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Estimating drug efficacy and viral dynamic parameters: HIV and HCV‡

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SUMMARY

Mathematical models have proven valuable in understanding the *in vivo* dynamics of human immunodeficiency virus type 1 (HIV-1), the virus that causes AIDS, and hepatitis C virus (HCV), the virus that causes hepatitis C infection. By comparing mathematical models with the data obtained from patients being treated with antiviral drugs, it has been possible to determine many quantitative features of these infections. The most dramatic finding has been that even though AIDS and hepatitis C are diseases that occur on a timescale of one or more decades, there are very rapid dynamical processes that occur on timescales of hours to days, as well as slower processes that occur on timescales of weeks to months. We show how dynamical modeling and parameter estimation techniques have uncovered these important features of HIV and HCV infection and subsequently impacted the way in which patients are treated with potent antiviral drugs. Published in 2007 by John Wiley & Sons, Ltd.

KEY WORDS: HIV; hepatitis C; HCV; viral dynamics; parameter estimation

INTRODUCTION

The use of mathematical modeling and statistical analysis of experimental data has helped revolutionize the field of human immunodeficiency virus (HIV) research. Human immunodeficiency virus type 1 (HIV-1) infects CD4+ T cells and causes their depletion. A healthy individual has a CD4+ T cell count of about 1000 cells/μL. In HIV-infected individuals, the T cell count falls and when it reaches 200 cells/μL a person is classified as having AIDS. The decline in T cells is slow, taking on average about 10 years in adults. Because of the central role of CD4+ T cells in immune regulation, their depletion has widespread deleterious effects on the functioning of the immune system and leads to the immunodeficiency that characterizes AIDS.

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The dynamics of HIV-1, measured in plasma, has also been intensively studied. After infection, the viral levels increase reaching a peak after a few weeks. The viral levels then fall and establish a set-point level that remains approximately constant for years. Because viral levels remain relatively unchanged for years, it was supposed by some that the viral infection was slow. This turned out to be incorrect. As we show below, modeling combined with statistical analysis of viral load data obtained from HIV-infected patients initiating antiretroviral (ARV) therapy revealed that HIV is a dynamic disease encompassing a number of different timescales, running from hours to days to weeks to months [1–6]. We will review how perturbation experiments, combined with mathematical modeling, led to the uncovering of these different timescales and to the recognition that they correspond to important biological processes underlying HIV infection. Further, the analysis of such *in vivo* perturbation experiments has helped elucidate the nature of various reservoirs for the virus.

Owing to the success in understanding HIV infection through the use of modeling, a similar approach was applied to understanding the dynamics of hepatitis C virus (HCV) infection [7–9]. While HCV does not cause immunodeficiency, it still causes a chronic infection that over many decades can lead to cirrhosis and liver failure. As in the case of HIV, when viral levels are measured in the blood they are largely unchanging over timescales of days, weeks, and months. Thus, again a set-point seems to be reached and it is through perturbation of this set-point *via* drug therapy that insights have been gained into the dynamics of HCV infection. Below we will present a simple model of viral infection and show how it has been applied to both HIV and HCV infections. It has only been through the estimation of parameters in these models that important quantitative insights have been gained into the underlying biological processes.

A MODEL OF VIRAL INFECTION

Cells that are susceptible to viral infection are called target cells, T. Upon interaction with virus these cells can become productively infected cells, I, which produce virus V.

Using a mass-action term to model viral infection, the following system of ordinary differential equations has been used to describe the infection process:

$$\begin{aligned} \frac{\mathrm{d}T}{\mathrm{d}t} &= \lambda - \mathrm{d}T - \beta V T \\ \frac{\mathrm{d}I}{\mathrm{d}t} &= \beta V T - \delta I \\ \frac{\mathrm{d}V}{\mathrm{d}t} &= pI - cV \end{aligned} \tag{1}$$

The model assumes that target cells are produced at a constant rate λ, although more general forms of production, such as proliferation, can be considered [10–12], without much change in the overall behavior of the model. These cells die at rate d per cell and are infected according to a mass-action law with infection rate constant β. Infected cells are lost at a rate δ per cell. Viral particles (virions) are produced at rate p per infected cell and cleared at rate c per virion.

