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# Dynamics and Evolution of HIV-1 During Structured Treatment Interruptions

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**Abstract**

**Our understanding of the way in which HIV responds to highly active antiretroviral therapy (HAART) has benefited greatly from the use of mathematical models of viral dynamics and evolution. In this paper, I review the role that these models may play in the design and analysis of studies of structured treatment interruptions (STIs). STIs are being investigated in several different contexts: to reduce drug toxicities; to boost HIV-specific immune responses; and to allow reversion of drug resistance mutations in highly drug-experienced patients. I illustrate how models can help to compare the dynamics and evolution of HIV in these different scenarios, and to assess the risks and benefits of STIs.**

**Key words**

**Structured therapy interruption (STI). Dynamics. Evolution. Mathematical models. HAART. HIV.**

## Introduction

Highly active antiretroviral therapy (HAART) of HIV infection can result in long-term suppression of viral loads to undetectable levels in the plasma, resulting in lower disease progression rates and lower infectivity. Mathematical models have added greatly to our understanding of viral dynamics under HAART[1]. These models can be used simply to describe the rate of decay of virus at different times following initiation of treatment, which is useful when comparing the efficacy of different drug regimens. They may also add to our understanding of the processes underlying viral dynamics if the model parameters have a biological interpretation. For example, the decay rate of plasma virus on HAART is not constant, but declines over time[2]. Different mathematical models have provided different explanations for this phenomenon, including the presence of long-lived infected cells[2], declining immune responses[3], and declining immune activation of uninfected cells, which result in reduced availability of target cells[4]. Due to the high replication and mutation rate of HIV, drug resistance may emerge during HAART and can result in viral rebound. Mathematical models have also been developed to help us understand this phenomenon. For example, Ribeiro and Bonhoeffer[5] concluded that evolution of drug resistance during therapy was predominantly due to mutations that exist prior to therapy rather than *de novo* mutations that appear during therapy. The timing of emergence of these mutations during therapy is affected by the population dynamics of the virus on therapy[6], the relative fitness of the mutations, both in the presence and the absence of therapy[7], and by stochastic effects due to the low frequency of these mutations prior to therapy[5,8].

However, HAART regimens are often complicated and incur serious side effects. While cessation of

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HAART may relieve these side effects, the virus rapidly rebounds, reflecting the long-term persistence of the virus and the inability of immune responses to contain viral replication. Structured treatment interruptions (STIs), where antiviral therapy of HIV-1 infected patients is discontinued multiple times, have been proposed as a treatment strategy that can overcome some of the problems associated with HAART. Short (a week or less) interruptions of therapy have been studied to reduce exposure to antiviral agents and hence reduce drug toxicities, while limiting the amount of viral replication[9]. Medium length interruptions (weeks to a month) have been studied to boost HIV-specific immune responses in patients with suppressed viral loads by allowing a limited exposure to autologous viral antigens. These studies have been performed in both acutely-infected[10,11] and chronically-infected[12-15] patients. Longer interruptions (several months) have been used to allow resurgence of wild-type virus in individuals with highly drug-resistant virus[16], which may improve the response to salvage therapy, at least in the short term[17].

There is also a great deal of interest in how HIV-1 evolves during therapy interruptions. A major concern is that allowing the virus to replicate increases the risk that drug resistance will evolve, either by the production of new drug-resistant mutations or by the re-emergence of mutations that pre-exist prior to therapy. Pre-existing resistance mutations may be present in latently infected cells after being produced during previous suboptimal drug regimens, or may be generated during therapy due to the high mutation rate of HIV. Evolution plays a central role in the use of STIs to allow reversion to wild-type virus in patients who are failing therapy due to the existence of drug resistance. As HIV has a high rate of evolution, genetic differences can be generated both over time and between tissues. This phenomenon can help us understand the reservoirs of virus that persist during suppressive therapy, and reseed the periphery during therapy interruption; genetic differences between virus rebounding in the plasma following interruption and virus present in a reservoir argue against that reservoir being responsible for reseeding the periphery.

