**Review**

**Mathematical modelling of HCV infection: what can it teach us in the era of direct-acting antiviral agents?**

*Anushree Chatterjee1,2†, Jeremie Guedj1,3,4†, Alan S Perelson1*†

1Theoretical Biology and Biophysics, Los Alamos National Laboratory, Los Alamos, NM, USA
2Center for Nonlinear Studies, Los Alamos National Laboratory, Los Alamos, NM, USA
3Present address: INSERM, UMR 738, F-75018 Paris, France
4Present address: Univ Paris Diderot, Sorbonne Paris Cité, UMR 738, F-75018 Paris, France

*Corresponding author e-mail: asp@lanl.gov
†These authors made an equal contribution to this work

**Introduction**

Chronic HCV infection affects nearly 3% of the worldwide population [1]. The goal of treatment is to achieve a sustained virological response (SVR), a marker of viral eradication, assessed by the absence of detectable virus 24 weeks after cessation of therapy. Treatment outcome with pegylated interferon (PEG-IFN) and ribavirin (RBV) is correlated with HCV genotype, and SVR is only achieved in approximately 50% of HCV genotype 1 patients, the most prevalent genotype in western countries [2]. In the last two decades, research on understanding the mechanisms of HCV replication has resulted in the development and the clinical implementation of a large number of direct-acting antivirals (DAAs). The recent approval of two protease inhibitors (PI), telaprevir and boceprevir, in combination with PEG-IFN/RBV, has raised expectations that SVR can be achieved in more than 70% of treatment-naive HCV genotype 1 patients [3,4]. Although representing an undisputable milestone in HCV therapy, the success of PI-based therapy is tempered by the existence of severe side effects and the emergence of resistance to treatment [5]. New drugs are still needed, and dozens of compounds targeting all stages of viral replication are currently in different phases of clinical development [6].

Mathematical modelling of viral kinetics aims at understanding and quantifying the biological mechanisms that govern the changes in the viral load and related biomarkers, such as alanine aminotransferase (ALT) levels, both before and after therapy [7]. Initiated in the mid-1990s to characterize the decline in HIV during antiretroviral therapy [8–10], it was later successfully applied to understand HCV RNA kinetics during therapy [11]. This approach has provided valuable insights into deciphering the modes of action of PEG-IFN/RBV and estimating key parameters of the HCV life cycle, thus offering an efficient tool for the prediction of treatment outcome [12–14]. Here, we review recent modelling efforts in the context of DAAs and show how these new models challenge some of the understanding of HCV obtained by analyzing the effects of PEG-IFN/RBV on viral load.

**The basis of HCV mathematical modelling: the biphasic viral decline during high daily dose IFN therapy**

In patients given high daily doses of standard IFN, HCV RNA generally declines in a biphasic manner, where a first phase lasting between 1 and 2 days leads to a 0–2 log fall in virus load, followed by a slower but...
persistent phase of decline. Similar to what had been done for HIV [9], Neumann et al. [11] developed the following mathematical model to explain the biphasic decline in HCV (Figure 1A):

\[
\begin{align*}
\frac{dT}{dt} &= s - dT - (1 - \eta)\beta VT \\
\frac{dI}{dt} &= (1 - \eta)\beta VT - dI \\
\frac{dV}{dt} &= (1 - \epsilon)pI - cV
\end{align*}
\]

where \(T\) and \(I\) represent the susceptible and the infected cell density, respectively, and \(V\) is the virus concentration in the serum. Susceptible cells are generated at a rate \(s\) and die at a rate \(d\). Infected cells, which are generated from the interaction of virus with target cells with rate constant \(\beta\), produce virus at per capita rate \(p\) and are eliminated at rate \(d\) per cell. At treatment initiation (that is, at \(t=0\)) the infection is assumed to be in steady state with a constant baseline viral load \(V_0\). Treatment affects the viral load after a pharmacological delay \(t_0\).