The average rate of viral production p can also be expressed as $p = N\delta$, where the burst size N is the total number of viral particles produced by an infected cell over its entire lifetime.

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Note that the average life span of an infected cell is $1/\delta$, and thus $N\delta$ is the average number of virions produced per unit time by an infected cell.

TREATMENT OF HIV

Two classes of ARV drugs are often used to treat HIV infection. One class is reverse transcriptase inhibitors (RTIs), which can effectively block the infection of target T cells by free virus and the other is protease inhibitors (PIs), which prevent HIV-1 protease from cleaving the HIV polyprotein into functional units, causing infected cells to produce immature virus particles that are non-infectious. In the presence of these two inhibitors, the model equations (1) are modified to become

$$\begin{aligned}\frac{\mathrm{d}T}{\mathrm{d}t} &= \lambda - \mathrm{d}T - (1-\varepsilon_{\mathrm{RT}})\beta V_{\mathrm{I}} T \\ \frac{\mathrm{d}I}{\mathrm{d}t} &= (1-\varepsilon_{\mathrm{RT}})\beta V T - \delta I \\ \frac{\mathrm{d}V_{\mathrm{I}}}{\mathrm{d}t} &= (1-\varepsilon_{\mathrm{PI}})pI - cV_{\mathrm{I}} \\ \frac{\mathrm{d}V_{\mathrm{NI}}}{\mathrm{d}t} &= \varepsilon_{\mathrm{PI}} pI - cV_{\mathrm{NI}}\end{aligned} \tag{2}$$

where $\varepsilon_{\mathrm{RT}}$ and $\varepsilon_{\mathrm{PI}}$ ($0 \leqslant \varepsilon_{\mathrm{RT}}, \varepsilon_{\mathrm{PI}} \leqslant 1$) are the efficacies of RTIs and PIs, respectively. V_{I} and V_{NI} are the concentrations of infectious and non-infectious virus particles, respectively, and $V = V_{\mathrm{I}} + V_{\mathrm{NI}}$ is the total amount of virus, which is measurable in plasma.

In a clinical trial of one of the PIs, ritonavir, David Ho, Aaron Diamond AIDS Research Center, Rockefeller University, NY, and colleagues sampled blood very frequently for seven days after administering the drug [3]. The patients in the trial were chronically infected with HIV-1 and had relatively constant HIV-1 levels. If we assume that a 100 per cent effective PI ($\varepsilon_{\mathrm{PI}} = 1$) is given to an infected individual, that the patient has an initial viral load V_0 that is at quasi-steady state ($pI(0) = cV(0)$), and that the uninfected target cells remain approximately at a constant level T_0 over the time period of interest, then the viral load at time t can be solved from model (2) with the initial conditions $I(0) = \beta V_0 T_0/\delta$, $V_{\mathrm{I}}(0) = V_0$, and $V_{\mathrm{NI}}(0) = 0$, yielding [3]

$$V(t) = V_0 \mathrm{e}^{-ct} + \frac{cV_0}{c-\delta}\left[\frac{c}{c-\delta}(\mathrm{e}^{-\delta t} - \mathrm{e}^{-ct}) - \delta t \mathrm{e}^{-ct}\right] \tag{3}$$

Using nonlinear regression analysis, estimates of the parameters c and δ were obtained by fitting equation (3) to experimental data [3]. (Because of pharmacokinetic and pharmacodynamic considerations drug given at time 0 takes some length of time to distribute throughout the body and enter infected cells. Thus a small delay, varying between 2 and 6 h, was introduced into equation (3) in the data fitting procedure [3].) An example of fits to data from three patients is shown in Figure 1(a). These estimates gave upper bounds for the half-life of the plasma virus ($t_{1/2} = \ln 2/c$) of about 6 h and for the half-life of productively infected cells ($t_{1/2} = \ln 2/\delta$) of about 1.6 days. These estimates were only upper bounds because therapy in reality is not 100 per cent effective and additional viral clearance and/or loss of productively infected cells is required to account for the decay of the residual virus being produced. In addition, some error is introduced by assuming that the number of target cells remained constant during therapy.

