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Rapid emergence of hepatitis C virus protease inhibitor resistance is expected

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Approximately 170 million people worldwide are infected with hepatitis C virus (HCV)[1]. Current therapy, consisting of pegylated interferon (PEG-IFN) and ribavirin (RBV)[2], leads to sustained viral elimination in only about 45% of patients treated[3]. Telaprevir (VX-950), a novel HCV NS3-4A serine protease inhibitor, has demonstrated substantial antiviral activity in patients with chronic hepatitis C genotype 1 infection[4-8]. However, some patients experience viral breakthrough during dosing[4-6], with drug resistant variants being 5%-20% of the virus population as early as day 2 after treatment initiation[4]. Why viral variants appear such a short time after the start of dosing is unclear, especially since this has not been seen with monotherapy for either human immunodeficiency virus or hepatitis B virus[9]. Here, using a viral dynamic model, we explain why such rapid emergence of drug resistant variants is expected when potent HCV protease inhibitors are used as monotherapy. Surprisingly, our model also shows that such rapid emergence need not be the case with some potent HCV NS5B polymerase inhibitors. Examining the case of telaprevir therapy in detail, we show the model fits observed dynamics of both wild-type and drug-resistant variants during treatment, and supports combination therapy of direct antiviral drugs with PEG-IFN and/or RBV for hepatitis C.

The HCV-encoded NS3-4A serine protease plays an essential role in the generation of components of the viral RNA replication complex[10], and thus has emerged as an important target in searching for direct antiviral agents that inhibit specific steps in the HCV life cycle[11]. Several inhibitors of the NS3-4A protease have been developed[12-14], of which telaprevir has shown promising results in early-phase clinical trials[4-8]. Monotherapy with telaprevir has induced a rapid viral load decline in patients infected with genotype 1 HCV, with a median reduction in HCV RNA levels ranging from 2 to 4.4 $\log_{10}$ after 2 weeks of treatment[5]. However, in some patients viral breakthrough occurred during the dosing period, which has been suggested to be associated with selection of HCV variants with decreased sensitivity to telaprevir[4-6]. Using a highly sensitive sequencing assay, Sarrazin et al.[6] identified mutations in the HCV NS3-4A protease catalytic domain that conferred different levels of drug resistance to telaprevir. These resistance mutations were further confirmed in a subsequent detailed kinetic analysis of HCV variants in 16 patients treated with telaprevir alone or telaprevir plus PEG-IFN-α-2a for 14 days[4]. Four of the eight patients that were treated with telaprevir monotherapy had viral rebound during the dosing period (Supplementary Fig. 1). Virus isolated from these patients at day 2 after treatment contained drug resistant strains with single nucleotide mutations at a frequency of 5-20%, which then increased in frequency at days 6 and 10, and were replaced by high-level resistant double mutant variants by day 13 (ref. 4). Although these mutant HCV variants may exist before treatment[15] due to the high viral replication rate of HCV[16] and its error-prone polymerase[17], their appearance at high frequencies such a short time after the start of dosing was not expected[18].

We developed a viral dynamic model to examine the frequency of the mutant virus in the total virus population before and after drug administration. The model includes both wild type (WT)

and drug resistant strains, and can be described by the following equations:

$$\begin{aligned} dT/dt &= s + \rho T(1 - T/T_{\max}) - dT - \beta V_s T - \beta V_r T \\ dI_s/dt &= \beta V_s T - \delta I_s \\ dI_r/dt &= \beta V_r T - \delta I_r \\ dV_s/dt &= (1-\mu)(1-\varepsilon_s) p_s I_s - cV_s \\ dV_r/dt &= \mu(1-\varepsilon_s) p_s I_s + (1-\varepsilon_r) p_r I_r - cV_r \end{aligned} \quad (1)$$

WT and drug resistant HCV virions, V_s and V_r, infect target cells, T, to create productively infected cells, I_s and I_r, at rates $\beta V_s T$ and $\beta V_r T$, respectively. Target cells are generated at rate s, die at rate d, and can proliferate with maximum proliferation rate ρ and carrying capacity $T_{\max}$. I_s and I_r are lost at per capita rate, δ, and are assumed to produce virions at rates p_s and p_r, respectively, which are then cleared with rate constant c. ε_s and ε_r represent the drug efficacies of telaprevir against the sensitive WT and the drug resistant virions, respectively, where $0 \le \varepsilon_s, \varepsilon_r \le 1$ with 0 corresponding to no treatment effect and 1 to a 100% effective drug. We assume that I_s with probability μ generates drug resistant virions. Backward mutation is neglected because it has a minor effect on the mutant frequency (L.R., R.M.R. and A.S.P., unpublished observation). We also neglect proliferation of infected cells since the estimate of the proliferation rate is small based on data fitting (will be discussed later).

Before treatment, both WT and drug resistant viral strains coexist at steady state levels in infected individuals (Supplementary Note S1). The frequency of the pre-existing mutant virus in the total virus population is $\Gamma = \mu/(1-r)$, where $r = R_r / R_s$ with $R_r = \beta p_r T_0/(c\delta)$ and $R_s = \beta p_s T_0/(c\delta)$ are the basic reproductive ratios of drug resistant and WT strains, respectively. Here $T_0 = T_{\max}\left(\rho - d + \sqrt{(\rho-d)^2 + 4\rho s/T_{\max}}\right)/(2\rho)$ is the target cell level in the absence of HCV infection. The ratio $r = R_r/R_s = p_r/p_s$ represents the relative fitness of drug resistant to WT in the

absence of treatment, and we expect $r<1$ because of resistance-associated loss of fitness[6]. Thus, although the two strains coexist before therapy, the drug resistant virus level is very low because $\mu<<1$ (ref. 17).

We assume that the target cell concentration remains at approximately its baseline value, $\overline{T}=c\delta/\left((1-\mu)\beta p_s\right)$, for a few days after drug administration[16, 19]. Furthermore, we ignore the term $\mu(1-\varepsilon_s)p_s I_s$ in the V_r equation because μ is very small and ε_s is close to 1 (telaprevir is very effective against WT virus[4, 5, 20]). We solve the simplified system for $V_s(t)$ and $V_r(t)$. The mutant frequency is $\Gamma(t)=V_r(t)/\left(V_s(t)+V_r(t)\right)$, which depends only on the parameters c, δ, μ, r, ε_s and ε_r (Supplementary Note S2).

To examine the change of the mutant frequency, $\Gamma(t)$, after treatment initiation, we need to determine the drug efficacy of telaprevir for each strain. The effectiveness of a drug can be related to its concentration, $C(t)$, by $\varepsilon_s(t)=C(t)^h/\left(IC_{50}{}^h+C(t)^h\right)$, where IC_{50} is the drug concentration needed to inhibit viral production by 50%, and h is the Hill coefficient[21, 22]. Based on the pharmacodynamics of telaprevir, the median ε_s exceeds 0.999 (ref. 20), which is consistent with the 3-4 $\log_{10}$ first-phase drop of plasma HCV RNA levels in patients undergoing telaprevir monotherapy[5]. Analogously, if mutation is associated with an n-fold increase of IC_{50}, then we have $\varepsilon_r(t)=C(t)^h/\left((n\cdot IC_{50})^h+C(t)^h\right)=\varepsilon_s(t)/\left(\varepsilon_s(t)+(1-\varepsilon_s(t))n^h\right)$.

We show in Fig. 1 the change of the mutant frequency expected using constant (median) ε_s and ε_r over a short time period after drug dosing. We consider that a single mutation may confer different levels of drug resistance to telaprevir[6]. For example, the mutation V36A/M confers ~3.5-fold resistance (Fig. 1a, b), whereas A156V/T confers ~466-fold resistance to telaprevir[6] (Fig. 1c,

d). In both cases, the mutant frequency undergoes a substantial increase from the pre-existing low level (< 1%) to > 5% within days after treatment initiation (Fig. 1a, c). This is in agreement with the observation that single mutants detected at day 2, such as V36A/M and A156V/T, account for 5-20% of the virus population[4] (Supplementary Fig. 1). Such a rapid and dramatic increase of the mutant frequency, however, does not necessarily imply that drug resistant HCV variants grow rapidly during treatment. In fact, further investigation of the simplified model (Supplementary Note S2) suggests that both WT and drug resistant strains undergo a two-phase viral decline (Fig. 1b, d; as we assume $T = \overline{T}$ over a short time period after drug dosing). Most interestingly, we find that the duration of the first-phase viral decline of WT virus is longer than that of drug resistant virus (Fig. 1b, d). If we denote by t_s and t_r the time at which the first-phase decline of WT and drug resistant virions ends, respectively, then we can show that $t_s > t_r$ (Supplementary Note S3). Moreover, the higher the fold-increase in resistance of the variants, the shorter the first-phase viral decline of the drug resistant virus (Fig. 1b, d and Supplementary Note S3). These results suggest that the substantial increase of the mutant frequency after drug administration is not due to a rapid mutant growth but rather due to a longer first-phase decline of WT virus, which unveils the pre-existing mutant HCV variants.