**Figure 1. Mathematical modelling of HCV kinetics**

(A) Schematic of the standard model for HCV infection described in the text. In the schematic \(V\) represents virions, \(T\), target cells and \(I\), infected cells. The parameter \(s\) is the rate of generation of target cells, \(c\) is the rate of clearance of virions, \(d\) is the death rate of infected cells, \(p\) is the rate of virion production from an infected cell, \(\beta\) is the rate of infection, \(\epsilon\) is the drug effectiveness in blocking virion production and \(\eta\) is the drug effectiveness in blocking viral infection.  

(B) Representative example of viral decline observed during daily interferon (IFN; triangle, in [9]) and the corresponding best-fit prediction using the standard model (black curve). A representative viral decline observed during treatment with telaprevir plus pegylated interferon (PEG-IFN)-2a for 14 days (diamond) [29] and best fit prediction using the standard viral kinetic model (grey curve) are shown. Compared to the decline generated by IFN therapy, the addition of telaprevir generates a larger first phase decline and a more rapid second phase decline. Studies with telaprevir alone show similar kinetic features but only for the first few days of therapy [28,29], as resistance rapidly develops during telaprevir monotherapy [30,40].
effectiveness of the drug in decreasing new infections is \( \eta \), whereas the effectiveness in blocking virion production is \( \varepsilon \), where the effectiveness is assumed to have a value between 0 (no effect of the drug) and 1 (100% effective). This model is often simplified by assuming that the susceptible cells, \( T(t) \), after treatment initiation remain constant and equal to their pretreatment value, \( T_0 \), and the parameter values do not change over time. Under these assumptions, Equation 1, can be solved to yield [11] Equation 2 and 3:

\[
V = \begin{cases} 
V_0 & t \leq t_0 \\
V_0 [A e^{-\eta (t-t_0)}] & t > t_0
\end{cases} 
\]  

(2)

where

\[
\lambda_{1,2} = \frac{c + \delta \pm \sqrt{(c-\delta)^2 + 4(1-\varepsilon)(1-\eta)c\delta}}{2}, \quad A = \frac{(\varepsilon c - \lambda_1)}{\lambda_1 - \lambda_2}
\]  

(3)

This model provides a simple conceptual framework for the understanding of the biphasic viral decline after treatment initiation (Figure 1B). Importantly, the assumption that the major effect of IFN is blocking viral production is necessary to reproduce a biphasic viral decline [11]. As a result of IFN reducing viral production, HCV RNA initially rapidly declines with a rate \( \approx 6 \) day\(^{-1} \) (varying between 3.6 and 11.2 day\(^{-1} \)), implying a viral half-life in serum (denoted as \( t_{1/2} \)) of about 2.7 h. Contrastingly, \( \varepsilon \) and \( \delta \) are very variable in the population, which is largely accounted for by HCV genotype, polymorphism in IL28B, ethnicity, baseline viral load, baseline interferon inducible protein 10 (IP-10) levels and histological factors [15–20].

Modelling changes in drug effectiveness

(PEG-IFN)

With frequent dosing, such as daily administration of standard IFN, the plasma concentration of drug is nearly constant [21]. Under such conditions the drug effectiveness \( \varepsilon \) should be nearly constant as assumed in the standard model. However, when drug is administered once a week, as is the case with PEG-IFN, the concentration of drug in plasma initially increases and then decreases over time, resulting in weekly fluctuations in HCV RNA levels. These fluctuations can be captured by relaxing the assumption of constant drug effectiveness and taking into account the pharmacokinetics and pharmacodynamics of PEG-IFN [22,23]. The average antiviral effectiveness and the kinetic parameters are similar to those found with standard IFN [22,23]. Some recent reviews discuss the modelling of drug effectiveness in detail [21,24].

