</td>
<td>80%</td>
</tr>
<tr>
<td>week 12</td>
<td>21/19 57% (37-75)</td>
<td>100% (80-100)</td>
<td>100%</td>
<td>68%</td>
</tr>
<tr>
<td>week 16</td>
<td>18/20 61% (39-80)</td>
<td>100% (81-100)</td>
<td>100%</td>
<td>74%</td>
</tr>
</tbody>
</table>

TMA, transcription-mediated amplification, LLD 5 HCV RNA IU/mL; PV, predictive value; NA, not applicable
PV of TMA negativity for SVR was defined as % of TMA negative patients with subsequent SVR
PV of TMA positivity for non-SVR was defined as % of TMA positive patients with subsequent non-SVR
Sensitivity = % of patients with SVR, TMA negative at this time point
Specificity = % of patients with non-SVR, TMA positive at this time point
Confidence intervals (95%) of PVs were calculated using the modified Wald method
Chapter 3

Prediction of non-SVR

All 16 treatment naive patients who were TMA positive at week 16 developed non-SVR (Table 2, Figure 5b). The mean time to reach a TMA negative status was significantly shorter in treatment naive patients who achieved SVR compared to those who became TMA negative but thereafter broke through or relapsed (5.9 ± 4.1 weeks vs 9.6 ± 3.4 weeks, respectively, P < 0.01, Mann-Whitney U test).

Table 2b. Table for the calculation of PV, sensitivity and specificity depicted in table 2a.

<table>
<thead>
<tr>
<th></th>
<th>SVR</th>
<th>non-SVR</th>
<th>total</th>
<th>PV for SVR (a/(a+b)*100)</th>
<th>PV for non-SVR (d/(c+d)*100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMA negative</td>
<td>a</td>
<td>b</td>
<td>a+b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TMA positive</td>
<td>c</td>
<td>d</td>
<td>c+d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>total</td>
<td>a+c</td>
<td>b+d</td>
<td>a+b+c+d</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

sensitivity of TMA negative and SVR (a/(a+c)*100)
specificity of TMA positive and non-SVR (d/b+d*100)

Figure 5. Prediction of SVR and non-SVR during treatment using TMA.

Figure 5a. PV (predictive value) of TMA negative test results for SVR, defined as the percentage of patients with a TMA negative test result that achieved SVR, during treatment in treatment naive patients and previous non-responders/relapsers. Figure 5b. PV of TMA positive test results for non-SVR, defined as the percentage of patients with a TMA positive test result that did not achieve SVR, during treatment in treatment naive patients and previous non-responders/relapsers.

TMA, Transcription-Mediated Amplification, LLD 5 HCV RNA IU/mL; SVR, sustained virologic response.
All 21 previous non-responders/relapsers who were not TMA negative at week 8 developed non-SVR (Table 2, Figure 5b). The mean time to reach a TMA negative status was significantly shorter in previous non-responders/relapsers with SVR compared to those with eventual breakthrough or relapse (4.3 ± 2.4 weeks vs 9.2 ± 5.6 weeks, respectively, P = 0.03, Mann-Whitney U test).

**Safety**

Thirteen patients (13%) dropped out during the study, 8 during the first 4 weeks and 5 between week 4 and 24. Five patients were hospitalized, 3 during the first 4 weeks (pancytopenia, gastroenteritis, ketoacidosis secondary to preexisting diabetes mellitus) and 2 between week 4 and 24 (new onset diabetes mellitus, pneumonia). 1 of the 13 dropouts stopped treatment at week 4 due to non-medical reasons. All hospitalized patients recovered. Three patients who stopped prematurely because of asthenia between 24 and 48 weeks of treatment were not considered dropouts, and were analyzed according to their virological response (1 BT, 2 REL).

Four patients developed diabetes mellitus during or shortly after treatment. Nine patients already suffered from diabetes mellitus before treatment, 1 patient with type 2 diabetes mellitus became temporarily insulin dependent during antiviral therapy.

All patients experienced known side effects associated with interferon or ribavirin. If necessary patients were referred to other specialists (i.e., dermatology (27%), psychiatry (31%), pulmonology (4%), neurology (7%), ophthalmology (12%), ear-nose-and-throat (7%), surgery (2%), and internal medicine (4%)).

The dose of interferon alfa was reduced during the first 6 weeks in 9 patients, and at week 8 in 1 patient. The dose of ribavirin was reduced in 28 patients (12 during the first 6 weeks, 27 during week 6 to 48). Dose reduction was not associated with subsequent non-SVR (P = 0.38, Fisher's exact test).

Incidence or recurrence of psychiatric disease during treatment was not associated with subsequent non-SVR (P = 0.37, Fisher's exact test). Thirteen (42%) of the 31 patients with a history of depression or other psychiatric disease did not have any psychiatric complaints during treatment, and conversely 13 (42%) of the 31 patients with psychiatric complaints during treatment did not have a history of psychiatric disease.

**Discussion**

In this high dose interferon induction study, the SVR rates in treatment naive genotype 1 and 4 patients (44%) and previous non-responders/relapsers (26%) were apparently not higher than described for patients treated with standard of care (peginterferon alfa and ribavirin for 24-48 weeks). However, high dose interferon induction enabled us to predict treatment outcome already within the first weeks of treatment. In fast-responders (≥ 3 log_{10} HCV RNA decline at week 4 of treatment) a significantly higher proportion of patients (47%) developed SVR compared to slow-responders (25%, < 3 log_{10} HCV RNA decline at week 4 of
treatment). In fast-responders the relapse rate in patients treated for 24 or 48 weeks was not significantly different (27% vs 20% respectively) indicating that a treatment duration of 24 weeks may suffice. In slow-responders none of the previous non-responders/relapsers but 40% of treatment naive patients achieved SVR.

