

Dynamics of hepatitis C virus infection

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Abstract

Viremia shows only minor fluctuations in untreated patients chronically infected with hepatitis C virus. The steady state situation of balanced viral production and clearance in untreated patients can be disturbed by active antiviral treatment. After initiating interferon- α therapy, a typical biphasic decline of viremia can be observed and analyzed. Evaluation of mathematical models of viral dynamics during the initial phase of antiviral treatment shows high turnover rates of pre-treatment viral production and clearance of about 10^{11} – 10^{13} virions each day and *in-vivo* half-lives of a few hours for free hepatitis C virions. During the first 24 to 48 hours of therapy, a dose-dependent first phase of interferon- α induced viral kinetics is characterized by a rapid exponential decline of serum viral load. Then viral decline enters a second phase of a relatively slow exponential decay during the following weeks of therapy which mainly reflects the death rate of infected hepatocytes. This second phase decay is predictive for the virologic end-of-treatment and even more the sustained response. Non-responding patients typically show constant viremia or even a rebound during this second phase.

Key words: Interferon-alfa, kinetic model, pegylated interferon, ribavirin.

Dynamics of viremia during antiviral treatment

For untreated patients chronically infected with hepatitis C virus (HCV), mean serum viral load is approximately 3.5×10^6 copies per milliliter^{1–3} or equivalently 1.4×10^6 IU/mL according to the WHO HCV RNA standard.⁴ Vire-

mia appears to be independent from the HCV genotype.⁵ Serum levels of HCV RNA show only minor fluctuations within individual, untreated chronically infected patients.⁶ The kinetics of HCV RNA are closely correlated with the kinetics of serum HCV core protein.⁷

Interferon- α

The kinetics of the viral decline in patients responding to interferon- α is characterized by a bi-phasic shape (*Figure 1*) following a delay of about 8–9 hours, likely to be the sum of interferon- α pharmacokinetics and pharmacodynamics as well as the intracellular delay of the viral life cycle.^{7–12} Almost all patients treated with interferon- α show a first phase of rapid dose-dependent viral decline for about 24 to 48 hours.^{10–12} The slopes and extent of decline in the first 24 hours significantly increase from 3 MU, 5–6 MU until 10 MU regimens, but no significant difference between the 10 and 15 MU regimens was observed.^{10–12} After about 48 hours, a second phase of slower viral decline starts which has large inter-patient variation. Initial studies showed no statistically significant correlation with the applied interferon- α schedules (5, 10, or 15 MU daily),¹¹ while more recent studies indicate that the second phase exhibits also some dose-dependency.¹³ According to the initial HCV RNA decline, patients can be categorized as null-responder if they show no first or second phase decline, flat partial responder if they show a first but no second phase decline, slow partial responders and rapid responders (*Figure 2*). Retreatment of non-responders to a standard interferon regimen (3–6 MU tiw) with daily 10 MU of interferon- α typically reveals a considerable first phase decline with no or only minor further decline during the second-phase and occasionally a rebound of viremia can be observed.^{14,15}

Therapeutic response to interferon- α has been shown to be significantly influenced by the genotype of HCV infecting the patient. Controlled clinical trials have demonstrated that end-of-treatment and sustained virologic response rates are approximately 2- to 3-fold greater in patients infected with HCV genotypes 2 or 3, compared with patients infected with genotype 1.^{1–3} Both, the first-phase and second-phase decay and the extent of viral decline after 24 hours have been shown to be HCV genotype dependent and larger for genotype HCV non-1 than for genotype HCV-1 patients.^{16,17}

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Interferon- β

Different turnover rates of hepatitis C virus clearance by different treatment regimen were also described using interferon- β .¹⁸ The treatment efficacy in patients infected with HCV-1 was significantly higher with twice daily i.v. administration of 3 MU interferon- β compared with once daily i.v. administration of 6 MU interferon- β . After the first injection peak levels of interferon were more than two times higher in patients who received 6 MU compared with patients who received 3 MU. After 4 weeks of treatment serum interferon levels were similar in patients treated with 6 MU qd and those treated with 3 MU bd. In addition, the 2'-5' oligoadenylate synthetase activity in serum between day 0 and 28 was similar in both cohorts, indicating that the observed difference in initial viral decline cannot be attributed to differences in 2'-5' oligoadenylate synthetase activity. A significant reduction of platelets and serum albumin together with a marked increase in serum aminotransferases and a high incidence of renal toxicity (proteinuria), however, appears to limit twice daily administration of interferon- β .¹⁸

