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Treatment of hepatitis C virus infection with interferon and small molecule direct antivirals: viral kinetics and modeling

Libin Rong and **Alan S. Perelson***

Theoretical Biology and Biophysics, Los Alamos National Laboratory, Los Alamos, New Mexico 87545

Abstract

Hepatitis C virus (HCV) infection remains a threat to global public health. Treatment with pegylated interferon (IFN) plus ribavirin leads to a sustained virologic response in about 50% of patients. New therapies using direct antiviral agents have the potential to cure patients unresponsive to IFN-based therapies. Mathematical modeling has played an important role in studying HCV kinetics. Using models one can evaluate the effectiveness of new treatment agents, estimate important parameters that govern virus-host interactions, explore possible mechanisms of drug action against HCV, investigate the development of drug resistance and study quasispecies dynamics during therapy. Here we review our current knowledge of HCV kinetics under IFN-based therapy and newly developed antiviral agents specifically targeted to attack HCV, and show how mathematical models have helped improve our understanding of HCV infection and treatment.

Keywords

Virus dynamics; interferon; ribavirin; small molecule inhibitors; drug resistance; combination therapy

1. Introduction

Chronic hepatitis C virus (HCV) infection has caused an epidemic with approximately 170 million people infected worldwide and three to four million people newly infected each year.^{1,2} The majority of newly infected individuals develop chronic infection. Natural history studies show that 5% to 20% of patients develop cirrhosis after about 20 years of infection.³ An increasing number of patients with cirrhosis will develop hepatocellular carcinoma. End-stage liver disease due to chronic HCV infection is the leading cause of liver transplantation in the western world.⁴

The current standard therapy for hepatitis C consists of pegylated interferon- α (IFN- α), administered once weekly, plus daily oral ribavirin (RBV) for 24 to 48 weeks.⁵ This combination therapy is quite successful in patients with HCV genotype 2 or 3 infection, leading to sustained virologic response (SVR, defined as the absence of detectable HCV RNA in a patient's blood 6 months after the completion of antiviral treatment) in about 80%-90% of patients treated. However, in patients infected with HCV genotype 1 or 4, only about a half of treated individuals achieve SVR.^{6,7} Further, the treatment is often associated with troublesome side effects and high costs. The HCV RNA level before initiation of therapy is another determinant of outcome in patients with genotype 1 infection. Patients with low baseline viral

*Corresponding author: Dr. Alan S. Perelson, Senior Fellow, Theoretical Biology and Biophysics, Mail Stop K710, Los Alamos National Laboratory, Los Alamos, New Mexico 87545, Telephone: 505-667-6829, Fax: 505-665-3493, asp@lanl.gov.

loads (e.g., $<8 \times 10^5$ IU/mL)⁸ have a better chance of SVR. The response rate can also be affected by host factors, such as age, race, gender, obesity, and degree of liver fibrosis.⁹ Mechanistic understanding of the association between treatment failure and each factor has not been established. Currently, there are no effective alternative therapies for nonresponders to prior IFN-based therapy.^{6,7} Therefore, improving existing therapies and developing new antiviral drugs with higher treatment efficacy and more favorable side-effect profiles are of great clinical relevance and importance.

Intensive effort has been invested in the development of specifically targeted antiviral agents for HCV.^{10,11} Increased knowledge of the molecular structure of HCV,^{10,12} its component proteins and the different phases of the viral life cycle^{13,14} has propelled development of specific small-molecule inhibitors of viral entry and replication. Two important targets are the HCV-encoded NS3-4A serine protease and the HCV RNA-dependent RNA polymerase.¹⁰ A number of protease inhibitors and polymerase inhibitors have been developed. Some of them have demonstrated potent antiviral activities in early clinical trials.^{15,16} However, emergence of drug resistant variants complicates monotherapy with these new direct antivirals.^{11,17,18} Combination of these agents with standard therapy may be an attractive strategy for improving SVR rates.¹⁹ Recent clinical trials have shown promising results for the combination of the protease inhibitor telaprevir^{20,21} or boceprevir²² with pegylated IFN plus RBV.

Mathematical models have been developed to analyze HCV kinetics under therapy.²³⁻³³ They have played an important role in evaluating the antiviral efficacy of treatment, improving our understanding of virus-host interactions, providing information about how antiviral drugs act against HCV, and in explaining different HCV RNA kinetics during therapy.²⁷⁻³³ In this paper, we review viral kinetics in HCV patients under the standard IFN-based therapy, and introduce general principles of viral dynamic modeling. We discuss HCV RNA kinetic profiles observed during therapy and recent developments of models proposed to explain them. We also include HCV kinetics in patients receiving newly developed small-molecule antivirals. Our overall goal is to show that mathematical modeling, in conjunction with experimental data, can provide insights into fundamental questions related to HCV infection and treatment.

2. Interferon-based antiviral therapy

Interferons are natural cellular proteins that can have different actions in humans, e.g., direct antiviral effect, inhibition of cell growth and control of apoptosis, and promoting immune responses.^{8,34} The effectiveness of IFN in HCV treatment can be significantly enhanced by the addition of RBV.^{6,7,35} The combination results in higher SVR rates than does IFN monotherapy, even in patients with advanced liver disease or those who either did not respond or relapsed after completion of IFN therapy.⁵ A significant advance in IFN therapy was the introduction of pegylated IFNs, which slow drug elimination, making it possible to maintain a stable serum concentration with once-weekly administration.³⁶ Two pegylated IFNs, PEG-IFN- α 2a and PEG-IFN- α 2b, are commercially available, and they have different pharmacokinetic properties. The duration of IFN-based therapy depends on a few factors, such as HCV genotypes and baseline viral loads. Currently, 24-week combination therapy is recommended for genotypes 2 and 3 patients and 48 weeks for genotypes 1 and 4. By monitoring HCV RNA levels at baseline and after treatment initiation, particularly at 4 and 12 weeks, the optimal duration of therapy can be further customized.³⁷ Careful studies of HCV kinetics during treatment may help the development in individualization and optimization of combination therapy.

