Second-Phase Hepatitis C Virus RNA Decline During Telaprevir-Based Therapy Increases With Drug Effectiveness: Implications for Treatment Duration

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Hepatitis C virus (HCV) RNA decay during antiviral therapy is characterized by a rapid first phase, followed by a slower second phase. The current understanding of viral kinetics attributes the magnitude of the first phase of decay to treatment effectiveness, whereas the second phase of decay is attributed to the progressive loss of infected cells. Here, we analyzed data from 44 patients treated with telaprevir, a potent HCV protease inhibitor. Using a viral kinetic model that accounts for the pharmacokinetics of telaprevir, we found the second-phase slope of viral decline to be strongly correlated with treatment effectiveness and to be roughly four-fold more rapid than has been reported with interferon-based therapies. Because telaprevir is not known to increase the death rate of infected cells, our results suggest that the second-phase slope of viral decline is driven not only by the death of infected cells, but may also involve other mechanisms, such as a treatment-effectiveness–dependent degradation of intracellular viral RNA. As a result of the enhanced viral decay caused by the high antiviral effectiveness of telaprevir, we predict that if drug resistance could be avoided by using an appropriate combination of antiviral agents, treatment duration needed to clear HCV might be dramatically shortened. Indeed, we predict that in 95% of fully compliant patients, the last virus particle should be eliminated by week 7 of therapy. If the remaining infected hepatocytes act as a potential reservoir for the renewal of infection, no more than 10 weeks of treatment should be sufficient to clear the infection in 95% of fully compliant patients. However, if patients miss doses, treatment duration would need to be extended. (HEPATOLOGY 2011;53:1801-1808)

Chronic hepatitis C virus (HCV) infection has a worldwide prevalence of approximately 3%.[1] Achieving a long-term, sustained virologic response (SVR), defined as undetectable HCV RNA in serum 24 weeks after the end of treatment, is the most effective way to prevent disease progression. Currently, treatment outcome with pegylated interferon (PEG-IFN) and ribavirin (RBV) is correlated with HCV genotype, and SVR is only achieved in approximately 50% of patients infected with genotype 1 HCV.

After the initiation of high doses of daily IFN with or without RBV, viral kinetics are characterized in most patients by a biphasic decline, where a rapid initial decline lasting for 1-2 days is followed by a slower, but sustained, second phase of viral decay (Fig. 1), where HCV RNA declines 0.42 $\log_{10}$ IU/mL/week, on average, with high variation among patients (standard deviation, 0.36 $\log_{10}$ IU/mL/week).[2,3] Mathematical modeling of viral kinetics has provided valuable insights for the understanding of the determinants of HCV RNA decay after treatment initiation.[4] In particular, it has been proposed that the second phase of viral decline is due to the loss of infected cells, and thus, the high variability in the second phase of viral decline could reflect the variability in the strength of the immune response.[2] Although several observations

Abbreviations: CE, constant effectiveness; DAA, direct-acting antiviral; HCV, hepatitis C virus; PEG-IFN, pegylated interferon; PK/PD, pharmacokinetics/pharmacodynamics; RBV, ribavirin; SOC, standard of care; SVR, sustained virologic response; VE, varying effectiveness.

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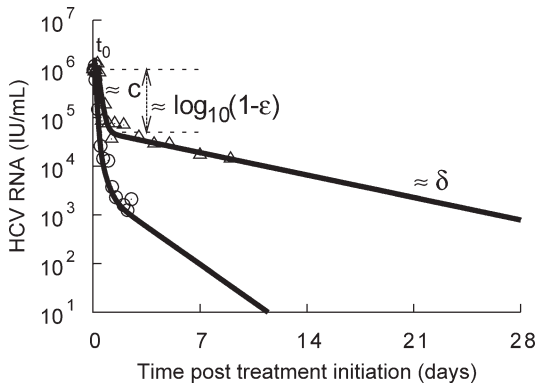