Pegylated interferon- α

The elimination half-life for unmodified interferon- α ranges from 4 to 10 hours, with peak serum concentrations occurring at 3 to 8 hours following subcutaneous administration. Thus, current antiviral treatment regimens require frequent administration of standard interferon- α with intervals ranging from three to seven times per week for periods of 24 to 48 weeks. Twenty-four hours following administration, there is little or no detectable interferon- α remaining in the serum.^{19,20} An increase of serum HCV RNA can therefore be observed on treatment-free days in patients receiving standard interferon- α three times a week.^{10,16}

Polyethylene glycols (PEG) are amphiphilic polymers with varying average molecular weights that can be chemically linked to proteins. Modification of proteins with PEG has resulted in decreased clearance, increased serum half-life and reduced immunogenicity for a number of proteins.²¹ Peginterferon is synthesized by the chemical conjugation of a PEG molecule with an average molecular weight of approximately 40 kDa or 12 kDa to interferon- α 2a (peginterferon- α 2a) or to interferon- α 2b (peginterferon- α 2b), respectively. The size of the PEG moiety results in sustained delivery and reduced clearance providing the possibility of once weekly dosing.^{22,23} While oscillations of serum HCV RNA are typically seen in patients treated with standard interferon- α , an intermediate increase of the viral load was not observed in the majority of patients treated with peginterferon- α 2a.¹⁶

Treatment of patients with chronic hepatitis C with peginterferon- α 2b (1.0 μ g/kg qw) for one year doubled sustained virologic response rates compared with a stan-

dard regimen of 3 MU interferon- α thrice weekly for one year (24% vs 12%).²⁴ Peginterferon- α 2a (180 μ g qw) was associated with a higher end-of-treatment virologic response at week 48 (69% vs 28%) and sustained virologic response 24 weeks after the end of treatment compared with unmodified interferon- α given three times per week at a dose of 6 MU for 12 weeks followed by 3 MU for the subsequent 36 weeks (39% vs 19%).³

The second-phase but not the first-phase initial decline of serum HCV RNA appears to be faster for patients treated with peginterferon- α (in particular for genotype HCV-1 infected patients) than for patients treated with standard interferon- α , although statistical significance was not achieved.¹⁶ Such an increase may be due to an enlarged death rate of infected cells during therapy with peginterferon- α but can also be explained by the elimination of oscillations in the initial decay of viral load in patients treated with peginterferon- α .¹⁶

Ribavirin

The synthetic purine nucleoside Ribavirin rapidly enters eukaryotic cells and after intracellular phosphorylation exhibits virustatic activity against a broad spectrum of DNA and RNA viruses.²⁵ Several studies have been conducted to evaluate ribavirin monotherapy in daily doses of 600-1,200 mg in the treatment of chronic hepatitis C.²⁶⁻²⁸ While all trials consistently showed a decrease in aminotransferase levels, no virologic end-of-treatment responses were observed. Moreover, the effect on aminotransferase levels was not maintained after the drug was discontinued.

The antiviral mechanisms of ribavirin in patients with chronic hepatitis C are unknown. Quantification of viremia revealed either a minor decline or constant HCV RNA levels after 3-6 months of ribavirin monotherapy compared with pretreatment values.^{26,27} Two studies investigated the initial antiviral effect of ribavirin (1,000-1,200 mg/day) in patients with chronic hepatitis C.²⁹⁻³¹ In the study by Lee et al. with daily sampling within the first week of treatment, a less than 0.5 log or 0.5-1.0 log decline in serum HCV RNA was observed in 5 and 10 patients, respectively, with no apparent difference to untreated patients.²⁹ Changes in HCV quasispecies according to single-strand conformation polymorphism band pattern within the initial four weeks occurred in only one patient treated with ribavirin and in three of the untreated patients. In the study by Pawlotsky et al., in which serum samples were obtained even more frequently within the initial treatment days, a mean decline of 0.3 log in serum HCV RNA was observed at day 2 in 6 patients treated with 1,000-1,200 mg ribavirin.^{30,31} Subsequent analysis of this study revealed that the decline in viral load between ribavirin and untreated patients achieved statistical significance.³¹