Suppression of the pre-existing mutant virus regardless of its drug resistance level, as shown in Fig. 1, is due to the assumption that target cells remain at a constant baseline level after drug administration. If we remove this assumption and describe the dynamics of target cells as in the T equation of model (1), then drug resistant virus is able to grow and ultimately dominate the virus population under certain conditions (Supplementary Note S4). The reproductive ratios of the two strains under treatment are $R_s' = (1-\varepsilon_s)R_s$ and $R_r' = (1-\varepsilon_r)R_r$. Because telaprevir is very effective against WT virus, R_s' usually becomes less than 1 during treatment and WT virus is successfully

suppressed. If mutation confers low-level drug resistance to telaprevir, then drug resistant virus will also be suppressed. However, if mutation confers high-level drug resistance, R_r' may be greater than 1 and the pre-existing drug resistant virus will outcompete the WT virus. We have applied our model (Eq. (1)) to an analysis of the experimental data obtained from the aforementioned four patients who had viral breakthrough during the 14 days of telaprevir monotherapy[4]. The model provides excellent fits to both the WT and drug resistant viral kinetics (Fig. 2). The estimated drug efficacy of telaprevir against WT virus is 0.9986 ± 0.0021, which confirms the previous result of telaprevir effectiveness[4, 5, 20]; whereas the estimated drug efficacy against telaprevir-resistant virus is 0.002 ± 0.001, supporting the notion that these HCV variants have significantly reduced susceptibility to telaprevir.

The model and its analysis can be employed to investigate the development of drug resistance during treatment with other HCV protease inhibitors as well as polymerase inhibitors. The HCV NS5B RNA-dependent RNA polymerase is crucial to viral RNA synthesis[10] and thus represents another attractive drug discovery target[11]. A number of NS5B polymerase inhibitors, including the non-nucleoside inhibitor HCV-796 and nucleoside inhibitors NM283 (prodrug of NM107), R1626 (prodrug of R1479), and R7128 (prodrug of PSI-6130), are currently undergoing clinical assessment[23]. Interestingly, during 14-day monotherapy studies, drug resistant variants appeared rapidly in HCV-796-treated patients[24] as in the study of telaprevir[4, 6]. However, there was no evidence of drug resistance with the nucleoside inhibitors NM283 (ref. 25), R1626 (ref. 26), and R7128 (ref. 27). Lack of resistance to these nucleoside polymerase inhibitors can be explained by the fitness disadvantage they induce. Dynamics of the mutant virus are determined by its fitness during therapy, i.e., $R_r' = (1-\varepsilon_r)\beta p_r T_0/(c\delta)$, which depends primarily on two factors: reduction of drug sensitivity (ε_r) and viral production capacity (p_r). Although many NS5B mutations, such as

S282T for PSI-6130 and S96T or S96T/N142T for R1479, confer a 3- to 5-fold loss in sensitivity to the nucleoside polymerase inhibitors, they also resulted in a greater than 85% reduction in replication capacity (corresponding to p_r)[28]. Thus, these viral strains seemed not to obtain enough fitness advantage over WT such that they could be selected from the pre-existing quasispecies. In contrast, the telaprevir and HCV-796 resistant variants generally have a higher replication capacity than the nucleoside inhibitor mutants[28], which gives them relatively higher fitness and thus makes them easier to be selected during treatment. Indeed, nucleoside polymerase inhibitors seem to have a higher genetic barrier to resistance than either protease or non-nucleoside polymerase inhibitors using the HCV replicon system[28], which highlights the clinical importance of nucleoside polymerase inhibitors in future HCV therapies.

The rapid appearance of drug resistant HCV variants suggests that treatment failure of monotherapy with a direct antiviral drug like telaprevir seems inevitable. The lessons from HIV-infection treatment indicate that combinations of drugs with different mechanisms of action also may be an attractive therapeutic strategy for hepatitis C (ref. 23). Growing evidence, from both *in vitro*[29] and clinical studies[4, 7, 8], has emerged to support this idea. All eight patients who received telaprevir plus PEG-IFN-α-2a had continued antiviral response during the 14-day dosing period[4] (Supplementary Fig. S2). Even in patients who had viral breakthrough following telaprevir alone, follow-up therapy with standard treatment (PEG-IFN + RBV) could inhibit growth of both WT and drug resistant virions[4]. We have also expanded our model by incorporating the combination effect of PEG-IFN-α-2a and telaprevir (Supplementary Note S5). The model provides excellent fits to the viral load data (Fig. 3; parameter estimates are listed in Supplementary Table S2). However, for Patient 3011 there appears to be a "shoulder phase" in which HCV RNA decays slowly or remains constant. A triphasic viral decline in Patient 3011 can be explained by incorporating proliferation of

infected cells into our model[30] (Supplementary Note S6). With combination therapy, the estimated total drug effectiveness against telaprevir-resistant variants (0.81 ± 0.12) is significantly ($P=0.006$) greater than that of telaprevir alone (0.002 ± 0.001). These results suggest that HCV variants with reduced sensitivity to telaprevir still remain sensitive to PEG-IFN, which strongly supports combination therapy of direct antiviral drugs like telaprevir with PEG-IFN and/or RBV for hepatitis C.

References

1. World Health Organization. Hepatitis C. Fact sheet No. 164. Revised October 2000. http://www.who.int/mediacentre/factsheets/fs164/en/index.html.
2. Manns, M. P. et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet 358, 958-65 (2001).
3. Fried, M. W. et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 347, 975-82 (2002).
4. Kieffer, T. L. et al. Telaprevir and pegylated interferon-alpha-2a inhibit wild-type and resistant genotype 1 hepatitis C virus replication in patients. Hepatology 46, 631-9 (2007).
5. Reesink, H. W. et al. Rapid decline of viral RNA in hepatitis C patients treated with VX-950: a phase Ib, placebo-controlled, randomized study. Gastroenterology 131, 997-1002 (2006).
6. Sarrazin, C. et al. Dynamic hepatitis C virus genotypic and phenotypic changes in patients treated with the protease inhibitor telaprevir. Gastroenterology 132, 1767-77 (2007).
7. Forestier, N. et al. Antiviral activity of telaprevir (VX-950) and peginterferon alfa-2a in patients with hepatitis C. Hepatology 46, 640-8 (2007).
8. Lawitz, E. et al. Antiviral effects and safety of telaprevir, peginterferon alfa-2a, and ribavirin for 28 days in hepatitis C patients. J Hepatol 49, 163-9 (2008).
9. Soriano, V., Perelson, A. S. & Zoulim, F. Why are there different dynamics in the selection of drug resistance in HIV and hepatitis B and C viruses? J Antimicrob Chemother 62, 1-4 (2008).
10. Lindenbach, B. D. & Rice, C. M. Unravelling hepatitis C virus replication from genome to function. Nature 436, 933-8 (2005).
11. De Francesco, R. & Migliaccio, G. Challenges and successes in developing new therapies for hepatitis C. Nature 436, 953-60 (2005).
12. Lamarre, D. et al. An NS3 protease inhibitor with antiviral effects in humans infected with hepatitis C virus. Nature 426, 186-9 (2003).
13. Perni, R. B. et al. Preclinical profile of VX-950, a potent, selective, and orally bioavailable inhibitor of hepatitis C virus NS3-4A serine protease. Antimicrob Agents Chemother 50, 899-909 (2006).