Fig. 1. Typical biphasic HCV RNA decay with daily standard IFN-α (triangles represent data from subject 2D in Neumann et al.[2]). After treatment initiation at time $t = 0$, viral load remains equal to its baseline value, V_0, for a short time, t_0. After that, a rapid, dose-dependent viral decline, lasting for 1-2 days (first phase), followed by a slower, but sustained, decline (second phase), is typically observed. According to theory,[2] the first phase of decline is due to the treatment effectiveness in blocking viral production, ε, and results in a decline of viral load at a rate close to the virion loss rate, c, with the magnitude viral decline depending on ε. With lower amounts of virus, less *de novo* infection occurs and the infected cells are not efficiently replaced, generating a second phase of viral decline at a rate determined mainly by the loss rate of infected cells, δ. By fitting this model to the data (black line), the parameters can be estimated[2] ($t_0 = 7$ hours, $c = 5.6$ day^{-1}, $\varepsilon = 0.95$, $\delta = 0.16$ day^{-1}). For comparison, the decline kinetics with telaprevir for a typical subject (circles represent data from subject 1 in the current study), and its best fit using the VE model ($t_0 = 2.4$ hours, $c = 12.2$ day^{-1}, $\varepsilon_1 = 0.9688$, $\varepsilon_2 = 0.9978$, $\delta = 0.51$ day^{-1}), is also displayed.

support the possibility that the immune response is involved in the second phase of viral decline,[2,5] no means exists to directly quantify the loss rate of infected cells *in vivo*, and the predictions made by mathematical modeling remain to be validated. Whatever the mechanisms involved in the second phase of viral decline, its determination is of great interest, because it can ultimately determine the length of time of treatment that needs to be given before all virus and infected cells are expected to be cleared.[3]

Direct-acting antivirals (DAAs) constitute a new stage in HCV therapy. These drugs inhibit specific HCV enzymes important for viral replication, such as the NS3 protease, and thus allow for a more profound antiviral effect than the current IFN-based therapy. Similar to what was observed with IFN-based therapy, HCV RNA after the initiation of protease-inhibitor therapy was found to decline in a biphasic manner, with, in most patients, a second-phase viral decline larger than 1 $\log_{10}$ IU/mL/week.[6-9]

In order to gain insights into the faster second-phase decline observed with HCV protease inhibitors, we reanalyzed data from 44 patients treated with telaprevir,[6] using a new viral kinetic model that accounts for the changes in drug pharmacokinetics/pharmacodynamics (PK/PD). Using the viral kinetic parameters found in this group of patients as a representative sample of naïve genotype 1 patients under telaprevir therapy, and assuming that drug resistance could be avoided, we estimated the treatment time needed to eliminate all virus and infected cells.

Patients and Methods

Data

We analyzed data from two phase 1 studies: the first with 28 subjects dosed with varying regimens of telaprevir monotherapy[10] and the second with 8 subjects dosed with telaprevir monotherapy and 8 subjects dosed with telaprevir plus pegylated-IFN-α2a (PEG-IFN).[11] Because resistant variants can emerge early, we focused on the first 2.5 days of data in order to avoid the possible perturbation of the HCV RNA decay due to the growth of drug-resistant variants.

Viral Kinetic Models

To explain the biphasic HCV RNA decline observed during daily IFN treatment, Neumann et al.[2] proposed the following model shown in Equation 1:

$$\begin{aligned} \frac{dI}{dt} &= bVT_0 - \delta I \\ \frac{dV}{dt} &= p(1-\varepsilon)I - cV \end{aligned} \qquad (1)$$