2.1 Viral kinetics in response to interferon

The typical response to IFN therapy is a biphasic decline in HCV RNA levels (Fig. 1) after a short time period (about 8-9 hours), which is believed to reflect the sum of IFN

pharmacokinetics/pharmacodynamics and the features of the HCV life cycle.^{32,38-40} The first phase decline in viral loads is rapid, dose-dependent, and usually occurs within the first 24 or 48 hours after treatment initiation. The slope of the first phase is not correlated with baseline viral load or alanine aminotransferase (ALT) level,³² a surrogate marker of liver cell necrosis. The first phase is followed by a second, slower phase, which seems not to be correlated with drug dose. However, the slope of the second phase decline demonstrates considerable inter-patient variability.³² Why does HCV RNA decline undergo a rapid first phase and then a significantly slower second phase? Why does the first phase decline depend on drug dose? Why does the second phase decline vary significantly in patients? Mathematical models have been developed and provided insights into these questions (Section 2.2).

A triphasic decline has been observed in a fraction of HCV patients after treatment with IFN or pegylated IFN.^{31,41,42} In a triphasic decline, after the rapid first phase, there is a second phase (referred to as the “shoulder phase”) with little or no HCV RNA decline, followed by a third phase with a renewed viral decline. Mathematical models, which we shall review (Section 2.5) can explain the triphasic viral decay under therapy.

2.2 Viral dynamic modeling

The general principles of viral dynamic modeling are briefly discussed below (see reviews^{43,44}). During chronic viral infection, the serum concentration of HCV RNA in untreated patients reaches a steady state or set-point value. At the set-point, there is an equilibrium in which the rate of viral production equals the rate of viral clearance. However, simply quantifying viral loads cannot determine the viral production or clearance rate. Initiation of therapy is able to disturb the dynamic equilibrium (i.e., interrupt viral infection/production and let viral clearance dominate), causing viral levels to fall. This provides a means to determine the rate of viral clearance and to estimate the number of virus particles produced each day at the pre-treatment steady state. This approach was initially used to study the dynamics of HIV infection,^{43,45,46} and later extended to the study of both HCV^{23-26,32} and hepatitis B virus infections.⁴⁷⁻⁵⁰

The basic model of HIV infection was adapted by Neumann et al.³² to study the kinetics of chronic HCV infection during IFN- α therapy. The model includes three variables (Fig. 2): uninfected target cells (T), productively infected cells (I), and free virus (V). s and d are the recruitment rate and per capita death rate of uninfected target cells, respectively. Viral infection is assumed to occur at a rate proportional to the product of the densities of virus and target cells (the law of mass action), i.e., at rate βVT , where β is the infection rate constant. Productively infected cells are lost, by either natural death or immune attack, at rate δ per cell. Virus is released from productively infected cells at rate p per cell and cleared at rate c . Lastly, IFN therapy is assumed to act by partially blocking viral production, with efficacy ε , and by reducing the infection rate, with efficacy η (Fig. 2), where ε and η are between 0 and 1. Before IFN therapy, both ε and η are 0. Once treatment is initiated, $\varepsilon > 0$ or $\eta > 0$ or both. The model is described by the following differential equations:

$$\begin{aligned}\frac{dT}{dt} &= s - dT - (1 - \eta)\beta VT \\ \frac{dI}{dt} &= (1 - \eta)\beta VT - \delta I \\ \frac{dV}{dt} &= (1 - \varepsilon)pI - cV\end{aligned}\quad (1)$$

To determine the mode of action of IFN- α against HCV, the viral load decline during the first two weeks of daily IFN- α 2b treatment with doses of 5, 10, and 15 IU was analyzed using the above model.³² If IFN acts solely by blocking new HCV infections ($\eta > 0$ and $\varepsilon = 0$), then HCV clearance and production would both continue at their pretreatment rate until infected cells begin to die. This would suggest a slow first phase decline. Moreover, it was shown that

blocking de novo infection could not account for the strong dose dependence observed during the first day of therapy and for the slower viral decline after 2 days.³² If the major effect of IFN is to block viral production ($\varepsilon > 0$), then a rapid dose-dependent HCV RNA decline is expected after treatment initiation. Further, if blocking is not 100% (i.e., $0 < \varepsilon < 1$), then model analysis showed that the viral decline will be biphasic. The slope of the first phase decline is determined by the viral clearance rate, c , and the drug efficacy, ε . The second phase decline reflects the loss rate of productively infected cells, δ , as well as the drug efficacy.³²

These results suggest that the rapid decay in HCV RNA levels is primarily due to IFN's inhibition of viral production. Subsequent studies using the HCV replicon system have confirmed that IFN is able to inhibit viral replication in a dose-dependent manner.^{51,52} When the viral load declines, the rate of de novo infection also declines. As a consequence, the level of infected cells cannot be maintained and a slow net loss of infected cells occurs. This net loss is predicted to account for the second phase viral decline.²⁵ Neumann et al.³² showed that the estimated infected cell death rate is inversely correlated with baseline viral load and positively correlated with baseline level of ALT, while a positive correlation was reported between baseline ALT level and anti-HCV cytotoxic T lymphocyte (CTL) frequency.⁵³⁻⁵⁵ These observations suggest that a CTL-mediated immune response may be responsible for the clearance of productively infected cells. Thus, the considerable variation in the estimates of infected cell death rate could reflect the observed differences in cellular immunity against HCV. Similar observations were reported in a study with pegylated IFN- α 2a.⁵⁶