14. Malcolm, B. A. et al. SCH 503034, a mechanism-based inhibitor of hepatitis C virus NS3 protease, suppresses polyprotein maturation and enhances the antiviral activity of alpha interferon in replicon cells. Antimicrob Agents Chemother 50, 1013-20 (2006).
15. Cubero, M. et al. Naturally occurring NS3-protease-inhibitor resistant mutant A156T in the liver of an untreated chronic hepatitis C patient. Virology 370, 237-45 (2008).
16. Neumann, A. U. et al. Hepatitis C viral dynamics in vivo and the antiviral efficacy of interferon-alpha therapy. Science 282, 103-7 (1998).
17. Domingo, E. Biological significance of viral quasispecies. Viral Hepatitis Rev 2, 247-261 (1996).
18. Pawlotsky, J. M. Treatment of hepatitis C: don't put all your eggs in one basket! Gastroenterology 132, 1611-5 (2007).
19. Dixit, N. M., Layden-Almer, J. E., Layden, T. J. & Perelson, A. S. Modelling how ribavirin improves interferon response rates in hepatitis C virus infection. Nature 432, 922-4 (2004).
20. Chu, H. M. et al. Pharmacokinetics of VX-950, and its effect on hepatitis C viral dynamics. Hepatology 42 Suppl 1, 694A (2005).
21. Powers, K. A. et al. Modeling viral and drug kinetics: hepatitis C virus treatment with pegylated interferon alfa-2b. Semin Liver Dis 23 Suppl 1, 13-8 (2003).
22. Shen, L. et al. Dose-response curve slope sets class-specific limits on inhibitory potential of anti-HIV drugs. Nat Med 14, 762-6 (2008).
23. Manns, M. P. et al. The way forward in HCV treatment--finding the right path. Nat Rev Drug Discov 6, 991-1000 (2007).
24. Villano, S. et al. Analysis of HCV NS5B genetic variants following monotherapy with HCV-796, a non-nucleoside polymerase inhibitor, in treatment-naive HCV-infected patients. Hepatology 44 Suppl 1, 607A (2006).
25. Godofsky, E. et al. A phase I/II dose escalation trial assessing tolerance, pharmacokinetics, and antiviral activity of NM283, a novel antiviral treatment for hepatitis C. Gastroenterology 126 Suppl 2, A681 (2004).
26. Roberts, S. K. et al. Robust antiviral activity of R1626, a novel nucleoside analog: a randomized, placebo-controlled study in patients with chronic hepatitis C. Hepatology 48, 398-406 (2008).
27. Reddy, R. et al. Antiviral activity, pharmacokinetics, safety, and tolerability of R7128, a novel nucleoside HCV RNA polymerase inhibitor, following multiple, ascending, oral doses in patients with HCV genotype 1 infection who have failed prior interferon therapy. Hepatology 46 Suppl 1, 862A (2007).
28. McCown, M. F. et al. The hepatitis C virus replicon presents a higher barrier to resistance to nucleoside analogs than to nonnucleoside polymerase or protease inhibitors. Antimicrob Agents Chemother 52, 1604-12 (2008).
29. Lin, K., Kwong, A. D. & Lin, C. Combination of a hepatitis C virus NS3-NS4A protease inhibitor and alpha interferon synergistically inhibits viral RNA replication and facilitates viral RNA clearance in replicon cells. Antimicrob Agents Chemother 48, 4784-92 (2004).
30. Dahari, H., Ribeiro, R. M. & Perelson, A. S. Triphasic decline of hepatitis C virus RNA during antiviral therapy. Hepatology 46, 16-21 (2007).

Supplementary Information is linked to the online version of the paper at

www.nature.com/nature.

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Figure Legends

Figure 1 | Model predictions of the mutant frequency and viral load decay profiles after drug administration. a, b, HCV mutant, e.g., V36A/M, induces ~3.5-fold increase in IC_{50} from WT and its relative fitness is $r \approx 0.98$ (ref. 6); **c, d**, HCV mutant, e.g., A156V/T, induces ~466-fold resistance with the relative fitness $r \approx 0.45$ (ref. 6). Model (S2) predicted a significant increase of the mutant frequency as illustrated in the left column. The right column shows a biphasic decrease of both WT (green dashed) and drug resistant (blue dashed) virions after drug dosing. t_s and t_r represent the time at which WT and drug resistant virions start the second-phase viral decline, respectively. Parameters used were $c = 6.2$ day^{-1} (ref. 16), $\delta = 0.14$ day^{-1} (ref. 16), $\mu = 10^{-4}$ per copied nucleotide[17], $\varepsilon_s = 0.9997$ (ref. 20), and the Hill coefficient $h = 2$ (ref. 21).

Figure 2 | Comparison between model predictions and patient data during telaprevir monotherapy. We employed the pretreatment steady-state values (Supplementary Note S1) as the initial conditions of the model including treatment. We fitted V_s (green dashed) and V_r (blue dashed) of model (1) to the WT (green up triangle) and drug resistant (blue diamond) viral load data simultaneously. Since we ignored the WT viral load data when they are below the detection

limit of the sequencing assay, i.e., < 5% (ref. 4), we included fitting $V_s + V_r$ (red solid) to the total viral load data (red square). The best-fit parameter values for each patient are listed in Supplementary Table S1.

Figure 3 | Comparison between model predictions and patient data during combination therapy. We fitted the model in Supplementary Note S5 to the viral load data from patients receiving both PEG-IFN-α-2a and telaprevir for 14 days. This model generalizes model (1) by incorporating an effect of PEG-IFN in partially blocking viral production. The fitting procedure and the symbols used are the same as those in Fig. 2. The best-fit parameter values for each patient are listed in Supplementary Table S2. Note that the two fits of the WT (green dashed) and total viral load (red solid) overlap in a few patients.

Figure 1

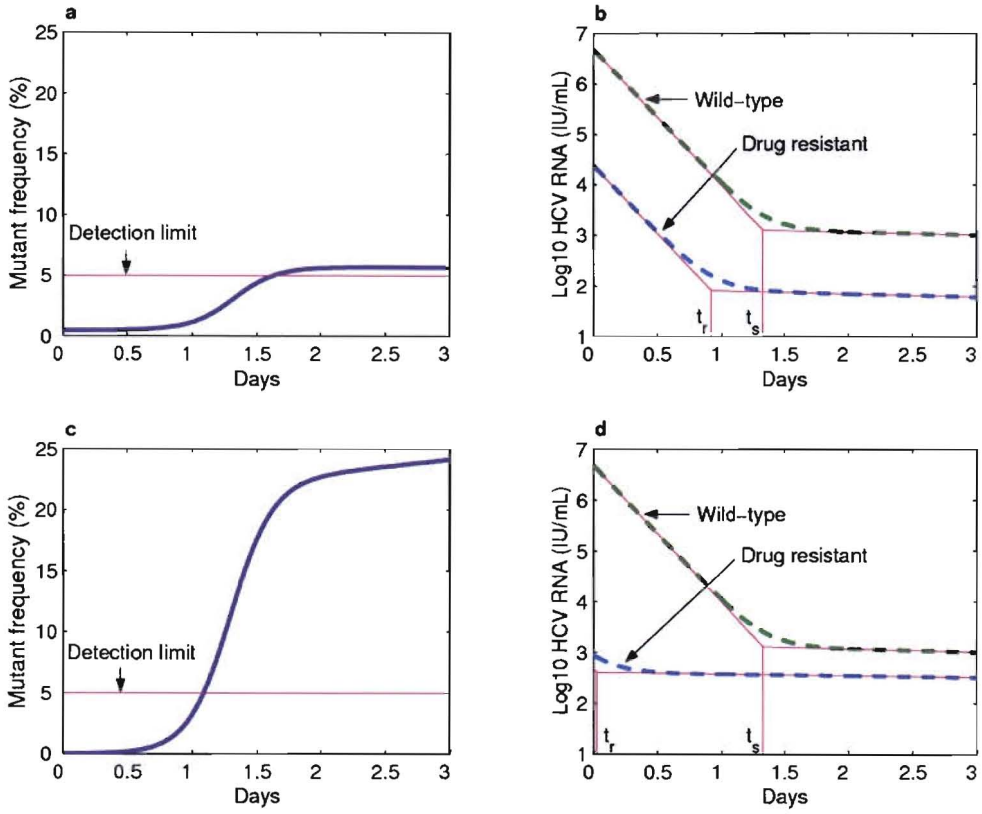

a
Mutant frequency (%)
Detection limit
Days
b
Log10 HCV RNA (IU/mL)
Wild-type
Drug resistant
t_r
t_s
Days
c
Mutant frequency (%)
Detection limit
Days
d
Log10 HCV RNA (IU/mL)
Wild-type
Drug resistant
t_r
t_s
Days