where I represents infected cells, V represents the virus concentration (measured as HCV RNA), and T_0 represents the target cell number at the start of therapy, which is assumed to be constant during the study time, b is the rate at which target cells are infected, and p is the viral production rate per infected cell in the absence of treatment. IFN is assumed to be effective after a delay time, t_0, and is assumed to reduce the average rate of viral production per cell from p to $p(1 - \varepsilon)$, where ε represents the constant effectiveness of IFN in blocking viral production, defined such that $\varepsilon = 0.9$ means that 90% of the viral production is blocked. As a result of this blocking, the model suggests that the initial rate of viral decline is due to the fast clearance of free virus, occurring with rate c. Furthermore, the model predicts that the second-phase slope is approximately $\varepsilon\delta$, where δ denotes the per capita rate of loss of infected cells.[2] Hence, for potent therapies for which ε is close to 1, the second-phase slope will be approximately δ. Because the model (Equation 1) assumes constant treatment effectiveness,

this model has been called the constant effectiveness (CE) model.[12]

Varying Effectiveness Model

With dosing every 8 or 12 hours, telaprevir plasma concentrations change, and an increase in drug area under the curve and in drug effectiveness after multiple doses has been reported.[13] To account for this feature, we introduce a function that allows the treatment effectiveness, ε, to change over time, t, according to Equation 2:

$$\varepsilon(t) = \varepsilon_1 + (\varepsilon_2 - \varepsilon_1)\left\{1 - \exp\left[-\left(\frac{t - t_0}{k}\right)\right]\right\}, \quad (2)$$

where ε_1 and ε_2 are the initial and final values of treatment effectiveness, respectively, and k defines the rapidity of change in effectiveness. This function smooths the variation in drug effectiveness and generates an effectiveness that increases with time (assuming $\varepsilon_2 > \varepsilon_1$) (Supporting Fig. S2), so as to account for the PK/PD of telaprevir (and PEG-IFN in patients treated with combination therapy). The use of this function (Equation 2) combined with the viral dynamics model (Equation 1) will be called the varying effectiveness (VE) model. Note that if the initial and final effectiveness are equal ($\varepsilon_1 = \varepsilon_2$) or if the changes in drug effectiveness are very rapid ($k \approx 0$), the VE model is equivalent to the CE model. In that respect, the CE model is a particular case of the VE model.

Treatment Effectiveness in Case of Partial Compliance to Treatment

We assume drug is given every τ time units. Before a dose is missed, $\varepsilon(t)$ is given by Equation 2. We assume each dose can be missed with an equal probability. When a dose is missed, we set $\varepsilon = 0$ until the next dose at time τ later. In reality, residual drug would be present and, depending on the drug PK/PD, effectiveness would decrease. Once dosing is continued, $\varepsilon(t)$ is again given by Equation 2, translated in time to the new start time of dosing.

In our simulation study, we will assume that drug is given three times a day and that, on average, one dose is missed every 2 days. Thus, $\tau = 8$ hours, and each dose can be missed with a probability of one in six.

Data Fitting and Statistical Methods

A nonlinear mixed-effects approach was used to estimate parameters, using MONOLIX software (http://software.monolix.org).[14] This approach allows one to borrow strength from the whole sample to estimate more precisely the mean value of the parameters in the population and their interindividual variation[15] (see Supporting Materials). After the population parameters were found, the estimated parameters $\hat{\beta}_i$ for each individual were deduced using empirical Bayes estimates.[15] As found in previous work,[6] one subject (subject 11) could not be fitted and was therefore not included in the analysis.

Time to Eliminate the Last Virus Particle

For each patient, SVR was considered as achieved at time τ_i once the predicted total HCV RNA $V(\hat{\beta}_i; \tau_i)$ was lower than one copy in the entire extracellular fluid volume, assumed to be 15 L, which corresponds to a viral concentration of 6.7×10^{-5} HCV RNA/mL. To be conservative, we chose $V(\hat{\beta}_i; \tau_i) < 3 \times 10^{-5}$ HCV RNA/mL. The time to clear the last infected cell was obtained similarly.