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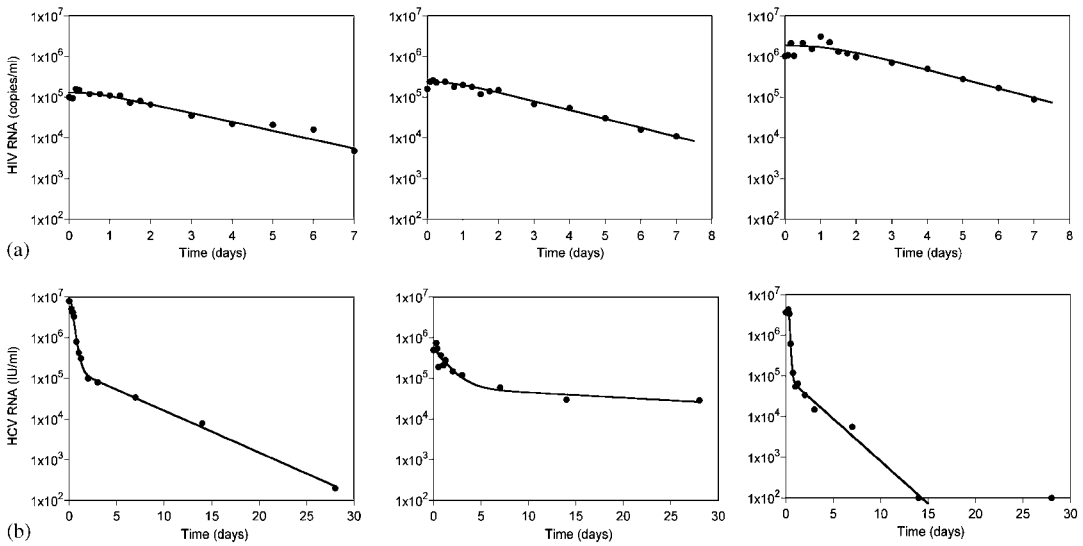

Figure 1. Fits of HIV and HCV dynamic models, equations (3) and (14), respectively, to patient data. (a) Three HIV-infected patients treated with the protease inhibitor ritonavir. Data taken from [3]. (b) Three HCV-infected patients treated with 10 MIU IFN given daily. Data taken from [13]. Note that the patients in (a) and (b) are different.

To address the issue of the accuracy of the parameter estimates, a number of approaches were taken. First, the Ho lab did an additional experiment in which they estimated the rate of loss of infectious virus V_I. According to the simple model (2) when the efficacy of the PI is 1, V_I should decay exponentially at rate c. This was checked for one patient and the decay rate of V_I was found to correspond to a half-life of approximately 6 h and this value was within the 95 per cent confidence interval estimated for c by nonlinear regression of the viral load data for this particular patient [3]. Second, to examine the errors introduced by linearizing the problem, i.e. assuming T_0 was constant or by assuming that the drug efficacy was 100 per cent, surrogate data were generated by numerically solving the nonlinear differential equations (2) and then fitting this surrogate data to equation (3). The drug's efficacy and the parameters c and δ were known for the surrogate data and using the linear approximation with 100 per cent efficacy led to an underestimation of the parameters as was expected. For example, when data were generated with $c=3\,\text{day}^{-1}$, $\delta=0.5\,\text{day}^{-1}$, and drug efficacies of 1.0, 0.99, 0.95, and 0.9, respectively, and then fit using model (3) that assumed a drug efficacy of 1.0, the estimates obtained were $c=3.000, 3.003, 3.015$, and $3.028\,\text{day}^{-1}$, respectively, and $\delta=0.500, 0.494, 0.470$, and $0.441\,\text{day}^{-1}$, respectively. Thus, c remained essentially unchanged and δ was underestimated. This implied that cell life spans are expected to be shorter than 1.6 days. (In fact, more recent experiments in which three or more potent drugs were given, and hence where efficacies are closer to 100 per cent, have shown that the infected cell life span may be approximately 1 day [14].)