Combination therapy interferon- α plus ribavirin

Recent studies indicated that combination treatment with interferon- α plus ribavirin considerably improves

end-of-treatment and sustained virologic response rates compared with interferon- α monotherapy.^{1,2} Kinetic analyses in patients treated with interferon- α and ribavirin have shown a synergistic antiviral effect of ribavirin both on the viral decline in the first week of therapy and in sustained response for HCV-infected patients with 3 MU interferon- α three times per week,^{1,31,32} whereas no significant synergy was observed at 3 MU interferon- α daily and at 6 MU interferon- α three times per week in patients with a profound initial virological response.^{12,31} The latter data were recently confirmed by Berg et al., showing no synergistic antiviral activity of ribavirin in patients treated with 6 MU tiw who achieved a virologic response at week 12 (i.e. HCV RNA below 1,000 copies/mL).³³ However, viral decline was clearly enhanced by ribavirin in the patients who had a less pronounced second phase decline and remained positive for HCV RNA at week 12.³³ Nevertheless, the anti-viral and/or immunomodulatory and/or anti-inflammatory effects of ribavirin in patients with chronic hepatitis C still need further clarification.

Interpretation and limitations of viral kinetic models

No or only minor fluctuations of serum HCV RNA levels⁶ enable the assumption of a steady state situation and therefore a balance between viral production and clearance in untreated patients infected with chronic hepatitis C. During antiviral treatment, serial measurements sample the time course of serum HCV RNA concentration to yield kinetic information on the dynamics of virus replication. Mathematical models analyzing the kinetics of HCV have been proposed by Zeuzem et al.,^{9,12} by Lam et al.,¹⁰ and by Neumann et al.¹¹ In general, models for the dynamics of viral replication are based on a biological process involving compartments of productively infected cells and free virus. Although mathematical definitions differ slightly between the different models, consensus is reached on both these compartments and transfer pathways being the fundamental objects in the underlying biological phenomena. Mathematical modeling and limitations of the mathematical models have previously been reviewed.³⁴

Mathematical modeling of the initial viral decay suggests that the very rapid first phase of initial decline on day 1 of treatment is due to direct, dose-dependent inhibition of virus production.¹¹ The second phase of initial decline is slower and mainly reflects the infected cell death rate in the mathematical model. A significant correlation between the second phase decay rate and baseline aminotransferase levels was observed which suits well with the conclusion of the mathematical model that the second phase decay represents the degradation rate of infected cells.^{11,16}

The antiviral mechanism of interferon- α after the first few days of treatment is less well identified. Even so the second-phase decline of viremia can be explained by continuing the direct inhibition of virus production over the

first day of treatment, however, it is not clear whether the antiviral effectiveness of interferon- α remains constant. Indeed, the oscillations of serum HCV RNA during treatment with standard interferon- α indicate non-constant effectiveness of unmodified interferon which is not modelled so far.¹⁶ The range of biological actions of interferon- α are wide and in addition to direct inhibitory effects include the protection of yet uninfected cells to become infected and several immunomodulatory effects. Interferon- α induces expression of class I major histocompatibility complex antigens and activates macrophages, natural killer cells, and cytotoxic T lymphocytes. Furthermore, interferon- α stimulates the production of type 1 T-helper (Th1) cells, which synthesize mainly IFN- γ and interleukin-2, and reduces that