A robust first and second phase viral decline has been shown to be important for treated patients to achieve an SVR.^{38,57} Lack of a good first phase viral decline implies that the IFN therapy is not potent enough to block viral production, while a poor second phase decline suggests that the immune response may not be effective in killing infected cells. These viral kinetic principles can be applied to explain different antiviral effects of IFN therapy in patients with different viral and host characteristics, such as genotype, race, obesity, and baseline viral load. For example, the efficacy of IFN in blocking viral production was found to be significantly higher in patients infected with HCV genotype 2 than those with genotype 1.⁵⁸ Patients with genotype 2 infection have larger estimates of the viral clearance rate and the infected cell death rate.⁵⁸ This may explain IFN therapy leads to a higher SVR rate in patients infected with genotype 2 than with genotype 1. It was estimated that African Americans more frequently had a lower IFN efficacy and a slower first phase decline than Caucasian Americans, which may explain why IFN therapy results in a higher SVR rate in Caucasian Americans.⁵⁹

2.3 Viral kinetics in response to pegylated interferon

Pegylated IFNs have a longer half-life (about 77 hours and 40 hours for PEG-IFN- α 2a and PEG-IFN- α 2b, respectively), better pharmacokinetic properties, and have yielded a better SVR rate in HCV patients than standard IFN.⁶⁰⁻⁶² Viral kinetic analysis has been performed to study the antiviral potency of pegylated IFNs.^{56,63} The viral decay after treatment initiation is generally biphasic, with a rapid dose-dependent first phase decline and a second, slower phase decline, similar to the results with unmodified IFNs. The first and second phase declines are also faster in patients infected with HCV genotypes 2 and 3 than with genotype 1.

Some patients treated with pegylated IFNs experienced a transient viral rebound after the initial rapid decline.^{33,63-65} This can be explained by the decrease in serum concentration of pegylated IFN toward the end of each 7-day dosing interval. In fact, it is of great interest and importance to incorporate pharmacokinetic/pharmacodynamic (PK/PD) properties into the viral kinetic studies of pegylated IFNs in HCV patients. A pilot study by Powers et al.³³ included PK/PD analysis of PEG-IFN- α 2b into the basic viral dynamic model (Eq. (1)), and examined the effect that changes in drug concentration and effectiveness can have on viral levels. The time-varying

effectiveness of a drug, $\varepsilon(t)$, can be related to its concentration, $C(t)$, by a simple equation (similar to the E_{max} model used in PD analysis⁶⁶)

$$\varepsilon(t) = \frac{C^h(t - \tau)}{IC_{50}^h + C^h(t - \tau)} \quad (2)$$

where IC_{50} is the drug concentration needed to inhibit viral replication by 50%, h is the Hill coefficient, and τ is the delay between the binding of IFN to cellular receptors and having an intracellular effect.

A simple “absorption and elimination model”³³ was employed to describe the change in the amount of drug in the blood (A):

$$\frac{dA}{dt} = k_a X - k_e A \quad (3)$$

where X is the amount of drug at the absorption site, k_a is the rate of absorption, and k_e is the elimination rate. X can be further calculated as $X = FD e^{-k_a t}$, where F is the bioavailability and D is the drug dose. Solving A from the above equation (3), and dividing by the volume of distribution, V_d , one can obtain the concentration of IFN in the blood:

$$C(t) = \frac{k_a F D}{(k_e - k_a) V_d} (e^{-k_a t} - e^{-k_e t}) \quad (4)$$

Here the first dose of pegylated IFN was assumed to be injected at time $t=0$. When the drug is given multiple times, a more complex expression of the drug concentration is obtained.⁶⁷ The time-varying drug efficacy, as calculated in Eq. (2), was integrated into the basic viral dynamic model (Eq. (1)). Assuming the level of uninfected cells is constant, the model is able to account for the observed viral rebound as the drug concentration declines between doses.³³

The model with time-varying drug efficacy was also used to analyze viral load data obtained from HIV/HCV coinfecting patients treated with PEG-IFN- $\alpha 2b$ plus RBV.⁶⁵ Both HCV RNA level and pegylated IFN concentration were measured frequently after the first 3 IFN doses. PK parameters, i.e., IFN absorption and elimination rate constants and the ratio FD/V_d , were estimated by fitting $C(t)$ to the serum concentration of PEG-IFN- $\alpha 2b$. The viral dynamic model with $\varepsilon(t)$ was fitted to both the initial viral decline and rebound at the end of the week, and the PD parameter IC_{50} was estimated based on the fitting. These PK/PD parameters were compared in sustained virologic responders and nonresponders.⁶⁵ It was found that pegylated IFN concentrations and PK parameters do not differ, while a few PD measurements, e.g., IC_{50} , the therapeutic quotient (the weekly average drug concentration divided by IC_{50}), and $C(7)/IC_{50}$, are different between sustained virological responders and nonresponders.⁶⁵ These parameters may be useful predictors of treatment outcomes during the first month of therapy.