Cumulative Distribution Function for the Time to Clear the Infection

Using the population approach described above, the distribution of each parameter in the population could be precisely estimated and the cumulative distribution function to eliminate the last virus particle or infected cell could be computed. To achieve it, $N = 10{,}000$ *in silico* patients were simulated according to the population parameters and their interindividual variation given in Table 1, and for each of them, the time, τ_i, to reach SVR, based on the time to eliminate the last virus particle or infected cell, was computed. The probability, $\hat{P}(t)$, to achieve SVR by time t was then determined by the fraction of *in silico* patients that achieved SVR by time t.

Results

Although both the CE and VE models provided good fits to the data at all drug doses used (Supporting Fig. S1), the VE model yielded significantly better fits when assessed by the Akaike information criterion, which allows one to compare the ability of models with different numbers of parameters to fit experimental data (Table 1). Because the VE model gave better fits, we only discuss results obtained with the VE model.

In principle, model parameters may vary according to treatment group. In particular, the parameters related to treatment effectiveness (e.g., k, ε_1, and ε_2) could be different in the telaprevir plus PEG-IFN, compared to the telaprevir monotherapy, group. However, no significant effect was found for any of the

Table 1. Viral Parameter Estimates Obtained Using the Constant (CE) and Varying Effectiveness (VE) Models

	$V_0 \cdot 10^6$ [mL^{-1}]	δ [day^{-1}]	c [day^{-1}]	ε_1	ε_2	t_0 [day]	k [day^{-1}]	AIC
				Population parameters (standard error)				
CE model	2.74 (0.46)	1.19 (0.065)	11.2 (0.54)	0.995 (0.00083)	–	0.088 (0.0059)	–	169.8
VE model	2.68 (0.45)	0.58 (0.11)	13.4 (0.87)	0.974 (0.0052)	0.999 (0.00028)	0.10 (0.0055)	0.35 (0.044)	147.4
				Interindividual variation (%)				
CE model	108	22.1	21.1	100	–	0 (fixed)	–	
VE model	109	24.5	24.8	73.6	61.3	0 (fixed)	63.1	

viral dynamic or drug effectiveness parameters (all P values >0.2).

We estimated that the initial treatment effectiveness, $\varepsilon_1 = 0.974$, increased and reached a significantly higher effectiveness, $\varepsilon_2 = 0.999$ ($P < 0.0001$), after approximately 1 day (Supporting Fig. S2). Furthermore, we estimated that there was a small delay, t_0, before drug became effective (see Patients and Methods), which was estimated to have nearly the same value in all the patients: $t_0 = 0.10$ days or 2.4 hours.

As reported previously,[6] we found that the mean value of δ was high, compared to what has been reported with IFN-based treatments (Fig. 1). However, our estimate of δ is much lower than what was found using the CE model (mean: 0.58 versus 1.19 day^{-1} in the CE model). Moreover, our estimated value of δ is similar in monotherapy patients (0.58 day^{-1}) and in patients receiving combination therapy (0.57 day^{-1}), thus resolving the apparent paradox of a slower