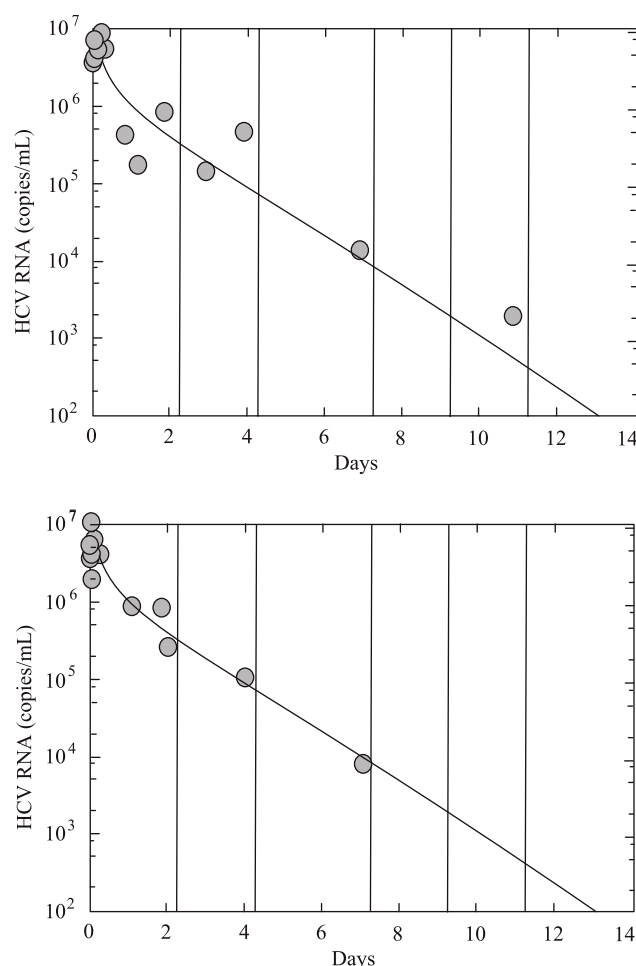


Figure 1. Hepatitis C viremia within the first 14 days of treatment in 2 individual patients. The patient shown above in the panel (A) was treated with standard interferon- α at a dose of 3 x 6 MU per week, while the patient shown below in the panel (B) received peginterferon- α 2a (180 μ g/week). Vertical lines indicate the time points of interferon- α or peginterferon- α s.c. application. Note the oscillation of HCV RNA in the patient treated with standard interferon- α tiw, which was apparently related to an increase of viremia on treatment free days. Lines are calculated from the best-fitting individual parameter sets derived from a biphasic exponential function.

of Th2 cells, which synthesize mainly interleukin-4 and interleukin-5. Interferon- α also has anti-inflammatory properties through the inhibition of peripheral production of interleukin-1, interleukin-8, and tumor necrosis factor α and the stimulation of interleukin-10 production.³⁵⁻³⁸ Most likely several of these biological actions of interferon- α contribute to the second phase of viral kinetics, probably with substantial interindividual variation. Mathematical modeling has not yet contributed to the understanding of the mechanism of action of ribavirin.

The better clearance of HCV-2,3 isolates during the first-phase decline may be due to a difference in interferon effectiveness and/or a faster free virion clearance rate.^{16,39} This clearance rate, which determines the serum half-life of the virions, may be enhanced by antibody-mediated viral clearance in addition to the intrinsic nonspecific clearance. Indeed, a number of studies have shown that antibody responses to the HCV envelope glycoprotein E2 are more vigorous and frequent in HCV non-1 compared with HCV-1 infected patients.⁴⁰⁻⁴² Furthermore, the more rapid genetic variation and evolution in the hypervariable region (HVR)-1 from HCV non-1 infected patients compared with HCV-1 also suggests a stronger immunological pressure on HCV non-1 isolates.⁴³

In addition, the second phase of initial viral decline is faster in HCV-2,3 compared HCV-1 infected patients.^{16,17} The faster second phase most likely reflects a greater degree of immune mediated recognition and killing of HCV-infected cells. The specific proliferative re-

sponse of CD4 cells is stronger and more enhanced by interferon therapy in patients infected with HCV-2 as compared with HCV-1 infected patients.⁴⁴ Whether indeed the decrease of infected cells depends on the HCV genotype will require more careful comparison between the CTL response in HCV-2,3 and HCV-1 infected patients. Current data do not support the hypothesis that the better response in HCV-2,3 infected patients could be explained by HCV genotype dependent differences in replication efficiency.^{5,17}

Conclusions on the viral kinetics over a longer time period cannot be absolute and an exact predictor on the duration of therapy cannot be derived from the models. Nevertheless, it is interesting that early viral kinetics (in particular the second-phase slope) shows statistically significant prediction for end-of-treatment and sustained viral responses.^{16,32,39}

Another important limitation of the mathematical models is that they are designed for fitting HCV RNA levels only in responding patients. The viral kinetics in patients for whom there is no decline in HCV RNA or for whom there is a rebound in viral load after a few days cannot be explained or fit by yet proposed models. This is mainly due to the fact that the evolution of viral fitness is not considered and might be additionally influenced by the simplifying assumption of a constant number of target cells during treatment. More advanced models and more intricate measurements are needed to explain and to fit the kinetics of viral load for rebounders. Possibly, also latently infected cells, in which the nonproducing period is sub-