Figure 2

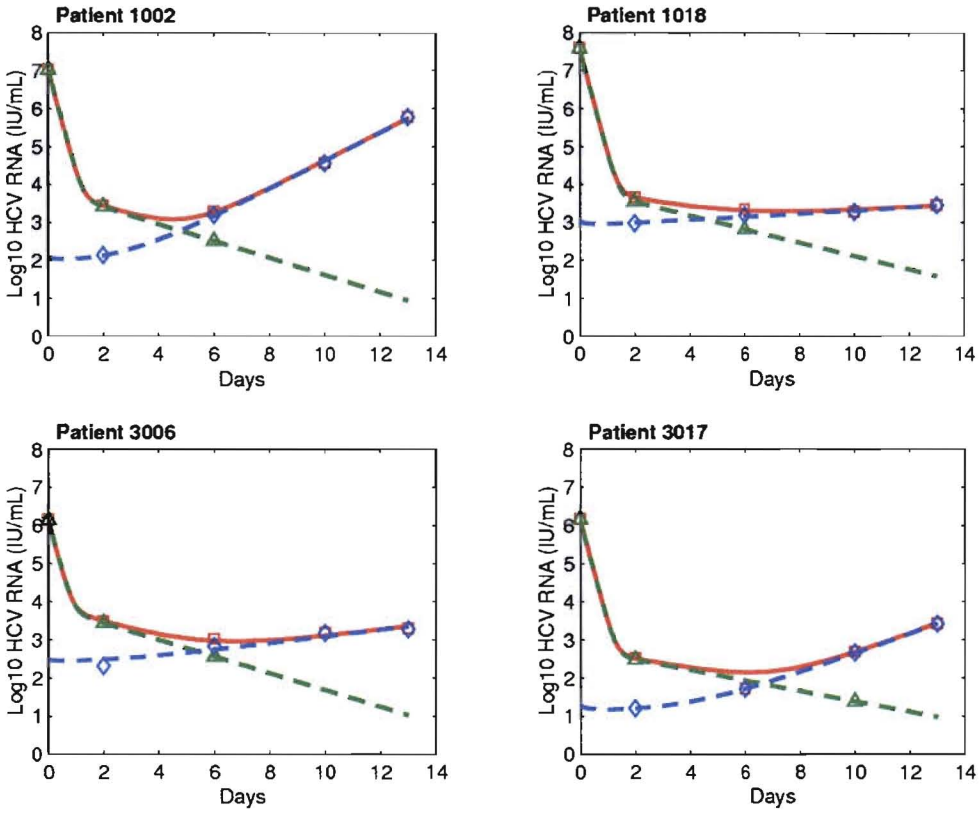
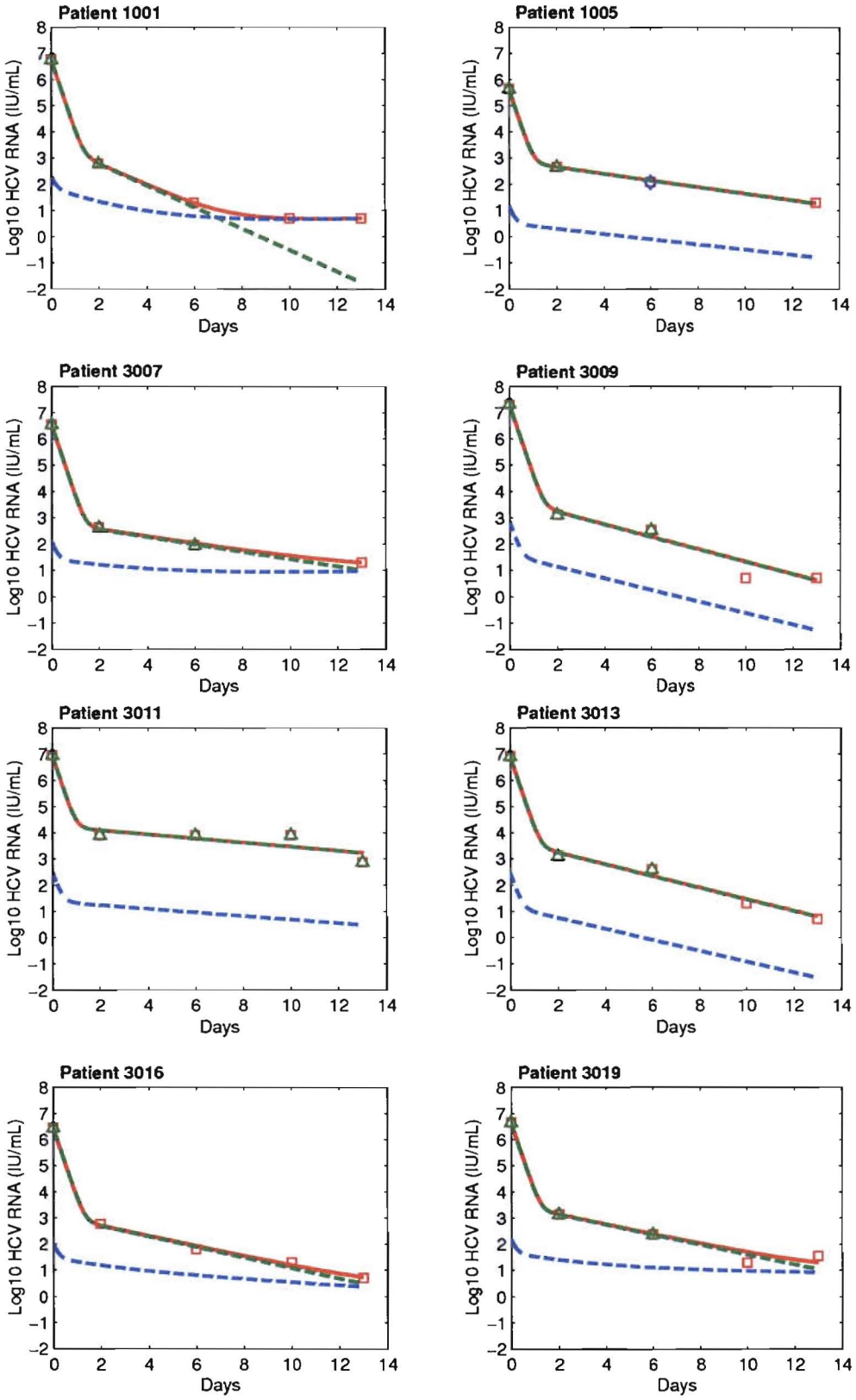

Patient 1002
Patient 1018
Patient 3006
Patient 3017
Log10 HCV RNA (IU/mL)
Days
0
1
2
3
4
5
6
7
8
0
2
4
6
8
10
12
14

Figure 3


Patient 1001
Patient 1005
Patient 3007
Patient 3009
Patient 3011
Patient 3013
Patient 3016
Patient 3019
Log10 HCV RNA (IU/mL)
Days

Supplementary Information

Supplementary Note S1

Before treatment ($\varepsilon_s = \varepsilon_r = 0$), model (1) reads:

$$\begin{aligned} dT/dt &= s + \rho T(1 - T/T_{\max}) - dT - \beta V_s T - \beta V_r T \\ dI_s/dt &= \beta V_s T - \delta I_s \\ dI_r/dt &= \beta V_r T - \delta I_r \\ dV_s/dt &= (1-\mu) p_s I_s - c V_s \\ dV_r/dt &= \mu p_s I_s + p_r I_r - c V_r \end{aligned} \qquad \text{(S1)}$$

Considering the predominance of WT virus before treatment and resistance-associated loss of fitness[6] (i.e., $R_s > \max(\frac{R_r}{1-\mu}, \frac{1}{1-\mu})$), the solutions of the above model converge to the steady state in which both WT and drug resistant strains coexist (L.R., R.M.R. and A.S.P., unpublished observation; also see Supplementary Note S4). The coexistence steady state is ($\overline{T}$, $\overline{I_s}$, $\overline{I_r}$, $\overline{V_s}$, $\overline{V_r}$), where

$$\overline{T} = \frac{c\delta}{(1-\mu)\beta p_s},\ \overline{V_s} = \frac{s + \rho\overline{T}(1 - \frac{\overline{T}}{T_{\max}}) - d\overline{T}}{\beta\overline{T}\frac{1-r}{1-\mu-r}},\ \overline{V_r} = \frac{\mu}{1-\mu-r}\overline{V_s},\ \overline{I_s} = \frac{\beta}{\delta}\overline{V_s}\,\overline{T},\ \overline{I_r} = \frac{\beta}{\delta}\overline{V_r}\,\overline{T}.$$

Here $r = R_r / R_s = p_r / p_s$ is the relative fitness of the mutant strain to WT. R_s and R_r are the basic reproductive ratios of WT and drug resistant strains, respectively, and are given by $R_s = \beta p_s T_0 / (c\delta)$ and $R_r = \beta p_r T_0 / (c\delta)$, where $T_0 = \frac{T_{\max}}{2\rho}\left[\rho - d + \sqrt{(\rho - d)^2 + \frac{4\rho s}{T_{\max}}}\right]$ is the level of target cells in the absence of viral infection. The frequency of the pre-existing mutant virus in the total virus population is $\Gamma = \frac{\overline{V_r}}{\overline{V_s} + \overline{V_r}} = \frac{\mu}{1-r}$.