In agreement with an earlier study [17], a fast-response (≥ 3 log_{10} decline in HCV RNA at week 4) was a weak predictor for SVR (PV 47%), but a slow response (< 3 log_{10} decline in HCV RNA at week 4) was a strong predictor of non-SVR in previous non-responders/relapsers, since all 12 patients did not develop SVR.

In a recent study by Zeuzem et al. [6] a 24 week treatment regimen was only possible, without compromising SVR rate, in patients with baseline HCV RNA < 600,000 IU/mL and RVR (HCV RNA negative by PCR at week 4). In our studies, SVR in fast-responders was independent of baseline viral load. Also, of the 19 fast-responders that achieved SVR after a 24 week treatment regimen in our studies, 4 patients would not have met the criteria for RVR, i.e., they were still HCV RNA positive by PCR at week 4, and 8 of these 19 patients were still HCV RNA positive by the more sensitive TMA at week 4. These differences between our results and Zeuzem's results are probably the result of the high dose interferon induction scheme applied in our studies.

In contrast to the results from Layden et al. [24], but in agreement with others [9, 27, 28], the utility of 1st phase viral kinetic parameters for prediction of SVR in individual patients was limited in our study. However, in previous non-responders/relapsers a decline in HCV RNA at day 1 < 0.7 log_{10} and/or HCV RNA load > 4.74 log_{10} IU/mL at day 1 had a 100% PV for non-SVR. Thus after one day high dose interferon induction can identify previous non-responders/relapsers who may benefit from retreatment. This early prediction of non-SVR is in agreement with the results from Layden et al. [24].

The utility of 2nd phase viral kinetics for prediction of SVR or non-SVR was also limited. This is probably related to the rapid initial HCV RNA decline in most patients due to the high dose interferon induction (18 MU/day) applied during the first 2 weeks of treatment. This decline is comparable to the rapid HCV RNA decline observed during administration of HCV protease inhibitors as monotherapy [29] or in combination with peginterferon alfa for 2 weeks [30]. Many patients in our study had HCV RNA levels below the lower limit of detection (615 IU/mL) of the quantitative bDNA assay after just 2 days of treatment. In these patients, in contrast to other studies [9, 24, 27, 28], the 2nd phase decline could not be assessed and therefore the utility of 2nd phase viral kinetics was limited in our study.

On the other hand our study demonstrated the value of repeated testing during the first 6 weeks of high dose interferon induction treatment for prediction of response. A negative TMA test within the first 6 weeks of treatment was a strong predictor of SVR. A positive TMA test at week 8 predicted non-SVR in previous non-responders/relapsers and a positive TMA test at week 16 predicted non-SVR in treatment naive patients.

The association between a TMA negative status within the first treatment weeks and SVR is in line with the observations of others that with standard treatment [20, 31] or consensus interferon [32] an HCV RNA negativity at week 4 was a strong predictor for SVR. Compared
to TMA, the predictive values of the less sensitive PCR and bDNA tests were lower at all time points in our study.

Our results also show that frequent assessment of HCV RNA with TMA changes the classification of “non-response”, “relapse” or “breakthrough” in patients with non-SVR. Some patients classified as TMA non-responders would have been classified as breakthrough or relapse using PCR. Conversely a number of breakthrough patients would have been classified as non-responders if we had not tested frequently with TMA.

We identified 10 patients who became HCV RNA negative by TMA, but thereafter showed reappearance of low level viremia at week 16 or 20. This turned out to be an early sign of impending treatment failure. These findings are described in detail in a separate article [33].

As in previous studies performed in our centre [11, 12], high dose interferon was associated with significant, but tolerable, side effects. Before treatment, all patients had been thoroughly informed about the possible adverse events. Furthermore, all patients visited the outpatient clinic at days 0, 1 and 2, and weeks 1, 2, 3, 4, 6, 8 and thereafter every 4 weeks until end of treatment. The 13% dropout rate is comparable to other studies [34], 8 of the 13 dropouts (61%) stopped during the first 4 weeks. Patients were promptly referred to other specialists if side effects occurred. Taken together, high dose interferon and ribavirin combination therapy was well tolerated, but it should be administered in centres that routinely exercise close patient monitoring and experienced multidisciplinary support [34].

In conclusion, high dose interferon induction followed by peginterferon based therapy allows early prediction of SVR and non-SVR in difficult-to-treat chronic hepatitis C patients. In patients with a \( \geq 3 \log_{10} \) HCV RNA decline at week 4 a 24 week treatment duration may suffice, regardless of baseline viral load, but this should be confirmed by other studies. Finally, the development of Specifically Targeted Antiviral Therapy for HCV (STAT-C) in combination with interferon holds great promise for these difficult-to-treat patients [29, 30, 35]. It may be that high dose interferon induction, as in this study, in combination with STAT-C will result in a rapid 1st and a sustained 2nd phase HCV RNA decline, preventing the development or selection of resistance mutations [30, 36] and enabling shorter treatment duration.

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Conflict of interest statement

Dr Reesink is a consultant for Schering-Plough, he has received unrestricted grants from Hoffmann-La Roche, Schering-Plough and Vertex Pharmaceuticals. Dr Beld is a consultant for Bayer Diagnostics.

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Chapter 3

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