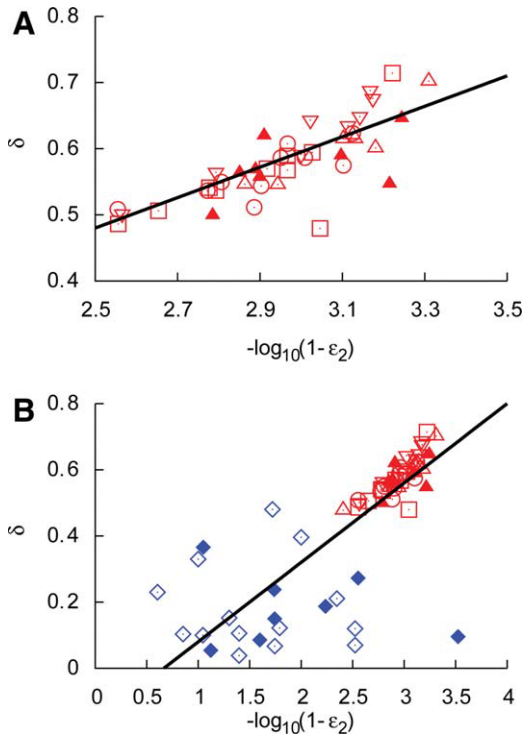

Fig. 2. Loss rate of infected cells increases with drug effectiveness. (A) Distribution of the loss rate of infected cells, δ, as a function of the final (log-transformed) effectiveness, ε_2, in patients dosed with telaprevir. Squares are telaprevir monotherapy 450 mg q8h, upper triangles are 750 mg q8h tablets (filled triangles when used in combination with PEG-IFN), reverse triangles are 750 mg q8h suspension, and circles are 1250 mg q12h suspension. The black line is the best-fit regression line ($r = 0.79$, $P < 0.001$). (B) Distribution of the loss rate of infected cells, δ, as a function of the final (log-transformed) effectiveness, ε_2, in patients dosed with telaprevir (red symbols), compared to values found in the literature for genotype 1 Caucasian patients treated with 10 MIU of IFN daily[2,3,18] monotherapy (blue diamond) or in combination with ribavirin (blue-filled diamond). The black line is the best-fit regression line ($r = 0.78$, $P < 0.001$), where one point, considered as an outlier, has not been taken into account.

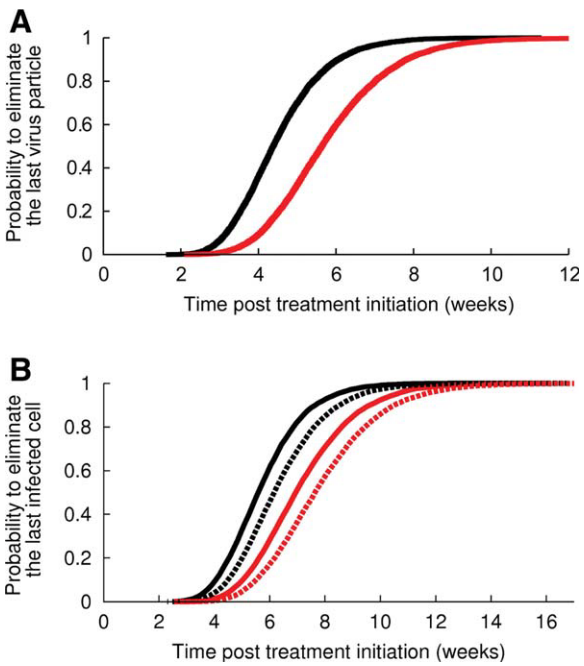

Fig. 3. Estimated cumulative probability distribution function (cdf) for the treatment duration needed to eliminate the last remaining (A) virus particle and (B) infected cell. The black line corresponds to perfect treatment adherence, whereas the red line represents the case of partial adherence of three doses per day, where one dose is randomly missed every 2 days (see Patients and Methods). Because the cdf for the time to eliminate the last infected cell depends on the unknown value for the viral production rate in the absence of treatment (p in Equation 1), the solid lines in (B) correspond to $p = 100$ virions/day and the dashed lines correspond to $p = 10$ virions/day.

second-phase decline when PEG-IFN was added to telaprevir that was previously reported.[6]

Because only the first 3 days of treatment were analyzed, we checked whether our estimates would remain unchanged when including later time points (days 6, 10, and 13) in patients treated with telaprevir plus PEG-IFN and in whom no resistant virus was detected.[16] Interestingly, we found no significant differences in this subset of patients in the loss rate of infected cells, δ, as compared to the original data set limited to 3 days of treatment ($P = 0.49$, t test), and the population parameters remained unchanged.