Supplementary Note S2

Assuming T remains at a constant level, $\overline{T} = \dfrac{c\delta}{(1-\mu)\beta p_s}$, and ignoring $\mu(1-\varepsilon_s)p_s I_s$ in the V_r equation of model (1), we have the simplified system:

$$\begin{aligned} dI_s/dt &= \beta V_s \overline{T} - \delta I_s \\ dI_r/dt &= \beta V_r \overline{T} - \delta I_r \\ dV_s/dt &= (1-\mu)(1-\varepsilon_s)p_s I_s - cV_s \\ dV_r/dt &= (1-\varepsilon_r)p_r I_r - cV_r \end{aligned} \tag{S2}$$

Solving it, we obtain $V_s(t) = C_1 e^{\lambda_1 t} + C_2 e^{\lambda_2 t}$ and $V_r(t) = C_3 e^{\lambda_3 t} + C_4 e^{\lambda_4 t}$. Here λ_i, $i = 1,2,3,4$, are the eigenvalues given by $\lambda_{1,2} = -\left(c+\delta \pm \sqrt{\Delta_1}\right)/2$ and $\lambda_{3,4} = -\left(c+\delta \pm \sqrt{\Delta_2}\right)/2$, where $\Delta_1 = (c+\delta)^2 - 4\varepsilon_s c\delta$ and $\Delta_2 = (c+\delta)^2 - 4\theta c\delta$ with $\theta = 1-(1-\varepsilon_r)r/(1-\mu)$. The coefficients C_i, $i = 1,2,3,4$, are given by

$$C_{1,2} = \frac{\sqrt{\Delta_1} \mp \left(c(1-2\varepsilon_s)+\delta\right)}{2\sqrt{\Delta_1}} V_s(0), \quad C_{3,4} = \frac{\sqrt{\Delta_2} \mp \left(c(1-2\theta)+\delta\right)}{2\sqrt{\Delta_2}} V_r(0),$$

where $V_r(0) = \mu/(1-\mu-r)V_s(0)$ based on the pretreatment steady state. The mutant frequency after drug dosing is a function of t, $\Gamma(t) = \dfrac{V_r(t)}{V_s(t)+V_r(t)} = \dfrac{C_3 e^{\lambda_3 t} + C_4 e^{\lambda_4 t}}{\sum_{i=1}^{4} C_i e^{\lambda_i t}}$, which depends only on the parameters c, δ, μ, r, ε_s and ε_r.

Considering $(c-\delta)^2 < \Delta_1 < (c+\delta)^2$, we have $\lambda_{1,2} < 0$. Next, we show that $(c+\delta)^2 > \Delta_2$. It suffices to show that $1 - \dfrac{(1-\varepsilon_r)R_r}{(1-\mu)R_s} > 0$, i.e., $\dfrac{R_r}{R_s} < \dfrac{1-\mu}{1-\varepsilon_r}$, which is satisfied because WT virus is more fit than drug resistant virus in the absence of treatment ($R_r < R_s$) and $\mu \ll 1$. It follows that $\lambda_{3,4} < 0$. Thus, both WT and drug resistant viral loads undergo a two-phase decline.

Supplementary Note S3

Because t_s represents the time at which the second-phase decline of WT virus begins, t_s is the time at which the two curves, $C_1 e^{\lambda_1 t}$ and $C_2 e^{\lambda_2 t}$, intersect. Thus, $t_s = \frac{\ln(C_1/C_2)}{\lambda_2 - \lambda_1} = \frac{\ln(C_1/C_2)}{\sqrt{\Delta_1}}$. Similarly, we have $t_r = \frac{\ln(C_3/C_4)}{\sqrt{\Delta_2}}$. We notice that $C_i > 0,\ i = 1,2,3,4$. Next, we show that $\Delta_1 < \Delta_2$.

Calculating the difference, we have $\Delta_2 - \Delta_1 = 4c\delta(1-\varepsilon_s)[\frac{1}{(1-\mu)}\frac{R_r'}{R_s'} - 1]$, where $R_s' = (1-\varepsilon_s)R_s$ and $R_r' = (1-\varepsilon_r)R_r$ are the reproductive ratios of WT and drug resistant strains during treatment[31], respectively. Because we assume that drug resistant virus is more fit than WT virus during therapy, we have that $R_r' > R_s'$. Thus, $\Delta_2 > \Delta_1$.

Calculating the difference between C_1/C_2 and C_3/C_4, we obtain

$$\frac{C_1}{C_2} - \frac{C_3}{C_4} = \frac{-c(1-2\varepsilon_s) - \delta + \sqrt{\Delta_1}}{c(1-2\varepsilon_s) + \delta + \sqrt{\Delta_1}} - \frac{-c(1-2\theta) - \delta + \sqrt{\Delta_2}}{c(1-2\theta) + \delta + \sqrt{\Delta_2}}.$$

Using the common denominator, we obtain the numerator, which can be simplified to $4c(\varepsilon_s\sqrt{\Delta_2} - \theta\sqrt{\Delta_1}) - 2(c+\delta)(\sqrt{\Delta_2} - \sqrt{\Delta_1})$. Because drug resistant virus is more fit than WT virus during therapy, we have $(1-\varepsilon_s)R_s < (1-\varepsilon_r)R_r$, i.e., $(1-\varepsilon_s) < r(1-\varepsilon_r)$. Thus, $\theta = 1-(1-\varepsilon_r)r/(1-\mu) < \varepsilon_s$ because μ is very small. Therefore, the numerator satisfies

$$\begin{aligned}
&4c(\varepsilon_s\sqrt{\Delta_2} - \theta\sqrt{\Delta_1}) - 2(c+\delta)(\sqrt{\Delta_2} - \sqrt{\Delta_1}) \\
&> 4c(\varepsilon_s\sqrt{\Delta_2} - \varepsilon_s\sqrt{\Delta_1}) - 2(c+\delta)(\sqrt{\Delta_2} - \sqrt{\Delta_1}) \\
&= 2(\sqrt{\Delta_2} - \sqrt{\Delta_1})[2c\varepsilon_s - (c+\delta)] \\
&> 0
\end{aligned}$$

The last inequality holds because telaprevir is very effective against WT virus (i.e., ε_s is close to 1) and virus has much faster dynamics than productively infected cells[16] ($c >> \delta$). Thus, $C_1/C_2 > C_3/C_4$. Also considering $\sqrt{\Delta_2} > \sqrt{\Delta_1}$, we have $t_s > t_r$. This implies that WT virus undergoes a longer first-phase decline than drug resistant virus.

Moreover, we can show that t_r is an increasing function with respect to ε_r, the efficacy of the protease inhibitor against the drug resistant strain. As ε_r decreases (i.e., a more resistant viral strain), $\theta = 1-(1-\varepsilon_r)r/(1-\mu)$ decreases and $\Delta_2 = (c+\delta)^2 - 4\theta c\delta$ increases. Rearranging C_3/C_4, we have

$$\frac{C_3}{C_4} = \frac{-c(1-2\theta)-\delta+\sqrt{\Delta_2}}{c(1-2\theta)+\delta+\sqrt{\Delta_2}} = \frac{2\sqrt{\Delta_2}}{c(1-2\theta)+\delta+\sqrt{\Delta_2}} - 1 = \frac{2}{1+f(\theta)} - 1,$$

where

$$f(\theta) = \frac{c(1-2\theta)+\delta}{\sqrt{\Delta_2}} = \frac{c(1-2\theta)+\delta}{\sqrt{(c+\delta)^2 - 4\theta c\delta}}.$$

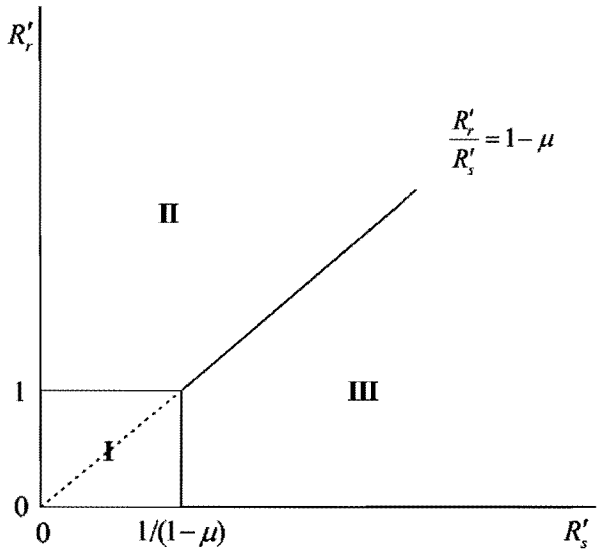
Taking derivative of $f(\theta)$ with respect to θ, we have

$$f'(\theta) = \frac{-2c[(c+\delta)^2 - 4\theta c\delta] + 2c\delta[c(1-2\theta)+\delta]}{[(c+\delta)^2 - 4\theta c\delta]^{\frac{3}{2}}}.$$

The numerator of the above faction can be simplified to $2c^2(2\theta\delta - c - \delta)$, which is less than 0 because $\theta\delta < c$ and $\theta\delta < \delta$. Therefore, as θ decreases, $f(\theta)$ increases. Consequently, C_3/C_4 decreases and $t_r = \frac{\ln(C_3/C_4)}{\sqrt{\Delta_2}}$ decreases. This shows that t_r is an increasing function of ε_r.

Supplementary Note S4

With a varying target cell number, model (1) describes the competition between the two HCV strains similar to that in an HIV-1 drug resistance model[31]. The relationship between R_s' and R_r' determines which strain will dominate the virus population during therapy. As shown in the following figure, both WT and drug resistant strains die out in Region **I**. In Region **II**, WT virus dies out and only drug resistant virus persists. In Region **III**, WT virus dominates the virus population, although the two strains coexist.