The modeling approach with PK/PD analysis needs to be considered in studying the antiviral effect of pegylated IFNs. Shudo et al.⁶⁸ showed that using a constant drug efficacy in the viral dynamic model may result in errors in the estimation of viral kinetic parameters. However, the integrated PK/PD modeling approach requires data on serum drug concentrations, which are normally not measured during therapy. A heuristic model with a time-varying efficacy, as

suggested in Bekkering et al.,⁴¹ can be an alternative method. A “decreasing effectiveness” model was used by Shudo et al.⁶⁹ to analyze HCV RNA data from patients treated with PEG-IFN- α 2b and RBV.

2.4 Viral kinetics in response to ribavirin

When used as monotherapy, ribavirin results in either a transient initial decline or no decrease in HCV RNA level.^{70,71} The addition of RBV to IFN- α , however, has dramatically improved the long-term outcome of therapy.^{6,7,35} In some patients, RBV induced an increase in the slope of the second phase decline, whereas in other patients, it did not significantly affect the second phase, although it decreased the frequency of viral rebound observed when only pegylated IFN was administered.⁷⁰ In patients with a triphasic decline after treatment with IFN or pegylated IFN, administration of RBV seemed not to affect the first phase decline, but enhanced the third phase decline.^{29,31}

The antiviral mechanisms of RBV's action against HCV have not been fully elucidated. A number of mechanisms have been proposed (see reviews^{8,72}), and mathematical models have been developed to test possible mechanisms. In one study,³¹ RBV was assumed to serve as an immune modulator. A model was developed based on this mechanism and used to study the effect of RBV on HCV kinetics.³¹ The model included an inflation factor that represents the increase in the death rate of infected cells after an initial delay (i.e., assuming a delayed enhancement of immune responses induced by RBV alone or in combination with IFN). Model results suggested that RBV did not have a noticeable effect on the first phase decline and the second shoulder phase. However, it significantly increased the slope of the third phase decline.³¹

Another study by Dixit et al.³⁰ tested the hypothesis that RBV acts by lowering the infectivity of HCV, possibly via mutagenesis but effects on the host cells also have this effect. They modeled this loss in infectivity by assuming that RBV causes a fraction ρ of newly produced virions to be noninfectious, and developed the following model:

$$\begin{aligned}\frac{dI}{dt} &= \beta V_I T - \delta I \\ \frac{dV_I}{dt} &= (1 - \rho)(1 - \varepsilon)pI - cV_I \\ \frac{dV_{NI}}{dt} &= \rho(1 - \varepsilon)pI - cV_{NI}\end{aligned}\tag{5}$$

where V_I and V_{NI} represent infectious and non-infectious virus, respectively. The model predicted that RBV does not influence the first phase viral decline, but increases the slope of the second phase decline in a dose-dependent manner if the efficacy of IFN (ε) is low. When ε is high, RBV does not influence the second phase decline either (Fig. 3). These predictions are in agreement with experimental results and resolve the seemingly conflicting observations that RBV influences the second phase viral decline in some patients but not in others.^{31,59,70}

Although two recent studies suggested that RBV can lead to an early and detectable increase in mutation rates in NS5B,^{73,74} consistent with the mutagenesis hypothesis, other studies have not detected an increase in mutation rates.⁷⁵ Whether RBV acts mainly as a mutagen or involves other mechanisms remains unclear. Further studies are needed to elucidate RBV's antiviral mechanisms against HCV. Such studies may determine if RBV is needed to be included in future therapies when potent direct antivirals become available and are used in combination therapies.⁷⁶

2.5 Triphasic viral decline

The typical response to IFN- α therapy alone or in combination with RBV is a biphasic viral decline, as shown in Fig. 1. However, a fraction of patients experience a triphasic viral decline with a shoulder phase (Section 2.1). For example, Herrmann et al.³¹ observed triphasic declines in 9 of 17 patients treated with pegylated IFN- α alone, in 4 of 7 patients treated with IFN- α and RBV, and in 8 of 10 patients treated with pegylated IFN- α plus RBV. Although they developed a model to explain the triphasic decline by assuming RBV acts as an immune modulator (the above section), the death rate of infected cells has to be close to zero in order to generate the shoulder phase.³¹ To overcome this problem, Dahari et al.^{29,77} showed that including the proliferation of both uninfected and infected cells in the basic viral dynamic model (Fig. 2) can account for a triphasic HCV RNA decline after treatment. The model is an extension of Eq. (1):

$$\begin{aligned}\frac{dT}{dt} &= s + r_T T \left(1 - \frac{T+I}{T_{\max}}\right) - \beta V_I T - dT \\ \frac{dI}{dt} &= r_I I \left(1 - \frac{T+I}{T_{\max}}\right) + \beta V_I T - \delta I \\ \frac{dV_I}{dt} &= (1 - \rho)(1 - \varepsilon)pI - cV_I \\ \frac{dV_{NI}}{dt} &= \rho(1 - \varepsilon)pI - cV_{NI}\end{aligned}\quad (6)$$

where r_T and r_I are the maximum proliferation rates of uninfected and infected cells, respectively, and $T_{\max}$ is the carrying capacity of the liver.