Modelling long-term viral kinetics: relaxing the assumption of constant target cells and the notion of critical drug effectiveness

Even when constant treatment effectiveness is a reasonable assumption, the viral load does not necessarily decline continuously, as predicted by the biphasic model. Viral rebound can occur due to increases in target cells and cannot be captured in viral kinetic models by relaxing the assumption of constant target cells. In this case, the long-term viral kinetics depend on the ability of treatment to overcome a certain threshold called the critical effectiveness \( \varepsilon_c \). If \( \varepsilon < \varepsilon_c \), enough new infections occur that the viral load eventually stops declining. Depending on the parameter values, there can be almost no decline (as seen in non-responders) or a biphasic decline where the second phase is flat (partial responders) or a biphasic decline followed by a rebound where viral load establishes a new set-point value (rebounders). Lastly, a second flat phase followed by a renewed decline of viral load has been reported in some patients [26,27]. This triphasic pattern can be captured by including the proliferation of infected cells [26]. However, little is known about the rate of hepatocyte proliferation in vivo during chronic HCV infection.

Direct-acting antivirals

DAAs have ushered in a new era of HCV treatment. Below we review recent viral kinetics studies of DAA therapy and discuss how models developed for DAAs
Table 1. Mean estimates of viral dynamic parameters obtained using the standard model (Equation 1)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>c, day⁻¹</th>
<th>δ, day⁻¹</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Telaprevir (VX-950) monotherapy</td>
<td>36</td>
<td>0.999</td>
<td>13.4</td>
<td>0.58 [29]</td>
</tr>
<tr>
<td>Ciluprevir (BILN 2061) monotherapy, 2×500 mg/day</td>
<td>8</td>
<td>0.9992</td>
<td>8.0</td>
<td>0.36 [62]</td>
</tr>
<tr>
<td>TMC-435, 200 mg/day</td>
<td>6</td>
<td>0.9993</td>
<td>6.25</td>
<td>0.25 [63]</td>
</tr>
<tr>
<td>Mericitabine (RG7128) monotherapy, 2×1,500 mg/day</td>
<td>8</td>
<td>0.98</td>
<td>6 (fixed)</td>
<td>0.03 [53]</td>
</tr>
<tr>
<td>Daclatasvir (BMS-79002) monotherapy, 10 or 100 mg/day</td>
<td>5</td>
<td>0.997</td>
<td>23.1</td>
<td>1.06 Guedj et al., unpublished observations</td>
</tr>
<tr>
<td>Silibinin monotherapy, 20 mg/kg/day dose</td>
<td>17</td>
<td>0.89</td>
<td>6 (fixed)</td>
<td>0.77 [57]</td>
</tr>
<tr>
<td>IFN-α2b monotherapy</td>
<td>14</td>
<td>0.91</td>
<td>5.95</td>
<td>0.14 [11]</td>
</tr>
<tr>
<td>IFN-α2b+RBV</td>
<td>31</td>
<td>0.92</td>
<td>8.0</td>
<td>0.14 [12]</td>
</tr>
</tbody>
</table>

aProtease inhibitors, c, viral clearance rate; IFN, interferon; RBV, ribavirin; e, drug effectiveness in blocking viral production; δ, infected cell death rate.

The rapid second phase decline observed with PIs suggests that the duration of therapy needed to clear drug-sensitive virus might be considerably shortened with PIs as compared to IFN-based treatments [29]. Conceptually, viral eradication can be considered as achieved when the predicted total HCV RNA is less than 1 copy in the entire extracellular fluid volume (which in a 70 kg human is about 15 l), corresponding to a viral concentration of 6.7×10⁻⁵ HCV RNA/ml [12]. Thus, for an individual with a baseline viral load above 10⁶ HCV RNA/ml viral eradication requires over a 10 log decline from baseline. A more
conservative assumption is to consider that SVR is achieved when the predicted number of infected cells is lower than 1 [13], which, typically adds another 2–3 logs of decline [29].

Since PIs exhibit a two- to fourfold more rapid second phase viral decline than IFN (Table 1), the treatment duration with a PI should be able to be roughly reduced two- to fourfold from that used with IFN. Using parameters obtained in 44 treatment-naive patients during the first 3 days of telaprevir treatment, Guedj et al. [29] estimated the empirical distribution of the time needed to achieve SVR. The model predicted that in fully compliant patients, the last virus particle could be eradicated within 7 to 8 weeks for 95% and 99% patients, respectively [29], whereas another 3 weeks would be necessary to clear the last infected cell. The latter estimate seems closer to the clinical observation where a higher rate of virological failure after week 12 (10% versus 5%) with drug-sensitive virus was observed in patients that had received telaprevir for 8 instead of 12 weeks [36]. However, with the emergence of highly resistant virus whose eradication essentially relies on PEG-IFN/RBV, treatment duration needs to be significantly extended [4].