To examine the effect of T varying, the empirical data on the increases of T in each patient over the 7 days of treatment can be used. To avoid having to estimate parameters in the T-equation, the measured changes were fit to either a linear increase in T or an exponential increase of T with time. Thus, in the equation for I, T was replaced say by $T(t)=T_0+bt$, where b was

estimated from the data on CD4+ T cell increases during therapy. Because of limited data, there was no significantly better fit with either the linear or exponential model [1]. The largest observed increase in T cell count was five-fold. Using this increase, $\varepsilon=1$, $c=3.0\,\text{day}^{-1}$, and $\delta=0.5\,\text{day}^{-1}$ to generate surrogate data and then fitting with equation (3) yielded estimates of $c=2.75\,\text{day}^{-1}$ and $\delta=0.499\,\text{day}^{-1}$. Thus, again only small changes in parameter estimates occurred, giving us confidence that our estimates of c and δ were reasonable.

Before this analysis was done nothing was known about the *in vivo* rate of clearance of HIV or the life span of productively infected cells. Thus, these estimates, although possibly crude, had startling implications. First, at the quasi-steady state in viral load before therapy was initiated, the rate of viral production had to equal the rate of virion clearance. The rate of clearance is cV_0. Since V_0 was measured in units of virus (or more precisely HIV RNA copies) per mL, multiplying by the total amount of extracellular fluid that the virus could be suspended in gave an estimate of the total rate of virion clearance. Doing this for each patient, with the amount of extracellular fluid estimated based on the patient's weight, allowed us to estimate that on average about 10^{10} virions are produced each day in a typical chronically infected patient [3]. Virions that are not released into the extracellular fluid are not included in this calculation and the fact that c is probably underestimated both imply that this production rate is a lower bound.

In order to better estimate c, a more direct experimental measurement was made using plasma apheresis. In this technique plasma and suspended virions are removed from the blood by a machine and fluids without suspended virions are returned to the patient. Thus, the procedure artificially increases the rate of virion clearance. The clearance rate by apheresis is known, as one can directly measure how many virions are removed per unit time. If this rate is fast compared with the rate of natural clearance c, then the plasma virus concentration should drop. Analogously, if c is large compared with the removal rate by apheresis, little effect should be seen. More precisely, if one assumes that virus is produced at rate P and cleared at rate cV, then before apheresis is started one can assume

$$\frac{\mathrm{d}V}{\mathrm{d}t}=P-cV \tag{4}$$

Once apheresis is begun,

$$\frac{\mathrm{d}V}{\mathrm{d}t}=P-cV-aV \tag{5}$$

where a is the known rate of plasma removal in units of volume per unit time and V is the concentration of suspended virions. If we assume that plasma viral load was in quasi-steady state at level V_0 at the start of apheresis and viral production remained unchanged during the apheresis process that lasted ~ 2 h, then $P=cV_0$ and equation (5) becomes

$$\frac{\mathrm{d}V}{\mathrm{d}t}=cV_0-(c+a)V \tag{6}$$

with solution

$$V(t)=V_0\left[\frac{c-a\mathrm{e}^{-(c+a)t}}{c+a}\right] \tag{7}$$

Fitting equation (7) to patient data with V_0 and a known allowed one to estimate c [15]. Only four patients were studied this way, and two were coinfected with HCV, but nonetheless the

measurements showed that c was much larger than we had previously estimated. The mean value of c for the four patients was $c=23\,\mathrm{day}^{-1}$. This implies that viral production and clearance rates may be as much as 10-fold higher than we previously estimated *via* drug perturbation experiments, i.e. of the order of 10^{11} viral particles produced and cleared each day.