Supplementary Note S5

We modify model (1) by incorporating the combination effect of PEG-IFN-α-2a and telaprevir. Assuming that interferon lowers the viral production rate by a factor $(1-\eta)$, where η is the effectiveness of interferon[16, 19], the model changes to

$$\begin{aligned} dT/dt &= s + \rho T(1-T/T_{\max}) - dT - \beta V_s T - \beta V_r T \\ dI_s/dt &= \beta V_s T - \delta I_s \\ dI_r/dt &= \beta V_r T - \delta I_r \\ dV_s/dt &= (1-\mu)(1-\varepsilon_s)(1-\eta)p_s I_s - cV_s \\ dV_r/dt &= \mu(1-\varepsilon_s)(1-\eta)p_s I_s + (1-\varepsilon_r)(1-\eta)p_r I_r - cV_r \end{aligned} \tag{S3}$$

Defining $\varepsilon^s_{total} = 1-(1-\varepsilon_s)(1-\eta)$ and $\varepsilon^r_{total} = 1-(1-\varepsilon_r)(1-\eta)$, ε^s_{total} and ε^r_{total} represent the total drug effectiveness against WT and drug resistant virions, respectively. Because $\varepsilon_r < \varepsilon_s$, we have $\varepsilon^r_{total} < \varepsilon^s_{total}$. We notice that the modified model is the same as the original model except that ε_s and ε_r are replaced with ε^s_{total} and ε^r_{total}, respectively.

Supplementary Note S6

As in Patient 3011, a triphasic viral decline (including a second "shoulder phase") has been observed elsewhere[32-34]. Dahari et al.[30, 35] have shown that a model including proliferation of both uninfected and infected cells can account for a triphasic HCV RNA decay. We incorporate proliferation of both uninfected and infected cells into our two-strain model and obtain

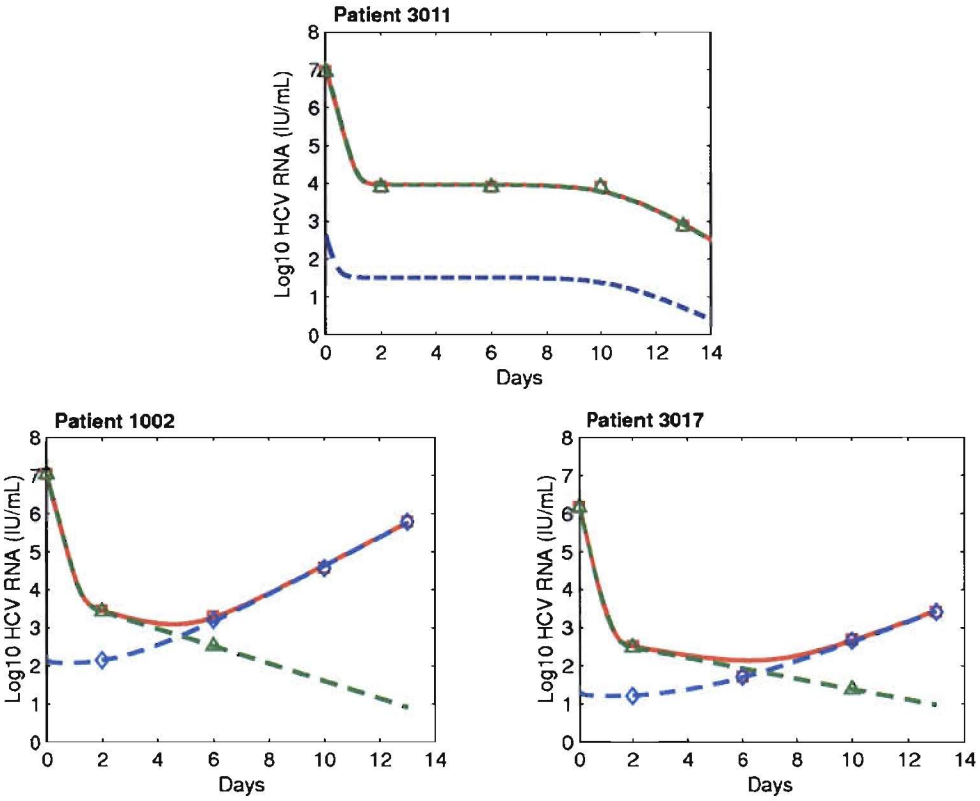
$$\begin{aligned} dT/dt &= s + \rho_T T(1-\frac{T+I_s+I_r}{T_{\max}}) - dT - \beta V_s T - \beta V_r T \\ dI_s/dt &= \rho_I I_s(1-\frac{T+I_s+I_r}{T_{\max}}) + \beta V_s T - \delta I_s \\ dI_r/dt &= \rho_I I_r(1-\frac{T+I_s+I_r}{T_{\max}}) + \beta V_r T - \delta I_r \\ dV_s/dt &= (1-\mu)(1-\varepsilon_s)p_s I_s - cV_s \\ dV_r/dt &= \mu(1-\varepsilon_s)p_s I_s + (1-\varepsilon_r)p_r I_r - cV_r \end{aligned} \quad \text{(S4)}$$

Here ρ_T and ρ_I are the maximum proliferation rates of uninfected and infected cells, respectively. The other parameters are the same as in model (1).

We fitted model (S4) to the viral load data of Patient 3011 (see below) using the same procedure as in Supplementary Table S2. It shows that the two-strain model with proliferation of both uninfected and infected cells can predict a triphasic viral decay after drug treatment. The root mean square (RMS) of the difference between data and fit is 0.19, which is smaller than the value (RMS=0.55) using model (S3). Using the F-test to compare the fitting results of model (S3) and model (S4) with additional parameter of infected cell proliferation, ρ_I, we found that there is a statistical trend (P=0.09) to support model (S4) when fitting the viral load data of Patient 3011.

We also employed model (S4) to fit the viral load data from patients on telaprevir monotherapy using the same procedure as in Supplementary Table S1. Two fits (Patients 1002 and 3017) are presented below. From these fits and parameter estimates, we obtained that the proliferation rate of infected cells is very small. This is in agreement with the observation that no

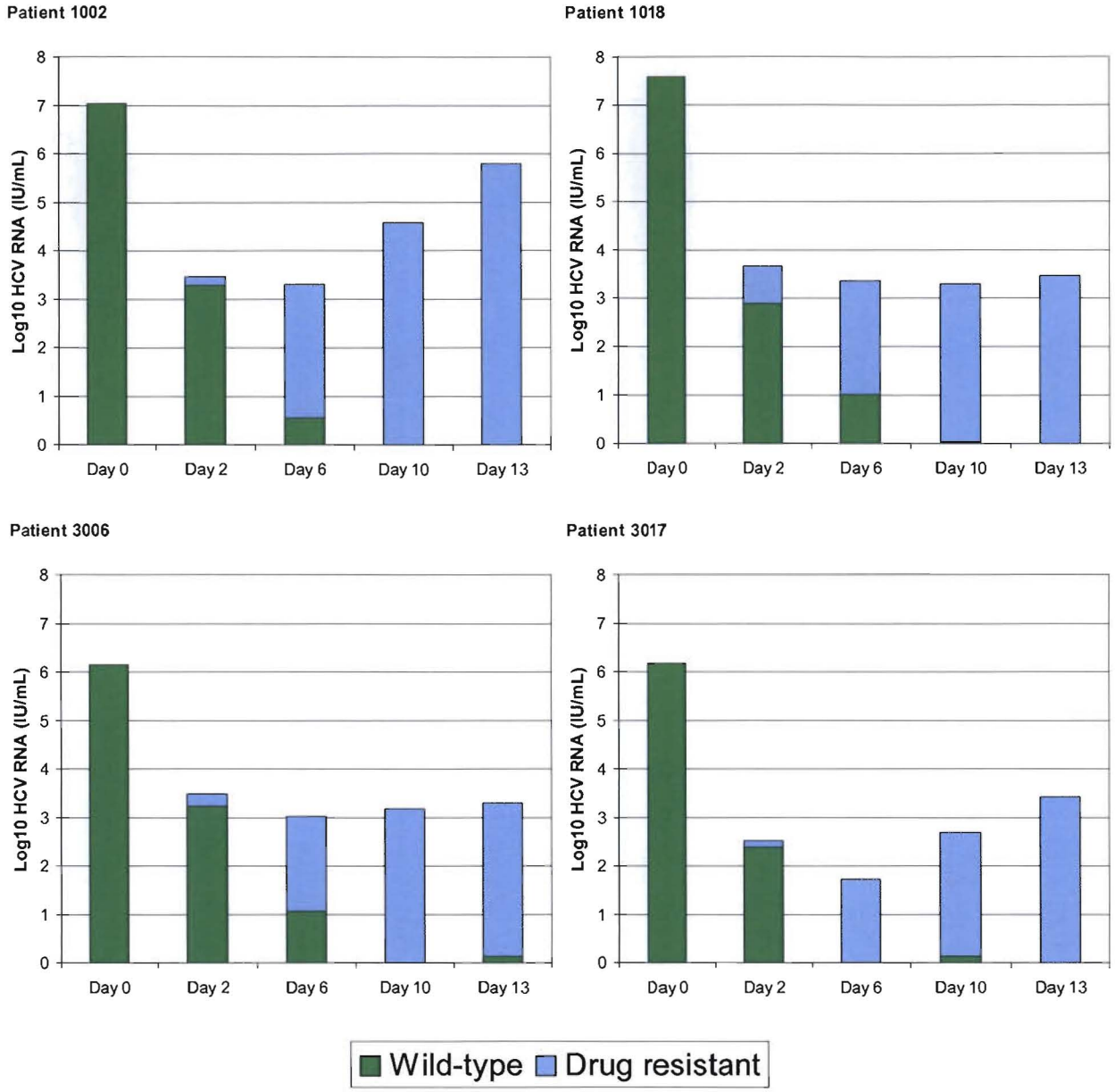
shoulder phase of either WT or drug resistant virus was seen except for Patient 3011. Therefore, to minimize the number of parameters, we employed model (1) in the main text to fit patient data, without considering proliferation of infected cells.