Mathematical modelling of emergence of drug resistance to telaprevir treatment

For the PIs telaprevir and boceprevir, emergence of drug resistant variants and viral breakthrough hinders their applicability as monotherapy agents [5]. The existence of resistant variants is primarily due to the high replication rate of HCV and the error rate (μ) of the NS5B RNA-dependent RNA polymerase (RdRp) estimated to be approximately 10^4–10^5 per nucleotide per replication cycle [37,38]. Using a conservative base substitution rate of μ=10^−5, the probability of 0, 1 and 2 mutations per replication cycle is estimated to be 91%, 8.7% and 0.42%, respectively [39]. Given that an infected patient typically produces 10^{10} virions/day [11], approximately 8.7×10^{10} and 4.2×10^9 mutants with single and double-nucleotide changes, respectively, that is, all possible single and double mutants, are expected to be produced every day. In absence of treatment, viral variants with lower replication fitness than wild-type are not competitive and therefore may not grow to detectable levels. However, in the presence of selective pressure, the pre-existence of these variants, even at low levels, may explain why patients treated with telaprevir monotherapy may exhibit a large proportion of resistant virus as early as day 2 of treatment [40].

A more quantitative understanding of the kinetics of growth and decline of resistant virus during and after therapy can be obtained by expanding the standard model (Equation1) to include viral competition. Rong et al. [39] showed that a model with two populations of virus (that is, drug-sensitive and drug-resistant) could provide a good fit for the viral kinetics observed in the first two weeks following the initiation of telaprevir as monotherapy or in combination with PEG-IFN. When the kinetics of several known variants are measured, for instance by clonal sequencing, a more comprehensive picture of viral competition can be obtained by using a model that directly includes the various viral variants (Figure 2A). Using such a model, Adiwijaya et al. [30] provided an estimate of the in vivo fitness of the principal single and double mutants observed during two weeks of telaprevir monotherapy. In a subsequent study [31], the authors applied their model to various Phase II/III studies where telaprevir was given in combination with PEG-IFN/RBV for longer times. Their model could predict the observed SVR rates for various regimens of telaprevir and PEG-IFN/RBV, suggesting that modelling might be a relevant approach to design treatment strategies.

An important assumption of these models [30,39] is that the growth of resistant virus is supported by the rapid elimination of infected cells and their rapid replacement by new susceptible cells. Although both of these models provide a good fit of the data, the origin of replication space needed to support the growth of virus remains to be determined. If the rapid decline of sensitive virus represents the intracellular elimination of virus rather than the elimination of infected cells per se, part of the replication space could come from infection with resistant virus of cells that were previously infected with wild-type virus [33].

Viral kinetics seen with the NS5A inhibitor daclatasvir (BMS-790052)

Although in vitro studies suggested an essential role of NS5A for both viral replication [41–43] and assembly/release of infectious particles [44–47], the lack of a known enzymatic function has long limited the search for compounds targeting HCV NS5A. An innovative screening approach identified daclatasvir (BMS-790052) as a potent NS5A inhibitor [48]. In a single ascending dose study, daclatasvir showed high antiviral effectiveness that decreased HCV RNA levels by approximately 3 orders of magnitude within 8–12 h after administration [48]. Although, in most patients, viral load rapidly rebounded due to drug elimination, five patients (one treated with 10 mg and four treated with 100 mg) had a sustained viral decline until day 3 post-dosing. In these patients, viral decline was biphasic, which allowed the use of the standard model to fit the data. Interestingly the mean antiviral effectiveness in blocking viral production/secrection, e, was found equal to 0.997. Further, the rapid viral decline was attributed
to high mean rates of elimination of free virus, $c$, and of infected cells, $\delta$, estimated to 23.3 day$^{-1}$ and 1.06 day$^{-1}$, respectively (Table 1; Guedj et al., unpublished observations).