The model shows that the flat second phase in flat partial responders (those who have a first phase decline followed by little or no second phase decline⁷⁸) and in triphasic responses can be a consequence of liver regeneration.²⁹ Furthermore, the model predicts that a triphasic decline occurs only in patients in which a majority of liver cells are infected before therapy, consistent with the observation that only a fraction of patients exhibit triphasic decline. Due to the inclusion of infected cell proliferation, the shoulder phase does not represent the death rate of infected cells, but rather a balance between death and generation of infected cells. Lastly, the slope of the third phase decline is close to the death rate of infected cells if the overall drug efficacy is high.²⁹ The model further supports that the last phase viral decline is enhanced by the addition of RBV due to its mutagenic effect, as suggested in Dixit et al.³⁰

3. Small-molecule inhibitors of viral enzymes

Recent advances in the understanding of the HCV genomic organization and the viral life cycle have made it possible for the development of new, specific antiviral drugs.^{13,14} Unlike traditional IFN and RBV, these new putative therapeutics target specific stages of the viral life cycle, the so-called specifically targeted antiviral therapy for hepatitis C (STAT-C).^{11,16,79,80} Many compounds are at the preclinical developmental stage, and some have entered clinical trials. Both in vitro models and early clinical studies have generated exciting results, regarding antiviral potency.⁸⁰ However, they have also raised concerns about the tolerability and the development of drug resistance during treatment.^{17,18} Based on the lessons from HIV combination therapy, a number of promising small-molecule drugs are being tested in combination with PEG-IFN- α with or without RBV.^{19,81}

3.1 HCV life cycle

The HCV life cycle (Fig. 4) starts with viral attachment, entry, and fusion, which involve both the HCV structural proteins and the receptor molecules at the surface of target cells. Two HCV envelope glycoproteins, E1 and E2, are essential for viral entry and fusion. A few cell surface molecules, such as CD81,⁸² the human scavenger receptor class B type 1 (SR-B1),⁸³

claudin-1,⁸⁴ and other putative receptor molecules⁸⁵ have been proposed to mediate HCV binding or HCV binding and internalization. After fusion of the viral and cellular membranes, viral nucleocapsid releases a single-stranded, positive-sense genomic RNA into the cell cytoplasm. This genome serves as a messenger RNA (mRNA) for synthesis of a large polyprotein (HCV polyprotein translation). It can also be a template for HCV RNA replication, or be packaged as a new genome in a progeny virus particle. At least two host cellular peptidases, i.e., host signal peptidase and signal peptide peptidase, are required for processing of the HCV structural proteins from the HCV polyprotein.¹⁴ A few viral enzymes, including the NS3-4A serine protease, are involved in the HCV polyprotein cleavage for generation of nonstructural proteins. This is followed by HCV RNA replication. The precise mechanism of HCV replication is largely unknown, but the process is thought to be semiconservative and asymmetric⁸⁶: the positive-strand genome RNA serves as a template for the synthesis of a negative-strand intermediate; the negative-strand RNA then serves as a template to produce multiple nascent genomes. NS5B RNA-dependent RNA polymerase (RdRp), a product of the polyprotein cleavage, is thought to catalyze HCV RNA replication. The last step of the HCV life cycle is viral assembly and release. Although the HCV core protein can self-assemble to generate nucleocapsid-like particles and virus seems to be released into the extracellular milieu by exocytosis,¹⁴ the exact mechanisms underlying HCV assembly and exportation are still poorly understood because of lack of appropriate study models. Inhibitors of different steps in the HCV life cycle (e.g., viral entry, RNA translation and post-translational processing, RNA replication, viral assembly and release) are currently at various stages of clinical development (see a recent review⁸⁰).

3.2 Protease inhibitors

The HCV-encoded NS3-4A serine protease has long been a desirable drug target. However, its shallow active binding site makes it difficult to develop small-molecule inhibitors. The first NS3-4A protease inhibitor to enter clinical trials was BILN 2061. It showed potent antiviral activity in patients with HCV genotype 1 infection.^{87,88} However, its clinical development has been halted due to drug-induced cardiotoxicity in animal models.⁸⁹ Despite this, the early study of BILN 2061 provided a proof of concept that NS3-4A inhibitors may lead to profound suppression of HCV replication. Moreover, in vitro studies provided information about the development of drug resistance to this class of drugs.⁹⁰⁻⁹²

Telaprevir (VX-950) is a reversible, selective, and specific peptidomimetic inhibitor of NS3-4A that is effective in inhibiting viral replication in HCV replicon cells.⁹³ It had a favorable pharmacokinetic profile with high exposure in the liver in several animal models,⁹⁴ and even in monotherapy induced a profound decline (3-4 log₁₀ IU/mL) of plasma HCV RNA levels in patients infected with HCV genotype 1 treated for two weeks.⁹⁵ However, some patients experienced viral breakthrough during the dosing period, which was believed to be associated with the selection of HCV variants with reduced sensitivity to telaprevir.⁹⁵ Sarrazin et al.⁹⁶ identified mutations that confer resistance to telaprevir using a highly sensitive sequencing assay. A subsequent study⁹⁷ provided a detailed kinetic analysis of HCV variants in patients treated with telaprevir for 14 days (see more discussions on drug resistance in Section 3.4).

Boceprevir (SCH 503034) is another HCV NS3-4A protease inhibitor. It has shown potent antiviral properties in vitro.⁹⁸ Phase I trials have suggested that the drug is not as potent as telaprevir at the doses tested so far. One-week monotherapy with SCH 503034 in patients who had previously failed to respond to PEG-IFN- α alone or PEG-IFN- α plus RBV induced an average HCV RNA load reduction of approximately 1 and 1.6 log₁₀ IU/mL at dose of 200 mg and 400 mg three times a day, respectively.⁹⁹ Drug resistant mutations emerged rapidly during

therapy.⁹⁹ In vitro studies also showed that several amino acid substitutions in the NS3 protease sequence confer resistance to boceprevir,¹⁰⁰ and cross-resistance to telaprevir.¹⁰¹

A few other NS3-4A protease inhibitors, such as TMC435350, MK-7009 and R7227, have entered phase I or II clinical programs.⁸⁰ Telaprevir and boceprevir are the two protease inhibitors that have progressed to the most advanced stage (phase III) of clinical development.