Comparison between predictions of model (S4) and patient data. Patient 3011 received combination therapy of PEG-IFN-α-2a and telaprevir for 14 days. The symbols used are the same as those in Fig. 2. The parameter values based on the fit are: $\beta = 7.94\times10^{-8}$ mL day^{-1} virion^{-1}, $\delta = 1.00$ day^{-1}, $\rho_T = 1.55$ day^{-1}, $\rho_I = 1.54$ day^{-1}, $\varepsilon_s = 0.99928$, $\varepsilon_r = 0.92$, $p_s = 17.82$ virions cell^{-1} day^{-1}. Note that in this case, ε_s and ε_r represent the effectiveness of combination therapy against WT and drug resistant virions, respectively. Patients 1002 and 3017 received telaprevir monotherapy during the 14-day study. The estimates of parameters for Patient 1002 are: $\beta = 3.93\times10^{-8}$ mL day^{-1} virion^{-1}, $\delta = 0.52$ day^{-1}, $\mu = 2.63\times10^{-6}$, $\rho_T = 0.85$ day^{-1}, $\varepsilon_s = 0.99943$,

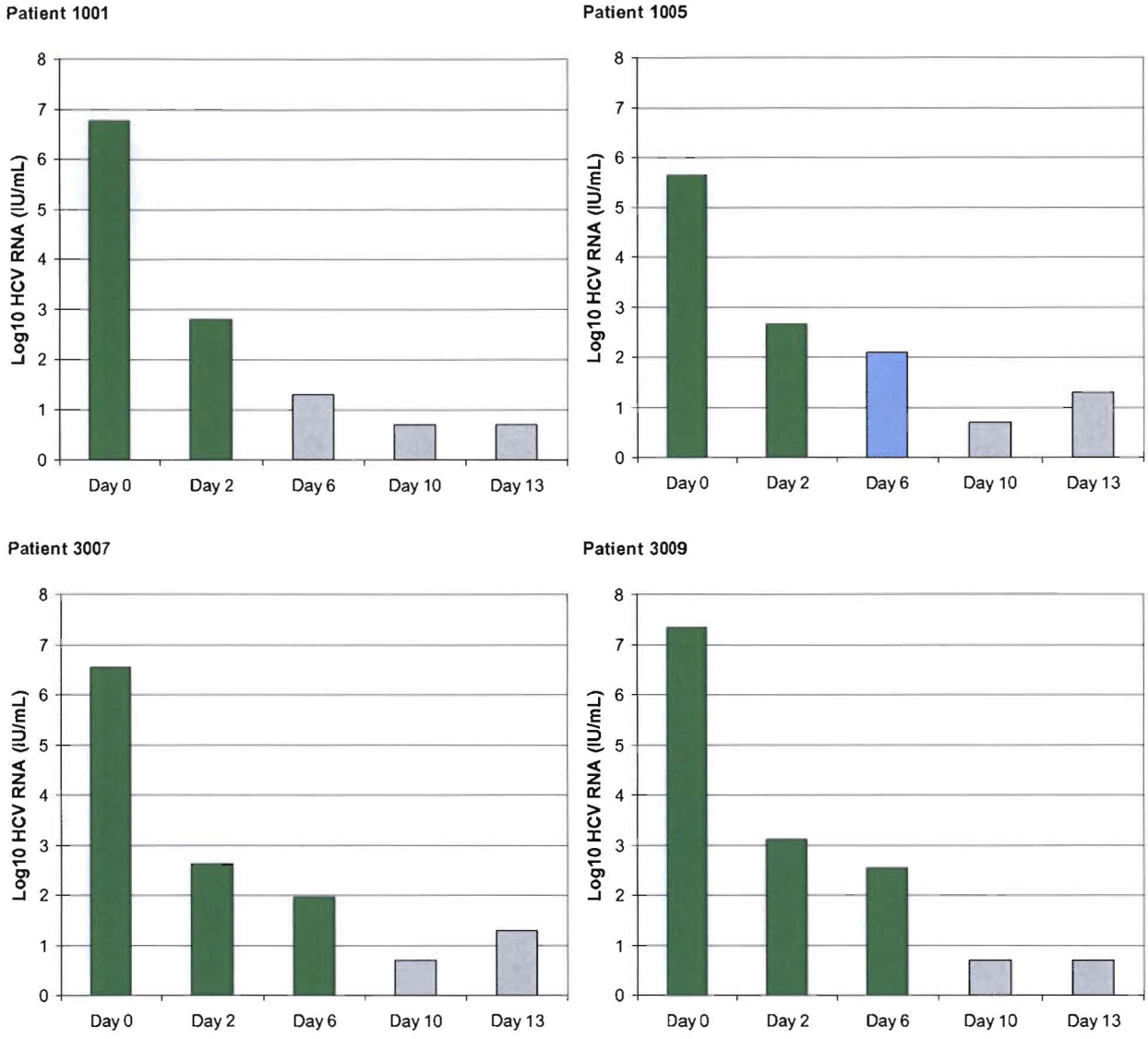
$\varepsilon_r = 0.002$, $p_s = 24.17$ virions cell^{-1} day^{-1}, $r = 0.81$. The RMS is 0.07, the same as that using model (1). The estimates of parameters for Patient 3017 are: $\beta = 1.88\times10^{-7}$ mL day^{-1} virion^{-1}, $\delta = 0.32$ day^{-1}, $\mu = 1.50\times10^{-6}$, $\rho_T = 0.57$ day^{-1}, $\varepsilon_s = 0.99965$, $\varepsilon_r = 0.004$, $p_s = 3.07$ virions cell^{-1} day^{-1}, $r = 0.87$. The RMS is 0.2, the same as that using model (1). Note that in the fits of Patients 1002 and 3017, the estimate of ρ_I is close to 0 ($\sim10^{-7}$ day^{-1}).