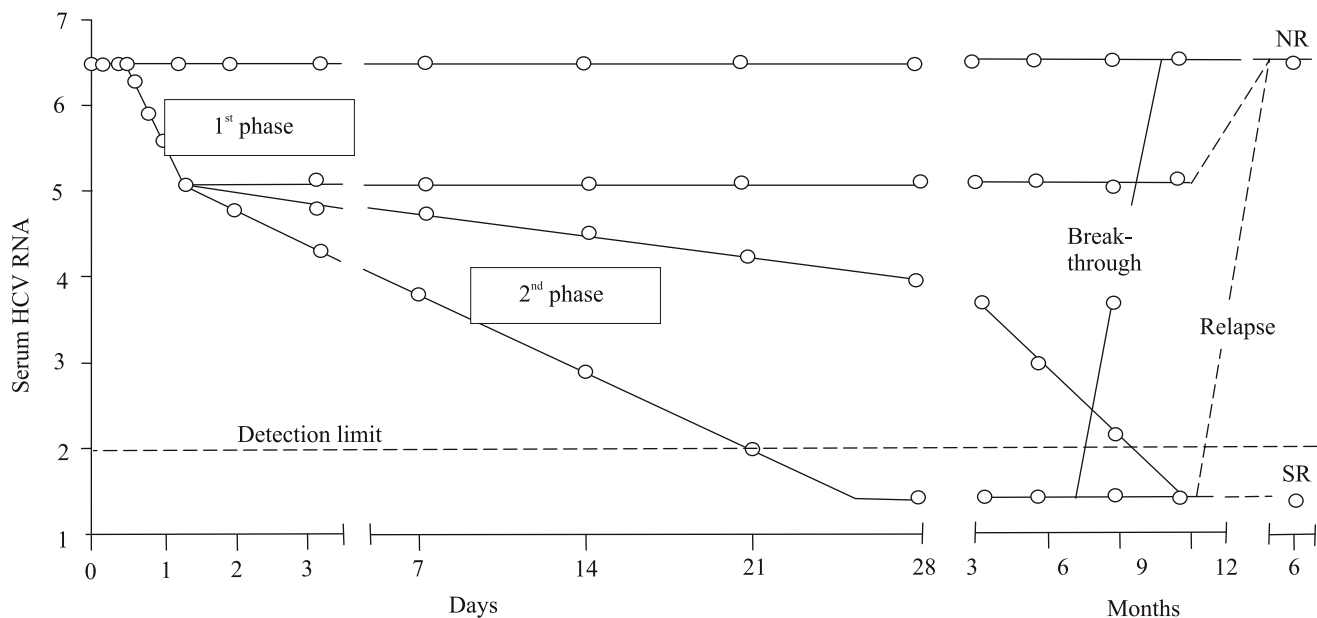


Figure 2. Model of serum hepatitis C viral load during antiviral therapy. After a lag phase of approximately 8-9 h, HCV RNA may or may not decline during the first 24-48 h (1st phase responder/non-responder) which can be followed by a slower second phase decline (2nd phase responder/non-responder). The HCV RNA decay during the second phase is highly variable (flat, slow, or rapid) and predictive for virologic end-of-treatment and sustained response. Some patients with an initial virological response may experience a break-through during therapy and some virological end-of-treatment responders may have a relapse after discontinuation of therapy.

NR, non-response; SR, sustained response.

stantially longer than for the productive infection cycle may need to be considered in hepatitis C virus infection. Such cells, if present, would serve as a reservoir capable of maintaining the infection even after the most effective antiviral therapy.

The most severe limitation originates from data being sampled exclusively from the virus compartment, due mainly to experimental and ethical difficulties. Mathematical modeling of other than the virus compartment and connected pathways based on such limited data quickly becomes less reliable. Although the currently proposed model represents a more detailed picture of the processes governing viral infection, it has to be stressed that all approaches do apply necessary simplifications.^{11,16,34} Rather than being accurate, the current state of HCV kinetic modeling more likely provides a basic yet not complete understanding of the underlying processes which allows to grasp trends and devise upper and lower bounds on the parameter sets. The hypotheses suggested by the model need further proving by experimental results. Nevertheless, such basic understanding of the kinetic processes and such parameter estimates are invaluable guidelines for therapy planning and comparison of different drugs.