The reason that the rate of viral production is so important is that HIV mutates as it replicates. Each replication cycle involves the virus entering a cell, reverse transcribing its RNA genome into a DNA copy *via* the action of the enzyme reverse transcriptase, and then reading out the DNA copy to produce new virions. The process of reverse transcribing a viral genome is very error prone and the error rate has been estimated as 3×10^{-5} per base copied. The HIV genome contains close to 10^4 bases; thus on average each DNA copy of the viral genome is expected to have 0.3 errors, and according to the binomial distribution the probability of exactly one, two, or three errors is 0.22, 0.033, and 0.0033, respectively, [16]. If 10^{11} virus particles are to be produced each day, and if each virally infected cell lives about 1 day while producing virus, then to maintain a steady-state level of production requires about 10^8 new infections to occur each day, assuming that each cell produces 1000 virus particles over its life span [17]. As each infection requires a reverse transcription event, it is clear that essentially every possible single point mutation will be made each day. In fact, with a genome of size 10^4 there are only 3×10^4 single mutation variants, i.e. each base can be changed to one of the three others since there are only four possible bases: A, C, T, and G. Thus, in 10^8 reverse transcriptions one expects 2.2×10^7 single errors to be made. With only 3×10^4 possibilities, each should occur about 1000 times per day. Doing the same calculation for double and triple mutants one finds that about 0.7 per cent of all possible double mutants and 7.4×10^{-8} of all possible triple mutants are created each day [16]. Thus, if one designs an antiviral drug that requires one or two point mutations in the virus to become resistant to the drug, such resistance will surely be observed in any sizeable population of treated patients.

Uncovering that HIV replicates extremely rapidly thus leads to the realization that the only way in which drug therapy could work for this infection would be if the development of drug resistance needed at least three mutations, which can be achieved in practice *via* a combination of at least three drugs. This strategy of triple combination therapy has worked and many HIV-infected individuals are now living with this virus. Thus, simple models when used to interpret quantitative experiments can yield important and practical results.

While models, such as the one given by equation (2), have proven valuable, they clearly are only approximations to reality. For example, the model totally ignores immune system effects. Antibody can enhance virion clearance or neutralize virus and thus reduce its infectivity. A cell-mediated immune response should help eliminate infected cells. Thus, the parameters, c, β, and δ, could in principle all be modulated by the immune response. If this were the case, then these constants should be replaced by functions of the immune response intensity. While some models have incorporated such effects, it is surprising that the model given by equation (2) and simple extensions [4] also with constant parameters have done a good job in summarizing both clinical treatment data [18, 19] as well as data on the kinetics of primary HIV infection [20].

Much additional work has been done in modeling HIV and in estimating parameters. Holte *et al.* [21] asked whether the loss of infected cells described by the term $-\delta I$ would be better described by the term $-\delta I^{\alpha}$, with $\alpha>1$ The logic behind this was that infected cells would stimulate a cell-mediated immune response that could enhance their clearance. If the intensity of the immune response were proportional to I, then using $\alpha>1$ might yield a better description of the kinetics. Testing this hypothesis on one set of clinical trial data, they estimated α to be 1.4 and showed that this was significantly different than $\alpha=1$. As estimates of the various viral kinetic parameters

accumulated from different modeling and data fitting studies, Bayesian approaches began to be used with parameter estimate ranges taken from the literature being used as prior's [22, 23]. Also, issues such as drug pharmacokinetics, drug resistance, and the issue of compliance with the drug regime were addressed [23].

MODELS OF HCV INFECTION

HCV infects about 170 million people worldwide and in the U.S. it is estimated that 3–4 million people are infected. HCV infection causes liver damage, e.g. cirrhosis, may lead to the development of liver cancer, and is the leading cause of liver transplantation in the U.S. As in the case of HIV, individuals chronically infected with HCV tend to establish a set-point level of viremia. Before the idea of modeling the dynamics of this infection was introduced, patients were sampled infrequently. However, the insights obtained into HIV by frequent sampling after the introduction of an antiviral drug led Thomas Layden's group at the University of Illinois Medical School in Chicago to repeat this type of experiment in HCV-infected patients [8, 9]. After injection of interferon-α (IFN), the standard treatment for HCV at the time HCV RNA was observed to decay in a biphasic manner, with decays of as much as 1.5 logs by 24 h after the initiation of IFN treatment, followed by a slower second-phase decline (Figure 1(b)). Interestingly, both phases of decline were approximately exponential and the magnitude of the first-phase decline depended on the IFN dose. To explain this rapid decay, Neumann *et al.* [9] hypothesized that IFN must interfere with the ability of infected cells to produce or release virus. This hypothesis has now been tested and validated in various *in vitro* systems. Modifying the basic viral dynamic model (2) to incorporate a partial block in virion production yields