Because the rate of second-phase viral decline was larger in this study using telaprevir than in previous studies using IFN-based therapies, we asked whether the high effectiveness of telaprevir could play a role. We found that δ was significantly correlated with the final treatment effectiveness, ε_2 ($r = 0.79$, $P < 0.001$) (Fig. 2A). Thus, for patients in whom drug effectiveness was higher, not only did the first phase bring viral levels down lower, but also the second-phase slope was larger. Adiwijaya et al.,[17] although they did not directly explore a correlation between ε and δ, found that allowing δ to increase with the telaprevir effectiveness, acccording to a relationship analogous to that shown in Fig. 2A, resulted in a better fit of their model to patient viral-load data. This finding not only supports the correlation we found, but shows its utility in data analysis.

Next, we asked whether this relationship between second-phase slope and treatment effectiveness was only true for telaprevir or whether it had wider applicability. To assess this, the relationship between drug effectiveness and δ was examined, both for the patients in this study and for patients from earlier studies involving treatment-naïve genotype 1 Caucasian patients receiving a high daily dose of IFN (>10 MIU).[2,3,18] Recent analyses have demonstrated an association between interleukin (IL)-28B genotype and slopes of viral decline.[19] Because the samples used here were not be tested for the IL-28B genotype, we restricted our analysis to Caucasians, for whom the chances to carry the favorable alleles are the highest.[19] Combining the data from these studies with that from the telaprevir studies, we encompass a much larger range of drug-effectiveness values. We still find a significant positive correlation ($r = 0.78$, $P < 0.001$) between drug effectiveness and δ (Fig. 2B). However, further analyses will be necessary to identify precisely whether polymorphisms in the IL-28B gene may affect the relationship between the first and second phases of viral decay in patients treated with IFN.

Interestingly, the second-phase slope in patients treated with telaprevir is much less variable than what was seen with IFN-based treatment. Because δ almost entirely determines the second phase of viral decline (see Patients and Methods), this finding suggests that duration of therapy needed to eliminate all virus and infected cells might be considerably shortened, as compared to IFN-based therapies. We evaluated empirically the distribution function of the time needed to achieve less than one virion in the extracellular body water (see Patients and Methods). We predict that with full patient compliance, 95% of patients could achieve viral clearance within 7 weeks and 99% within 8 weeks (Fig. 3). This time could be significantly delayed, if all drug doses are not taken. For patients taking three doses a day, we estimated that if 16% of doses are randomly missed (i.e., one every 2 days, on average), the time needed to eradicate the virus in 95 and 99% of patients would increase to 9 and 11 weeks, respectively (Fig. 3). If more drug doses are missed or if the missed doses are clumped together, as in a weekend drug holiday, a longer time to eradication should be anticipated (not shown).

Under treatment, each cell, on average, may generate less than one HCV RNA per day. Furthermore, the clearance rate of virions is much faster than that of cells, and thus when all viruses have been cleared, some infected cells may still be present. If SVR is defined as the time to eliminate all infected cells, SVR could be delayed. Because only HCV RNA is observed, the estimated number of infected cells is based, in part, on the rate of viral production per infected cell under treatment, $p(1 - \varepsilon)$ in Equation 1. Because only the ratio $(1 - \varepsilon)$ of the viral production before and during treatment can be estimated, but not the viral production rate itself (p in Equation 1), we considered the values, $p = 10$ virions/day and $p = 100$ virions/day, that cover the range of p values found in a previous study in patients treated with telaprevir.[20] With lower rates of viral production per infected cell, p, more infected cells are needed to explain the observed level of viremia in patients and hence the longer the time needed to eradicate the last infected cell. Based on these values of p, 2-3 additional weeks of treatments would be needed in order to eradicate all infected cells (Table 2).