Quantitative results

Calculations showed a minimum virus production and clearance per day in patients with chronic hepatitis C of approximately 10^{11} - 10^{13} virions per day and an *in vivo* half-life of the free virus in the order of a few hours (*Table I*).^{9,11} However, the relative proportion of infectious and defective hepatitis C virions is unknown. Interestingly, the same estimate for half life of HCV free virus was obtained from different kind of models, either of viral kinetics during the an hepatic phase of transplantation^{45,46} or during apheresis.^{47,48} The high turnover rate of HCV can explain the rapid generation of viral diversity and the opportunity to develop resistance to the host immune surveillance and to antiviral therapy.³⁷

Analyzing the second-phase decline slopes suggests a broad inter-individual variation with a high turnover rate for infected cells in some patients (*Table I*) and slow turn-

over in other patients. The half-life of infected cells is estimated to range between 1.7 to more than 70 days and the daily turnover between less than 1% up to 33% of infected cells.^{11,12,34} Assuming that 5% to 40% of hepatocytes are productively infected and that rapid death of infected cells gives rise to lower infection levels, a substantial proportion of total hepatocytes are killed and replenished every day (*Table I*). Several lines of evidence support this level of hepatocyte turnover. The hepatocyte turnover can be estimated by surrogate parameters such as aminotransferases which are released due to direct virus-related cytopathic and/or immune-mediated processes. In HCV-infected patients responding to interferon- α , the rapid decline of HCV RNA after initiation of treatment is accompanied by a similar decline of both serum alanine aminotransferase and serum aspartate aminotransferase towards normal levels. The calculated half-life of infected cells and of aminotransferases are similar, indicating that indeed hepatocytes have high turnover rates.¹² The liver of HCV-infected patients is infiltrated by a large number of cytotoxic T lymphocytes. A high expression of Fas antigen as an inducer of apoptosis is observed in liver tissue of patients with chronic hepatitis C. Finally, the regeneration of the liver, e.g. after partial resection, is extremely rapid. A precedent for a high hepatocyte turnover rate exists in patients with chronic hepatitis B, where it has been estimated that approximately 10^9 hepatocytes are killed and replenished every day.⁴⁹

Because eradication of the virus from the host will rely on the elimination of all infected cells, high turnover rates of infected cells improve the possibility for viral eradication within reasonable treatment periods. However, as in hepatitis B, cytokine-dependent virus clearance without cell death must also be considered.^{50,51}

Therapeutic implications

The effectiveness of interferon- α in blocking virion production/release was shown to be dose-dependent, ranging from 70% with 3 MU to 96% with 10-15 MU of interferon- α .¹⁰⁻¹² Ribavirin appears to enhance the initial viral decline in particular in patients treated with low interferon- α doses and those not already responding optimal to interferon- α .^{12,31-33} Peginterferon- $\alpha 2a$ at a dose of 180 mg qw showed an effectiveness comparable to 6 MU standard interferon tiw.¹⁶ Therefore, the enhanced virologic response rates observed with pegylated interferon- α are most likely related to the chemical modification allowing constantly maintained antiviral pressure.

Subcutaneous application of standard interferon- α three times per week leads to rapidly increasing and subsequently decreasing serum interferon- α levels with an infinite peak-to-trough ratio. In theory, the peak-to-trough ratio can be reduced but not be abolished by daily administration. However, there are no published data on serum interferon- α concentrations after daily admin-

Table I. Dynamics of the hepatitis C virus.

| | |
|-------------------------------|---------------------------------------|
| <i>Free Virus</i> | |
| Baseline viral load (plasma) | $4 \times 10^9 - 6 \times 10^{11}$ |
| Daily production (per person) | $4 \times 10^{10} - 1 \times 10^{13}$ |
| Daily turnover | 97% - 99.9% |
| Half-life | 1.5 - 4.6 hours |
| <i>Infected cells</i> | |
| Daily turnover | < 1% - 33% |
| Half-life | 1.7 - >70 days |

istration. As already pointed out, patients treated with 3-10 MU standard interferon- α thrice per week but not patients treated with peginterferon show an intermittent increase of viral load only 24 hours later and subsequently during treatment free days.^{10,16} Together with the initial interferon- α dose-dependent decline in viral load, HCV kinetics therefore suggest the consideration of more aggressive initial dosing regimens using daily injections of standard interferon- α or pegylated interferons.