In order to understand the high estimate of $c$ and $\delta$ and how this might be related to daclatasvir’s mode of action, (Guedj et al., unpublished observations) extended the standard model to incorporate essential features of intracellular viral replication that may be targeted by daclatasvir. In this multiscale model (Figure 3A), levels of intracellular viral RNA (vRNA, denoted $R$) depend on the time a cell has been infected (denoted $a$) and are governed by the following equation:

$$\frac{dR}{da} = (1-\epsilon_i)\alpha \cdot \mu R - (1-\epsilon_j)\rho R$$  \hspace{1cm} (4)$$

where $\alpha$, $\mu$ and $\rho$ are the (intracellular) rates of vRNA production, degradation and assembly/secertion as
virions into the circulation, respectively. This model is then coupled to the standard model, given by Equation 1, by replacing the rate of viral production, $pI$, by a rate given by $\rho R(a)I(a)$ for a cell of age $a$ and integrated (or summed) over all possible ages of infection, $a$. Thus, in this new model, the rate of virion production depends on the amount of vRNA within a cell. Unlike the standard model, the effects of blocking vRNA production (that is, reducing $\alpha$) and reducing assembly/secretion of virus (that is, reducing $\rho$) can be distinguished, and under treatment the model is given by Equation 4, where $\epsilon_\alpha$ and $\epsilon_\rho$ are the effectiveness of the drug in blocking vRNA production and viral assembly/secretion, respectively.

This model provides a more precise understanding of the determinants of early viral decline (Figure 3B). What is commonly called the ‘first phase’ of viral decline is governed in this model by two different processes: blocking of virion assembly/secretion, which induces an almost immediate decline of HCV RNA in the circulation with a rate $c$ that reflects clearance of virus particles (phase 1a), and blocking of vRNA production, which induces a slower viral decline reflecting the fact that infected cells containing less vRNA will
produce fewer virus particles (phase 1b). According to the model, to observe both phases, a drug needs to block both assembly/secretion and vRNA production with high efficacy. If a drug has only a modest effect in blocking assembly secretion, as is the case for IFN then only one phase will be observed early on, which is consistent with clinical observations.

Within the context of this new model, the rapid early biphasic decline seen over the first two days of therapy suggests that daclatasvir has a dual mode of action, that is, that it efficiently blocks viral assembly/secretion and vRNA production. If a drug does not effectively block virion assembly/secretion then the continuing release of virus after drug is administered will counterbalance virion clearance and lead to an underestimate of the true virion clearance rate; therefore, the virion clearance rate estimated with daclatasvir, which corresponds to a virion half-life of 45 min is likely to represent an improved estimate of the HCV half-life (Guedj et al., unpublished observations).

Interestingly, analysis of viral decline in six patients in the anhepatic and early reperfusion stages after liver transplantation, when virus production is negligible, provided a similar estimate of the virion half-life [49]. Large volume apheresis, where the virion clearance is artificially enhanced, can also be used to estimate the virion half-life [50]. To our knowledge, only one study used this approach and they only analysed two patients both HIV–HCV-coinfected [50]. They found half-lives of 1.7 h and 3 h, hinting that clearance could be slower in infected patients. Clearly more data will be needed to confirm these half-life estimates.

With IFN, a single early phase of viral decline is seen, which is significantly slower than that seen with daclatasvir. (Guedj et al., unpublished observations) attribute this to a low effectiveness of IFN in blocking virion assembly/secretion.

Several aspects of this new model still need to be explored. First, the intracellular model (Equation 4) is linear and only includes vRNA. This modelling choice was made in order to allow one to estimate parameters from clinical data. The model ignores HCV proteins, which are needed for HCV replication and virion assembly, and negative strand HCV RNA. Because multiple proteins are needed, their inclusion in a model is likely to induce high degrees of non-linearity in the processes of vRNA production and export. Second, the model was developed to study the first 2 days of viral decline following one dose of daclatasvir. To fit longer-term viral kinetics the model may need to be modified, for example, by relaxing the assumption that vRNA production occurs at a constant rate during therapy. As a consequence of the low genetic barrier of daclatasvir, information about long-term viral declines will need to be attained from analysis of data obtained from combination therapy studies. This will also be true for the study of other DAAs with a low barrier to resistance.