$$\frac{\mathrm{d}V}{\mathrm{d}t} = (1-\varepsilon)pI - cV \tag{8}$$

where ε is the effectiveness of therapy in blocking virion production. If over the time of the first-phase decline one assumes that the number of infected cell I is approximately constant and equal to its pre-treatment value and that the viral level was at its set-point value, then $pI = cV_0$ where V_0 is the pre-treatment viral load. Using this relationship in equation (8) yields

$$\frac{\mathrm{d}V}{\mathrm{d}t} = (1-\varepsilon)cV_0 - cV, \quad V(0) = V_0 \tag{9}$$

with solution

$$V(t) = V_0[1-\varepsilon+\varepsilon \mathrm{e}^{-ct}] \tag{10}$$

Fitting this equation *via* nonlinear least-squares regression to experimentally obtained HCV RNA data allows one to estimate c and ε. Using this approach, Neumann *et al.* [9] showed that $c \sim 6\,\mathrm{day}^{-1}$, in agreement with the estimates made by apheresis of HIV/HCV coinfected patients [15] and that ε increased with the IFN dose from approximately 0.8 at 5 MIU to 0.95 at 10 MIU daily. Why HCV is cleared at a different rate than HIV is not known, but the apheresis experiments clearly showed that in the same patients HCV is cleared slower than HIV [15]. Further, as in the case of HIV, knowing how rapidly the virus is cleared allows one to estimate the rate of viral production needed to maintain a set-point viral load. For HCV this calculation shows that about 10^{11}–10^{12} virions are made per day [9]. HCV mutates when it replicates about as fast as HIV; thus, HCV

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forms a diverse quasi-species that presents challenges for treatments under development using small molecule inhibitors of HCV protease and polymerase.

Besides revealing the potential for drug resistance to new agents, this modeling showed that the effectiveness of a given dose of IFN or other inhibitors [24] of virion production could be evaluated *in vivo* using data collected over 1–2 days. Clearly, long-term outcome depends on more than just drug efficacy. Factors, such as compliance, immune response, and the possibility of drug resistance also play roles. However, without an effective drug, therapy is likely to fail. Modeling of the sort demonstrated here allows pharmaceutical companies to rapidly and inexpensively test candidate drugs.

Over longer periods of time the assumption of a constant number of infected cells is probably invalid and the following model has thus been used to fit data:

$$\begin{aligned} \frac{\mathrm{d}I}{\mathrm{d}t} &= \beta V T_0 - \delta I \\ \frac{\mathrm{d}V}{\mathrm{d}t} &= (1-\varepsilon) pI - cV \end{aligned} \tag{11}$$

where T is assumed constant and equal to T_0, its pre-treatment quasi-steady value. Note, from equation (11) with $\varepsilon = 0$, corresponding to pre-treatment, that at steady state

$$T_0 = \frac{\delta c}{\beta p} \tag{12}$$

In order to solve equation (11), let $y = pI$. Then, making this change of variable and using equation (12), one obtains

$$\begin{aligned} \frac{\mathrm{d}y}{\mathrm{d}t} &= \delta(cV - y) \\ \frac{\mathrm{d}V}{\mathrm{d}t} &= (1-\varepsilon) y - cV \end{aligned} \tag{13}$$