In naive patients chronically infected with HCV genotype 1, HCV RNA determinations showed a mean log reduction by week 4 of 1.06 (3 MU interferon- α tiw), 2.6 (3 MU interferon- α qd plus ribavirin), 3.1 (5 MU interferon- α qd plus ribavirin), and 3.6 (10 MU interferon- α qd plus ribavirin). From linear regression analysis the predicted interval for HCV RNA negativity was less than 12 weeks for the 5 MU and 10 MU cohorts suggesting that such schedules are likely to maximize the number of patients achieving an initial virological response, i.e. undetectable HCV RNA by a sensitive molecular test at week 12 of therapy.¹³

Clinical trials have shown that higher initial virologic responses can be achieved in induction therapies and that these responses can be at least partially maintained at the end of treatment and the follow-up period.^{52,53} In a large multicenter study patients with chronic hepatitis C were randomized to receive either standard therapy with 3 MU interferon- α tiw plus 1.0-1.2 g ribavirin or high dose induction with 10 MU qd for 2 weeks, 5 MU qd for 6 weeks, 3 MU qd for 16 weeks, followed by 3 MU tiw for 24 weeks together with ribavirin (1.0-1.2 g according to body weight). High dose induction therapy showed higher initial virologic response rates in particular in HCV-1 infected patients. Several patients in the group receiving the induction therapy showed a virologic breakthrough when the interval of interferon- α injections was switched from qd to tiw after 24 weeks of therapy. Together with a higher drop-out rate due to an enhanced side effect profile of the high dose induction treatment the increase in the initial virologic response was not maintained at the end-of-treatment and the end of the follow-up period according to "intent-to-treat" analyses.⁵³

The highest virologic response rates in patients with chronic hepatitis C can currently be achieved by combination therapy peginterferon- α plus ribavirin. Treatment of patients with pegylated interferon- α 2a (180 μ g qw) and ribavirin (1,000-1,200 mg qd) for 48 weeks or peginterferon- α 2b (1.5 μ g/kg qw) and ribavirin (800 mg qd) for 48 weeks achieved sustained virologic response rates of 56% and 54% compared with 45% and 47% in the control group (standard interferon- α 2b 3 MU tiw plus 1.0-1.2 g ribavirin for 48 weeks), respectively.^{54,55} Sustained virologic responses in HCV-1 infected patients were 46% and 42%, respectively.^{54,55} It remains to be seen and investigated in clinical trials whether induction therapies with high-

er doses of pegylated interferons can further increase sustained virologic response rates in particular in HCV-1 infected patients.⁵⁶

Virologic relapse will occur when infected, replication-competent cells remain and/or are not controlled by the host immune system. Assuming that complete viral eradication can indeed be achieved in chronically HCV-infected patients, the antiviral success will depend on the efficacy of the drug, the half-life of infected cells, and noncytotoxic cytokine-dependent curative processes. Thus, one possible approach to improve sustained virologic response rates is prolongation of interferon- α treatment, especially for non-rapid initial responders. Furthermore, combination therapy interferon- α plus ribavirin has been shown to reduce virologic relapse rates, however, the pharmacological mechanisms are not yet well defined.^{1,2,57} Recent trials suggest that the antiviral efficacy of ribavirin is dose dependent, in particular in HCV-1 infected patients.^{54,58}

The predictive value of the initial decline of serum HCV RNA exceeds the significance of HCV genotype and pre-treatment viremia as predictors of successful interferon- α treatment.^{32,59} Assessment of HCV kinetics offer the possibility to tailor therapy individually according to the initial decline of viral load. It is anticipated that the second phase decline after the first couple of days of antiviral treatment will provide the major predictive information and therefore the necessary frequency of sampling will be realistic even in a clinical setting. Early identification of virologic non-responders will allow to intensify treatment in these patients using either higher doses of (peg) interferon or in the future possibly immunomodulatory drugs (e.g. other cytokines, histamine, therapeutic vaccines) or direct antiviral drugs such as ribozymes and enzyme inhibitors (protease, helicase, RNA-dependent RNA polymerase inhibitors).

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