3.3 Polymerase inhibitors

The HCV NS5B RdRp is indispensable to viral RNA synthesis¹³ and represents another attractive drug discovery target.¹⁰ Inhibitors of the RdRp can be divided into two categories: nucleoside/nucleotide inhibitors, which target the enzyme catalytic site, and non-nucleoside inhibitors, which target the allosteric site, separate from the catalytic site.³⁷

A number of nucleoside analogues have been developed, including NM283, R1626, R7128, and IDX184. NM283 (prodrug of NM107) is the first nucleoside polymerase inhibitor, showing potent antiviral activities in both the HCV replicon system and HCV infected chimpanzees.¹⁰² When administered to HCV genotype 1 patients as monotherapy for 2 weeks, NM283 induced a dose-dependent viral decline.³⁷ However, its clinical development has been placed on hold since a phase II trial suggested that its treatment benefits did not outweigh its gastrointestinal side effects.¹⁰³

R1626 is a prodrug of R1479, a potent inhibitor of HCV replication in vitro. When R1626 was administered at various doses (500 mg, 1500 mg, 3000 mg, or 4500 mg twice daily) for 14 days, it induced 0.32, 1.2, 2.6, and 3.7 log₁₀ IU/mL reductions in serum HCV RNA levels, respectively.¹⁰⁴ Doses up to and including 3000 mg twice daily were well tolerated, but higher doses (4500 mg) were associated with increasing adverse events. No resistance was observed after 14 days of treatment with R1626.¹⁰⁴ It is currently in phase II development.

R7128 is a prodrug of PSI-6130. A multiple dose study (750 mg once daily, 1500 mg once daily, 750 mg twice daily) was performed to assess safety, tolerability, pharmacokinetics, and antiviral activity of R7128 in HCV genotype 1 infected patients who had failed prior IFN therapy.¹⁰⁵ R7128 has induced dose-dependent reductions (0.9, 1.5, 2.1 log₁₀ IU/mL, respectively) in HCV RNA levels. The drug was well tolerated as monotherapy. Mutations associated with drug resistance have not been reported. More clinical studies are ongoing to evaluate the antiviral potency of R7128 in combination with pegylated IFN- α and RBV. A new nucleotide analogue, IDX184, has recently entered phase I development due to its promising preclinical results.¹⁰⁶

The non-nucleoside polymerase inhibitors are drugs that bind allosterically on the enzyme surface to disturb its structure and function. A few compounds have been developed. However, some of them, e.g., R803, HCV371, and HCV086, have not shown sufficient antiviral activities.³⁷ HCV-796 is a more potent non-nucleoside inhibitor of the HCV RdRp. In a clinical dose-ranging study, patients with the highest exposure achieved a 1.4-1.5 log₁₀ HCV RNA level reduction below baseline.¹⁰⁷ However, viral breakthrough occurred in most patients after a few days of administration. It has been shown to be associated with selection of drug resistant mutations, such as the amino acid substitution C316Y, which confers significant resistance to HCV-796, but remains susceptible to IFN and RBV in vitro.¹⁰⁷ A subsequent phase II study suggested that the combination of HCV-796 and pegylated IFN had greater efficacy and reduced the selection of drug resistant variants.¹⁰⁸ However, the clinical development was halted in late 2007 because of concerns about liver toxicity.⁸⁰

Another non-nucleoside polymerase inhibitor, VCH-759, induced an HCV RNA reduction of 2.5 log₁₀ IU/mL in patients receiving 800 mg three times a day for 10 days in a phase I study.

¹⁰⁹ Viral breakthrough was observed in a subset of patients. Drug resistant mutations, such as M423T/V and L419M, were associated with about 20-fold increases in replicon IC₅₀ values.

¹¹⁰ VCH-759 has recently entered phase II development.

3.4 Drug resistance to direct HCV antivirals

Although direct acting small-molecule HCV enzyme inhibitors have shown promising antiviral effects in early clinical trials, emergence of drug resistant variants may complicate the treatment of HCV patients with these STAT-C agents.¹⁸ Because of high-level viral replication³² and the error-prone nature of HCV RNA polymerase,¹¹¹ amino acid mutations in the NS3 and NS5B proteins can be generated before therapy. Indeed, the viral population exists as a complex mixture of genetically distinct, but closely related, variants, referred to as a quasispecies.^{112, 113} Normally, these mutations are associated with a fitness cost. Thus, they are present at low concentrations compared with the dominant wild-type virus before therapy. After treatment initiation, these minor viral populations with reduced sensitivity to the administered drug may gain a fitness advantage and begin to grow. A few factors may influence the selection and evolution of drug resistant variants, e.g., the number of mutations needed to confer resistance, the level of resistance conferred by mutation(s), the prevalence of viral variants before therapy, intrinsic replication capacity of variants, and replication space (availability of uninfected target cells).