Hence, as a consequence of the quasi-steady-state assumption, the only parameters that enter are c, δ, and ε. With the quasi-steady-state initial conditions $y(0) = pI(0) = cV(0)$ and $V(0) = V_0$, one can show that

$$V(t) = V_0(A\mathrm{e}^{-\lambda_1 t} + (1-A)\mathrm{e}^{-\lambda_2 t}) \tag{14}$$

and

$$I(t) = \frac{cV_0}{p}(B\mathrm{e}^{-\lambda_1 t} + C\mathrm{e}^{-\lambda_2 t}) \tag{15}$$

where

$$\begin{aligned} \lambda_{1,2} &= \tfrac{1}{2}(c+\delta \pm \sqrt{(c-\delta)^2 + 4(1-\varepsilon)c\delta}) \\ A &= \frac{\varepsilon c - \lambda_2}{\lambda_1 - \lambda_2}, \quad B = -\frac{\lambda_2}{\lambda_1 - \lambda_2}, \quad C = \frac{\lambda_1}{\lambda_1 - \lambda_2} \end{aligned} \tag{16}$$

Equation (14) predicts that after therapy is initiated HCV RNA should decrease monotonically following a double exponential with rates given by the eigenvalues λ_1 and λ_2. A biphasic or double-exponential decline has been observed in almost all patients who respond to therapy [9, 13, 25].

Fitting equation (14), where t was replaced by $t-\tau$, τ being a pharmacologic delay, to HCV RNA decline data obtained from HCV-infected patients treated with IFN allowed Neumann and colleagues to estimate c, ε, δ, and τ [9, 13, 25]. An example of such fits to data from three patients is shown in Figure 1(b). Measurements of $I(t)$ have not been made since this would involve performing sequential liver biopsies.

More recent modeling has relaxed the assumption of T being constant [26–28], but now additional uncertainty enters as there are more parameters to estimate. Also, the appropriate equation for describing target cell kinetics is still unknown. The targets of HCV infection are mostly hepatocytes and these cells can proliferate, but their proliferation rate has not been directly measured. Further, HCV is a non-cytolytic infection, and thus infected cells may also be able to proliferate and yield progeny that are infected. Whether this is in fact the case is still unknown, making modeling of infected cell proliferation somewhat speculative.

The initial model of HCV infection [9] was based on therapy involving IFN given daily. A more recent formulation of IFN, called pegylated-IFN (peg-IFN), is eliminated from the body slowly allowing once a week dosing. With this larger dosing interval, drug levels vary during the week, and presumably the drug efficacy ε also varies with time. Powers *et al.* [29] and Talal *et al.* [30] thus modeled HCV treatment with peg-IFN by assuming

$$\varepsilon(t)=\frac{C(t)^n}{\mathrm{EC}_{50}^n+C(t)^n} \tag{17}$$

where $C(t)$ is the peg-IFN concentration, EC_{50} is the effective concentration 50, i.e. the concentration that yields $\varepsilon=0.5$, and n is a constant integer called the Hill coefficient. The concentration of peg-IFN was measured frequently and fit to a standard one-compartment pharmacokinetic model so as to provide an estimate of $C(t)$ [30]. Then the numerical solution of equation (11) in which ε was replaced by $\varepsilon(t)$ given by equation (17) was fit to the HCV RNA data [30]. Although the HCV RNA level fell and then rebounded in some patients, this rebound was shown to occur when the drug concentration was low relative to the EC_{50}, and the non-monotonic behavior could be fit by this model with time-varying drug effectiveness, and the parameters c, δ, n, and EC_{50} estimated [30]. In general, models with time-varying drug effects are more accurate than models assuming constant efficacy, and such models have also been used to fit HIV treatment data [23, 31]. However, to use such models requires more data, such as drug concentrations, that are not always available.

CONCLUSIONS

We have provided examples of viral dynamics models and parameter estimation for two viral infections HIV and HCV. Similar techniques have also been used to characterize the *in vivo* dynamics of hepatitis B virus [32–35], cytomegalovirus [36, 37], and influenza [38]. A new field called viral dynamics has been emerging with the goal of modeling viral infections and treatment within single hosts. Results obtained on both HIV and HCV have influenced treatment. In the case of HIV, the recognition that the virus replicates and hence mutates extremely rapidly led to the introduction of triple combination therapy. In the case of HCV, modeling and parameter estimation techniques have helped usher in a new perspective of using viral kinetics to access drug efficacy and to determine the length of time a patient needs to be treated [39–41]. As more recognition of this field is obtained, we look forward to seeing a more quantitative perspective brought to the analysis and treatment of infectious diseases.

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