Third, the new estimate of HCV’s half-life of 45 min seems incontrovertible, since measurement of the rate of viral decline during the first hours after administration of drug yields this half-life. However, whether this is a consequence of daclatasvir blocking virion assembly/secretion (revealing a previously underestimated rate of virion clearance), or whether daclatasvir has other modes of action that lead to an off-target enhancement of the viral clearance rate, needs to be examined.

**Modelling HCV kinetics with the nucleoside polymerase inhibitor meritabine (RG7128)**

As discussed previously, the rapid and efficient blocking of viral production attributed to IFN and PIs causes a rapid decline of HCV RNA during the first 1–2 days of treatment; however, when the nucleoside polymerase inhibitor meritabine (RG7128) was given to 32 patients as monotherapy for 14 days, a much slower viral decline was observed (Figure 4A) [51]. In addition, about 40% of the patients in this study did not show the typical biphasic pattern, but rather had a monophasic viral decline, regardless of dosing group (Figure 4A). Because meritabine, as with all nucleoside polymerase inhibitors, needs to be triphosphorylated in order to become an active drug, one can ask whether the observed slow viral decline is related to the rate at which active drug is generated intracellularly.

Guedj et al. [51] showed that a slow viral decline can be reproduced in the standard viral dynamic model by allowing the drug effectiveness, e, to gradually increase over time until some final effectiveness is reached, and that such a model agrees well with the viral kinetic data obtained during meritabine monotherapy [51]. Using this model the final effectiveness reached by meritabine with twice daily dosing was found to be high (mean 750 mg and 1,500 mg, 98.0% and 99.8%, respectively; P=0.018).

Interestingly, the pyrimidine nucleotide PSI-7977 induces a faster first phase of viral decline [52] compared to meritabine, despite the fact that the active species of PSI-7977, a uridine triphosphate, is the same uridine triphosphate produced by meritabine [53]. This suggests that the first phosphorylation of meritabine could be limiting the rapid buildup of meritabine’s antiviral effectiveness [54]. Interestingly, ribavirin, which also needs to be triphosphorylated, when given as monotherapy, also induces a monophasic viral decline [55]. Thus, the assumption that ribavirin needs time to become fully effective might also explain why, in combination with IFN, ribavirin was found to enhance the second but not the first phase of viral decline [12,32].
Mode of action of silibinin using viral kinetic modelling

Because of the high cost and toxicity of the currently approved anti-HCV drugs, finding a low-cost natural product that has anti-HCV activity would be highly desirable. Silymarin, an extract made from the seeds of the milk thistle plant, has been used since the time of the ancient Egyptians for the treatment of liver ailments. More recently silibinin (SIL), the major active component of silymarin, has undergone clinical trials to assess its activity against HCV. In a pivotal study, SIL monotherapy demonstrated a strong antiviral effectiveness against HCV genotype 1 or 4 in 25 patients that were administered 10, 15 or 20 mg/kg over 4 h infusions each day for seven days [56].

To understand the mechanism of action of SIL, Guedj et al. [57] modelled HCV RNA kinetics measured in this study using the standard model of viral kinetics. Viral load data could be well-fitted (Figure 4B) by

![Figure 4. Mathematical models of HCV kinetics in response to direct-acting antiviral treatment](image-url)

(A) A monophasic decline of measured HCV RNA (diamond) and best-fit prediction of the viral kinetic model (grey curve, primary y-axis) [51] during a 14-day treatment with mericitabine monotherapy (1,500 mg twice daily). Follow-up showed viral rebound as drug was eliminated from the body. In the model of Guedj et al. [51] the effectiveness of mericitabine (1,500 mg twice daily) changes with time during treatment (black curve, secondary y-axis). It is the slow build-up of effectiveness that is responsible for the monophasic decay. (B) A biphasic decline of measured HCV RNA and best-fit prediction of the viral kinetic model during a 7-day treatment with silibinin (SIL) monotherapy (20 mg/kg/day) [57]. Patients demonstrated both biphasic (diamonds, grey curve) and monophasic (circles, black curve) viral declines.
assuming that SIL blocks viral production only with a dose-dependent effectiveness $\varepsilon = 0.69$ for $10 + 15$ mg/kg/day and $\varepsilon = 0.91$ for 20 mg/kg/day [57].