Discussion

Using a new viral kinetic model that allowed for an improved description of the changes in antiviral treatment effectiveness, the second phase of viral decay was found to be very rapid, compared with second phases

Table 2. Estimated Time to SVR for 95 and 99% of Treated Patients

	Length of Treatment (in Weeks) Needed to Clear HCV Based on Eliminating*		
	Last Remaining Virus	Last Infected Cell ($p = 100$ Virions/Day)	Last Infected Cell ($p = 10$ Virions/Day)
Full compliance			
95% cured	7	9	10
99% cured	8	10	11
Three doses/day: one missed every 2 days, on average			
95% cured	9	11	12
99% cured	11	13	14

*Times (rounded up to the nearest week) to eliminate the last remaining virus or the last remaining infected cell are estimated based on the distribution of first- and second-phase viral RNA declines observed with telaprevir. Because the number of infected cells needed to produce the observed levels of HCV RNA depends on the rate of virion production per cell, p, we provide estimates based on two biologically reasonable values of p. We also consider the effect of full compliance (all drug doses taken) and partial compliance (one-sixth of missed drug doses).

observed in patients treated with IFN alone, with no differences according to treatment regimen. More precisely, we estimated that telaprevir induced a four-fold more rapid second-phase viral decline than IFN-based therapy.[2,3] Because the current understanding of HCV RNA decay attributes the second phase of viral decline to the loss rate of infected cells, our result suggests that either cell death is enhanced or mechanisms of infected cell loss other than cell death may be operating. Yet, because no elevation in alanine aminotransferase, a surrogate marker of liver cell death, was reported during telaprevir-based therapy, the assumption that the enhanced loss rate of infected cells reflects an elevation in the cell death is unlikely.

The current explanation of HCV RNA decline under therapy comes from studies using moderately potent IFN treatment. In that context, assuming that, after a short delay, the viral production rate per infected cell is reduced under treatment by a constant factor, $(1 - \varepsilon)$, has provided excellent fits to viral kinetic data from a variety of studies. Nevertheless, as a result of their very high pressure on intracellular replication, the new direct antiviral agents might be able to continuously reduce levels of intracellular viral RNA and, consequently, the viral production per infected cell in a treatment-effectiveness–dependent manner. This may also be the case for IFN, if its effectiveness is high enough. Although this remains speculative, some experiments using the replicon system support the suggestion that intracellular viral RNA not only initially declines by the factor $(1 - \varepsilon)$, but then continues to decline under protease inhibitor[21] or IFN[22] treatment. If the rate of viral production per infected cell is constantly reduced during therapy, the second slope of viral decline may reflect not only the rate of loss of infected cells, but also the rate at which viral production declines in infected cells.[23] Hence, the higher chance for attaining SVR observed in patients with an initial rapid viral response[24] could not only be due to a better immune response, but also to the progressive elimination of intracellular replication complexes resulting from a more potent antiviral treatment.

No matter what the biological mechanism, the rapid second-phase decline observed with telaprevir suggests that the duration of therapy needed to clear the infection might be considerably shortened, as compared to IFN-based therapies. Based on the extrapolation of the kinetics of decline estimated in our population study, we estimated that eradication of all virus particles could be reached within 7-9 weeks in 95% of patients. If SVR is considered to be achieved when the last infected cell has been cleared, rather than when the last virus is eliminated, an additional 2-3 weeks of therapy may be needed. This estimate is based on the current modeling assumption that the level of viral production under treatment in infected cells is reduced by a constant factor. In the framework of a model considering intracellular viral RNA, the progressive vanishing of viral replicative intermediates could lead to the "curing" of infected cells before infected cells die, which would reduce the time to SVR closer to the estimate, based on the last remaining virus particle. Also, our model is deterministic and thus does not consider explicitly the random nature of each possible event (e.g., cell infection, cell death, and virus clearance). Although an approach that includes the randomness of these processes would more accurately capture the probability distribution function for the time to HCV eradication at the individual level, it would not change the distribution function at the population level, where the law of large numbers applies and which was our primary object of study.