The selection and kinetics of drug resistant HCV variants have been studied in patients treated with STAT-C drugs, particularly the protease inhibitor telaprevir. In a study with telaprevir monotherapy, the four patients with genotype 1a infection experienced viral breakthrough during the 14-day dosing period.⁹⁷ Virus isolated from them at day 2 after therapy contained low levels of single mutants, which increased in the virus population at days 6 and 10. They were replaced by more resistant double mutants by day 13 and during the first follow-up week with standard therapy⁹⁷ (Fig. 5). The rapid emergence of drug resistance to protease inhibitors can be partially explained by the structure of the NS3 enzyme. The active site for protease inhibitors is a long shallow groove.¹⁹ Thus, even a single point mutation in this enzyme might be sufficient to hinder the binding of these drugs. Sequencing analysis of samples obtained from telaprevir treated patients has shown that single mutants, such as T54A, V36A/M, R155K/T, and A156V/T, are able to confer different levels of drug resistance to the drug.⁹⁶ Many studies have reported the selection of HCV variants resistant to other protease inhibitors using the replicon system.^{90-92,100,114}

One can employ a two-strain mathematical model (Fig. 6) to study the development of drug resistance during treatment with HCV protease inhibitors, and compare model predictions with experimental data obtained from patients treated with telaprevir monotherapy.⁹⁷ Model analysis shows that the wild-type virus always undergoes a more profound decline than drug resistant virus during a short time period after treatment with potent antivirals like telaprevir (L. Rong, H. Dahari, R.M. Ribeiro, and A.S. Perelson, unpublished results). Such a substantial decline of drug sensitive virus can uncover pre-existing drug resistant HCV variants. Over a longer time interval, the tradeoff between the reduced susceptibility to the protease inhibitor and resistance-associated fitness loss of the mutant virus determines which strain(s) will dominate the virus population.

In contrast to the rapid appearance of drug resistance during treatment with protease inhibitors^{97,99} or non-nucleoside polymerase inhibitors,^{107,109} there is no clear evidence of drug resistance against the nucleoside polymerase inhibitors NM283,¹¹⁵ R1626,¹⁰⁴ and R7128.¹⁰⁵ Lack of drug resistance to these nucleoside analogues can be explained by decreased replication fitness. The active site of the NS5B polymerase is a highly conserved region in all HCV genotypes,¹⁹ and thus any amino acid mutations in this region may inhibit the ability of the virus to replicate. Recent in vitro studies have also confirmed mutation-associated loss of

replication capacity. NS5B mutations, such as S282T for PSI-6130 and S96T or S96T/N142T for R1479, result in a greater than 85% reduction in replication capacity.¹¹⁶ Interestingly, they only confer a 3- to 5-fold loss in sensitivity to nucleoside polymerase inhibitors in the replicon system.¹¹⁶ The combined effect of reductions in replication capacity and drug susceptibility leads to a fitness disadvantage of these viral variants. This may explain why no drug resistant virus against these nucleoside analogues has been observed in clinical trials to date.

In summary, the development of drug resistance to HCV protease inhibitors and non-nucleoside polymerase inhibitors is a major concern. Nucleoside polymerase inhibitors seem to have a higher genetic barrier to resistance than either protease or non-nucleoside polymerase inhibitors in the replicon system, which highlights the clinical importance of nucleoside analogues in future HCV therapies.

3.5 Combination therapy

Combination antiretroviral therapy has significantly changed the management of HIV infection. Antivirals from distinct classes with different modes of action have been shown to greatly suppress viral load, prevent or delay the development of drug resistance, and thus prolong treatment benefits by slowing disease progression. Because of many similarities between the HIV and HCV diseases, e.g., high-level viral replication, high mutation rate and large viral population size, combination therapy seems to be an attractive strategy for treatment of hepatitis C.

Currently, many newly developed direct antivirals are being tested in combination with pegylated IFNs with or without RBV. The non-nucleoside polymerase inhibitor HCV-796 was combined with PEG-IFN- α in a phase II trial. During the 14-day treatment, the HCV RNA level reduction was greater than that with PEG-IFN- α alone (3.3-3.5 log₁₀ vs 1.6 log₁₀ IU/mL) and no viral relapse due to emergence of drug resistance was observed.¹⁰⁸ Evaluation of the combination effect of pegylated IFNs with nucleoside polymerase inhibitors, such as R1626 and R7128, is ongoing.^{117,118} Early results were encouraging, showing synergistic reductions in serum HCV RNA levels and higher rates of early virologic response (see a review⁸⁰).

The protease inhibitors boceprevir and telaprevir have been extensively tested in combination with pegylated IFNs. The antiviral effect of boceprevir (SCH 503034) seemed to be strictly additive to that of PEG-IFN- α 2b. The combination has demonstrated substantial antiviral activities in HCV genotype 1 patients who were previously nonresponders to PEG-IFN +/- RBV.⁹⁹ Clinical studies have been performed to evaluate the combination effect of telaprevir and pegylated IFNs. During a 14-day therapy, all patients receiving telaprevir plus PEG-IFN- α -2a had continued antiviral response.⁹⁷ Even in patients who had viral rebound following telaprevir alone, follow-up therapy with standard treatment can suppress growth of both wild type and telaprevir-resistant virus.⁹⁷ The aforementioned two-strain model (Fig. 6) can also be used to analyze the kinetics of HCV RNA levels in patients under combination therapy and fit to data. Estimates of the total treatment efficacy confirmed that HCV mutants with reduced sensitivity to telaprevir still remain sensitive to pegylated IFNs (L. Rong, H. Dahari, R.M. Ribeiro, and A.S. Perelson, unpublished results). Recently, two clinical trials showed that the addition of telaprevir to standard therapy could significantly improve SVR rates in patients infected with HCV genotype 1.^{20,21} The duration of therapy can be reduced from 48 weeks to 24 weeks while maintaining an improved SVR in most patients, particularly in those who had not been treated previously.²¹ The response rate is lower with the regimen that did not include RBV,²⁰ which further supports RBV's role in preventing virologic relapse.¹¹⁹