A rapid second phase between day 2 and 7 was observed in all patients (mean 0.29 log IU/ml/day), which is comparable to estimates made from the early decline seen with telaprevir (0.25 log IU/ml/day) [29]. This demonstrates that a drug can generate a rapid second phase decline even if it has a modest first phase decline; thus, the relationship that was established [28,29] between the amplitude of the first phase decline and the slope of the second phase decline in patients treated with telaprevir may be drug specific.

Further, 40% of patients exhibited a monophasic viral decline with a rate of viral decline over the first 2 days that led to a $<1$ log decline and which was roughly similar to the rate of decline after day 2 seen in all patients (Figure 4B). This observation contradicts the hypothesis that SIL only blocks viral production, as the absence of a profound first phase of viral decline should, in this case, result in a large number of new cell infections that should hamper the second phase of viral decline. Indeed assuming that SIL inhibits both viral production and viral infection yielded a statistically improved fit to the patient data. This suggested that SIL potentially acts via both these modes of action, though blocking viral production was the more pronounced effect, which is consistent with in vitro experimental data [58–61]. However, the fact that blocking viral infection has a much less pronounced effect than blocking viral production did not make it possible to estimate this effect precisely. Moreover, although SIL concentrations in serum did not increase over time, the hypothesis that the monophasic viral decline results from a gradual increase in drug effectiveness intracellularly, as predicted for mericitabine, could not be ruled out.

Conclusions

In the new era of DAA-based therapy, mathematical models hold the potential to provide biologically relevant explanations of HCV kinetics under therapy, and may offer a convenient method for rationally designing combination therapy. As far as we are aware this has not yet been achieved, but kinetics studies, such as those reported here, are the first step in understanding the properties of drug combinations.

Although the conceptual framework of the biphasic model brought valuable insights into the origin of viral declines observed during IFN-based treatment [11,12], extensions of this model are needed to understand the patterns of viral decline during DAA therapy. Here we have shown that under telaprevir monotherapy and in combination with PEG-IFN/RBV, second phase slopes are up to 4x faster than seen with IFN-based therapies. In addition, the second phase slope increases with increasing drug effectiveness, as measured by the magnitude of the first phase log decline. Patients treated with the NS3A inhibitor daclatasvir (BMS-790052) show a first phase decline that is fourfold faster than that seen with IFN-based therapies, suggesting that the HCV half-life in serum may be 45 min rather than the 2–3 h previously estimated. Also, the analysis of viral declines seen with daclatasvir suggest that the standard first phase viral decline seen over the first two days of therapy may have two parts, a phase 1a decay due to virion clearance and a phase 1b decay due to loss of intracellular viral RNA. Viral kinetics observed during a 14 day study with the nucleoside polymerase inhibitor mericitabine and in a 7 day study with the natural product silibinin are frequently monophasic rather than biphasic. Data on other DAAAs is needed to provide a more complete picture of viral kinetics with the different agents that might be combined into novel therapies.

In the future, we expect to see more sophisticated and detailed models being developed that take into consideration both intracellular and serum HCV dynamics. In this regard, studying viral kinetics during DAA therapy using in vitro systems may provide further insights into the mechanisms of action of DAAAs [35]. A better understanding of replication space is needed in order to explain the rapid growth of drug resistant variants as observed during telaprevir monotherapy. Models of the future will hopefully include consideration of how HCV spreads spatially in the liver and include cell-to-cell spread. Such models will be needed to accurately assess the effects of entry inhibitors, which could be another component of a DAA cocktail. Lastly, understanding the viral kinetic aspects of host targeted therapies is another frontier that modellers need to address.

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