Although Fig. 2 shows a positive correlation between treatment effectiveness and second-phase slope, δ, one should not assume that the second-phase slope would continue to increase as drug combinations become increasingly effective. In principle, at some point, the rate of loss of the infected state would be limited by host cell processes, such as the intrinsic rate at which replication complexes decay, and thus would no longer increase with therapy effectiveness. Also, other viral kinetics studies will be necessary to determine whether the relationship in Fig. 2 is true for other protease inhibitors.

The second slope of viral decline has been reported for two other protease inhibitors—TMC-430 and danoprevir—and both studies reported a δ value roughly two times slower.[8,9]

Another limitation of our calculation of treatment duration is that we assume no loss of drug effectiveness throughout the course of treatment. With this assumption, the rate of second-phase decline is predicted not to decrease during treatment. Is this assumption reasonable with current therapeutic strategies? Based on the high turnover rate of virus and the high error rate of the HCV RNA–dependent RNA polymerase, it has been predicted that all possible single- and double-virus mutants are present at treatment initiation.[20] Thus, to avoid resistance emergence, combination therapy would be needed. Because a single-nucleotide substitution could be sufficient to confer resistance to protease inhibitors, the first treatment strategies that are expected to gain regulatory approval would be based on using a protease inhibitor (telaprevir or boceprevir) in combination with the standard of care (SOC). Because only approximately 50% of genotype 1 patients respond sufficiently strongly to the SOC to attain SVR, approximately 50% of genotype 1 patients treated with the current generation of protease inhibitors and SOC may not have a potent enough regimen to fully suppress the growth of protease-inhibitor resistance variants. This should also be the case in the majority of patients who already have failed prior regimens with SOC. Although resistant virus may not grow rapidly enough to cause viral breakthrough,[23] they can slow the second-phase decline, as suggested by the relationship between ε and δ in Fig. 2, and hence lead to a need for a longer treatment duration. Consistent with this argument, posttreatment relapse with resistant virus has been seen in patients treated with telaprevir and SOC for 12 weeks.[25,26] Nucleoside polymerase inhibitors present a high genetic barrier to resistance,[27] but their antiviral activity has tended, so far, to be much lower than protease inhibitors.[27] Using a protease inhibitor and a second DAA constitute the natural next step of anti-HCV treatment strategies. Recent results showed high rates of rapid viral response, with no or low prevalence of resistance emergence for up to 4 weeks when the second DAA was a polymerase inhibitor and up to 12 weeks when the second DAA was an NS5A inhibitor.[28-31] However, the fact that a resistance-related viral breakthrough occurred in some patients when SOC agents were not added to these cocktails hints that resistant virus may not be suppressed, but only reduced when two DAAs are used.[28,29,32] Most likely, to attain SVR in 95% of treatment-compliant patients with a 10-week course of therapy would require treatments with three or more DAAs, including RBV. Clearly, at present, there are no approved regimens that meet our criteria of high potency and a high enough barrier to resistance.

Even if resistance was avoided by using an appropriate combination of DAAs, other factors might affect our prediction. First, the ability of IFN-sparing antiviral strategies to reach every viral population residing in the liver or in extrahepatic reservoirs is unknown. Second, the combination of several DAAs might increase toxicity and thus the adherence to treatment. How this may impact treatment duration has only been touched on in this study, and more data are needed to understand how the lack of adherence to treatment may favor the appearance and persistence of resistant virus.

Thus, attainment of SVR in less than 10 weeks in 95% of fully compliant patients would require combination drug regimens (1) that have a genetic barrier high enough so that resistance is avoided, (2) that have high drug penetration into all anatomical sites that contain infected cells, and (3) for which the pharmacokinetics of the drugs in the regimen allow the effectiveness of the regimen against viral production to be maintained at high levels throughout the course of treatment.

In summary, our finding that the second-phase slope increases with the effectiveness of therapy and our expectation that a combination of DAA agents will suppress the growth of drug-resistant variants holds open the promise that more effective therapies that use combinations of DAA agents may, one day, lead to SVR with treatment durations of 2-3 months.

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