As more directly acting drugs edge towards the market and early reports show encouraging antiviral results of combination therapy, we anticipate that the way in which HCV is treated is about to change. Combinations using multiple STAT-C agents alone or with standard treatment

regimens may improve response rates in HCV genotype 1 patients, with a shortened duration of treatment. However, a few issues remain to be considered. First, even with the most advanced combination therapy (telaprevir + pegylated IFN + RBV), about one third of patients with HCV genotype 1 infection have not achieved SVR.^{20,21} Moreover, combination therapy may increase more adverse effects. For example, the addition of telaprevir to therapy with pegylated IFN and RBV was associated with an increase in the rate of treatment discontinuation, primarily due to the side effect of rash.²¹ When R1626 was combined with pegylated IFN, profound haematological toxicity was observed.¹²⁰ Second, the role of new STAT-C agents in the treatment of nonresponders to prior IFN-based therapy or patients who experienced viral relapse has to be investigated. Third, although viral breakthrough was infrequent (about 7%) in patients receiving telaprevir + PEG-IFN- α 2a + RBV, similar drug resistant variants against telaprevir as in monotherapy⁹⁷ were observed in patients at the time of breakthrough.²¹ The long-term clinical consequences of the selection and persistence of these HCV variants in patients who do not achieve SVR remain unclear and will require more long-term follow-up studies.

4. Conclusions

The current IFN-based therapy achieves SVR in about a half of patients treated. Assessment of HCV kinetics provides the possibility to tailor therapy according to the viral load decline after treatment initiation. Mathematical models that were developed to analyze experimental and clinical data have improved our understanding of HCV infection and treatment.

The development of new antiviral drugs such as specifically targeted small-molecule compounds is ongoing. Some of them have shown promising results in clinical trials. Two HCV NS3-4A protease inhibitors have progressed to phase III studies. A number of HCV polymerase inhibitors are in phase I or II trials and entry inhibitors are under development. Emergence of drug resistance is a major concern with these new drugs. Mathematical models can be used to study the development of drug resistance and explain clinical phenomena during treatment, e.g., the rapid emergence of drug resistance is expected with HCV protease inhibitors and non-nucleoside polymerase inhibitors, but not with nucleoside polymerase inhibitors.

Newly developed direct antiviral drugs may be added to treat HCV infection in the future. However, it is likely that IFN-based therapy will remain the cornerstone of the treatment for a long time, due to its efficiency in preventing or delaying HCV drug resistance against directly acting drugs. Combination of antivirals showing additive potency, lacking cross resistance and with a good safety profile is an ideal option to improve treatment benefits. Mathematical models may be used to explain viral kinetic profiles observed with new drugs, test possible mechanisms for novel biological/clinical phenomena, and to optimize combination therapy (e.g., determining optimal drug doses and treatment duration). We expect modeling can provide additional insights into HCV pathogenesis and help in the development of more effective treatment strategies.

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Figure 1.

Biphasic HCV RNA decline after IFN treatment of chronic hepatitis C. Before initiation of therapy, the viral load is at the set-point level. The first phase decline lowers HCV RNA levels 1-2 logs during the first 1-2 days of treatment. It is followed by a slower second phase viral decline during the subsequent weeks of therapy.

Figure 2.

Schematic representation of the basic viral dynamic model. There are three variables: target cells (T), productively infected cells (I), and free virus (V). s , r_T and d are the recruitment rate, maximum proliferation rate and death rate of target cells, respectively; β is the infection rate of target cells by virus; r_I is the maximum proliferation rate of infected cells; δ is the death rate of infected cells; p is the viral production rate; c is the viral clearance rate; ϵ is the drug efficacy in reducing viral production; η is the drug efficacy in blocking viral infection.

Figure 3.

The effects of RBV on HCV RNA decline in combination therapy with IFN. When IFN efficacy is small ($\epsilon=0.5$), RBV enhances the second phase decline in a dose-dependent manner (ρ is the drug efficacy of RBV). When IFN efficacy is large ($\epsilon=0.95$), RBV has negligible influence on both the first and the second phases.

Figure 4.

HCV life cycle. (1) After viral binding, entry and fusion, nucleocapsid enters into the cytoplasm of the host cell; (2) Nucleocapsid releases a positive-strand RNA genome (uncoating); (3) HCV translation for synthesis of a large polyprotein, which is then cleaved by enzymes for generation of viral proteins; (4) HCV RNA replication, catalyzed by the NS5B polymerase, a product of the polyprotein cleavage; (5) Packaging and assembly of progeny virions; (6) Viral release.

Figure 5.

Drug resistance profiles after monotherapy with telaprevir. The four HCV genotype 1a patients experienced viral rebound during a two-week dosing period. The limit of detection for the sequencing assay is 100 IU/mL, and the limit of HCV RNA detection is 10 IU/mL. Note here day 0 is the time of initiation of telaprevir therapy, whereas in the original study^{97,121} the first dose of telaprevir was administered at day 2.

Figure 6.

Schematic representation of the two-strain model. In addition to target cells (T), there are four variables: drug sensitive virus (V_s), drug resistant virus (V_r), cells infected with drug sensitive virus (I_s), and cells infected with resistant virus (I_r). p_s and p_r are the viral production rates of the two strains; ϵ_s and ϵ_r are the drug efficacies of telaprevir in reducing viral production; μ is the mutation rate. The other parameters are the same as those in Fig. 2.