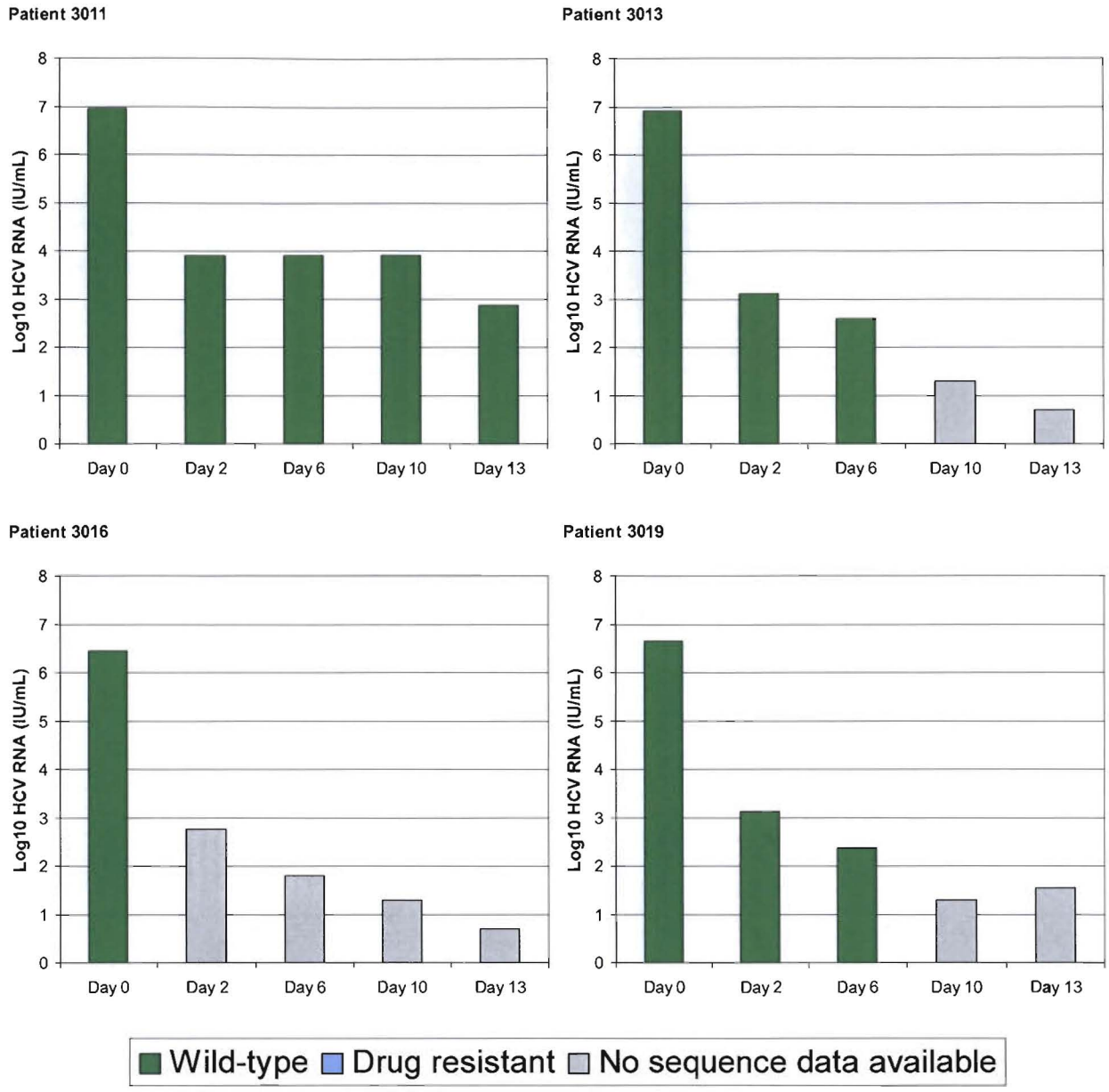
Supplementary Figure S1



Drug resistance profiles during telaprevir monotherapy. We plot the plasma HCV RNA levels and their composition (WT + drug resistant) in four patients who received only telaprevir and had viral breakthrough during the 14-day dosing period[4]. All HCV variants (single and double mutants) were lumped into one drug resistant strain. Note the x-axis here differs from that given in a related graph (Fig. 3) in (ref. 4). In that graph, day 2 denoted the initiation of telaprevir therapy[4, 7].

Supplementary Figure S2





Drug resistance profiles during combination therapy of telaprevir and PEG-IFN. We plot the plasma HCV RNA levels and their composition in eight patients who received both telaprevir and PEG-IFN-α-2a and had continued antiviral response during the 14-day treatment[4]. The limit of detection for the sequencing assay is 100 IU/mL and the limit of HCV RNA detection is 10 IU/mL. Note that in (refs. 4, 7) day 1 denoted the initiation of PEG-IFN therapy and day 2 denoted the initiation of telaprevir therapy.

Supplementary Table S1

Best-fit parameter values obtained from comparisons of model predictions (Eq. (1)) with experimental viral load data from four patients who were given telaprevir alone and had viral breakthrough during the 14-day treatment (Supplementary Fig. S1). Using Berkeley-Madonna, Version 8.3.9 (http://www.berkeleymadonna.com), V_s and V_r were fitted to the WT and drug resistant viral load data simultaneously. Due to lack of data on target cells, we fixed d, T_{max} and s. The half-life of uninfected hepatocytes was estimated to be a few hundred days in animal studies[36, 37]. Because of the uncertainty and possible changes in humans, we chose d to 0.01 day^{-1} (i.e., assume the half-life of uninfected hepatocytes is ~70 days) for all patients. Supposing that there are maximally 2×10^{11} hepatocytes in a normal human liver[38,39] and HCV can distribute throughout the 15 liters of extracellular fluid in a person with the average weight of 70 kg, we set $T_{max} = 2\times10^{11}/15000 = 1.3\times10^{7}$ cells/mL. Since changing s does not have a noticeable effect on our fits, we set $s = 0$. Because the viral clearance rate c is determined by the first-phase viral decline, we estimated c as done in Neumann et al.[16] using the median change of frequent plasma HCV RNA measurements during the first several days after telaprevir treatment[4]. We fixed the estimate $c = 6.28$ day^{-1} for all patients. Note that the estimate of μ is less than that in (ref. 17) (i.e., $10^{-5} - 10^{-4}$) because here it represents the average mutation rate of both single and double mutants.

Patient	ρ (day^{-1})	δ (day^{-1})	μ (10^{-6})	ε_s	ε_r	β (10^{-8} mL day^{-1} virion^{-1})	p_s (virions cell^{-1} day^{-1})	r
1002	0.63	0.52	1.11	0.99943	0.001	4.00	21.39	0.90
1018	2.05	0.41	4.48	0.99982	0.002	1.72	17.16	0.84
3006	0.50	0.51	8.65	0.99540	0.003	11.85	3.21	0.96
3017	0.46	0.32	2.54	0.99965	0.003	23.22	2.69	0.81
Average ± SD	0.91 ± 0.76	0.44 ± 0.09	4.20 ± 3.28	0.99860 ± 0.00210	0.002 ± 0.001	10.20 ± 9.71	11.11 ± 9.58	0.88 ± 0.07

Supplementary Table S2

Best-fit parameter values obtained from comparisons of model predictions (Eq. (S3)) with experimental viral load data from eight patients who were given telaprevir plus PEG-IFN-α-2a during the two-week therapy (Supplementary Fig. S2). Because of the limited data, we fixed, based on the previous fits, the following parameters for all patients: $d = 0.01$ day^{-1}, $T_{max} = 1.3 \times 10^7$ cells/mL, $s = 0$, $\mu = 4.2 \times 10^{-6}$, $r = 0.88$, $c = 6.28$ day^{-1}.

Patient	ρ (day^{-1})	δ (day^{-1})	ε^s_{total}	ε^r_{total}	β (10^{-8} mL day^{-1} virion^{-1})	p_s (virions cell^{-1} day^{-1})
1001	0.37	0.95	0.99950	0.67	4.54	38.50
1005	0.22	0.29	0.99832	0.78	5.14	3.21
3007	0.45	0.32	0.99982	0.77	10.14	8.43
3009	1.90	0.55	0.99980	0.95	3.10	13.14
3011	0.60	0.18	0.99810	0.92	2.84	5.61
3013	1.54	0.51	0.99950	0.95	4.92	6.85
3016	0.44	0.46	0.99962	0.70	9.67	6.29
3019	0.45	0.44	0.99938	0.70	7.07	10.37
Average ± SD	0.75 ± 0.62	0. 46 ± 0.23	0.99930 ± 0.00066	0.81 ± 0.12	5.93 ± 2.78	11.55 ± 11.30

Supplementary References

31. Rong, L., Feng, Z. & Perelson, A. S. Emergence of HIV-1 drug resistance during antiretroviral treatment. Bull Math Biol 69, 2027-60 (2007).
32. Bekkering, F. C., Neumann, A. U., Brouwer, J. T., Levi-Drummer, R. S. & Schalm, S. W. Changes in anti-viral effectiveness of interferon after dose reduction in chronic hepatitis C patients: a case control study. BMC Gastroenterol 1, 14 (2001).
33. Herrmann, E., Lee, J. H., Marinos, G., Modi, M. & Zeuzem, S. Effect of ribavirin on hepatitis C viral kinetics in patients treated with pegylated interferon. Hepatology 37, 1351-8 (2003).
34. Sentjens, R. E., Weegink, C. J., Beld, M. G., Cooreman, M. C. & Reesink, H. W. Viral kinetics of hepatitis C virus RNA in patients with chronic hepatitis C treated with 18 MU of interferon alpha daily. Eur J Gastroenterol Hepatol 14, 833-40 (2002).
35. Dahari, H., Lo, A., Ribeiro, R. M. & Perelson, A. S. Modeling hepatitis C virus dynamics: liver regeneration and critical drug efficacy. J Theor Biol 247, 371-81 (2007).
36. Macdonald, R. A. "Lifespan" of liver cells. Autoradio-graphic study using tritiated thymidine in normal, cirrhotic, and partially hepatectomized rats. Arch Intern Med 107, 335-43 (1961).
37. Macsween, R. N. M. et al. Pathology of the Liver (Churchill Livingstone, 1987).
38. Mackay, I. R. Hepatoimmunology: a perspective. Immunol Cell Biol 80, 36-44 (2002).
39. Sherlock, S. & Dooley, J. Diseases of the Liver in Humans and Biliary System (Blackwell, 2002).