There have been numerous reviews and commentaries of therapy interruptions that have focused primarily on the clinical aspects of STIs[18-22]. I take a different approach, where I consider how mathematical models may add to our understanding of factors underlying the dynamics of viral rebound during therapy interruptions, and how viral dynamics may affect the way in which HIV evolves during STIs. These models provide a structure within which we can compare and contrast different STI studies applied in different contexts.

## Stages in the dynamics of viral rebound

For ease of explanation, I will break down the dynamics of viral rebound during a single therapy interruption into three stages, illustrated schematically in figure 1:

1. The delay stage. In individuals with viral loads suppressed below detectable levels, there is a delay between therapy interruption and viral loads reaching detectable levels.
2. The growth stage. Following the viral load reaching detectable levels, there is a period of exponential growth.

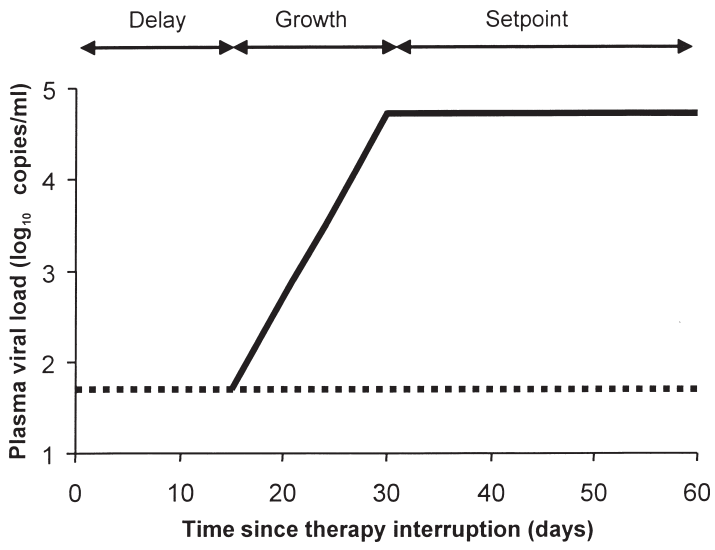


**Figure 1.** *Viral dynamics following therapy interruption in an individual whose viral loads are suppressed under HAART.*

3. The setpoint stage. Following exponential growth, the viral load reaches an equilibrium, reflecting a balance between viral production and clearance.

## Dynamics and evolution during the delay stage

In individuals with suppressed viral loads on HAART, there is a delay between interruption of therapy and the virus reaching detectable levels. Figure 2 shows the timing of this delay using a Kaplan-Meier plot, based on data from 11 chronically-infected individuals who were suppressed under HAART, and who underwent multiple therapy interruptions as part of the Autovac trial[13,24,25]. The frequent sampling of viral loads in this study allows the time to viral rebound to be estimated accurately. Using parametric survival analysis, the mean delay is 14 days, with a great deal of variation about the mean (2.5 and 97.5 percentiles are 6 and 31 days respectively). There was a highly significant difference between patients in their time to viral rebound ($p < 10^{-7}$). More robust HIV-specific immune responses may contribute to longer delays before viral rebound, and hence therapeutic vaccination during HAART may increase the time to viral rebound following therapy interruption. Given that most studies of STIs in chronically infected individuals have demonstrated a decrease in viral growth rates over successive STIs[12,13,26], we might expect that the delay would also increase over successive STIs. However, there was no significant difference between the times to viral rebound across the four STIs ($p = 0.47$). It has been hypothesized[25] that reseeding of viral reservoirs could lead to a decrease in the delay between therapy interruption and viral rebound over successive STI cycles, which may compensate for the increase in the delay due to lower viral growth rates. Testing this hypothesis is hampered by our lack of knowledge about the reservoir from which rebounding virus emerges.

Even after accounting for the number of STI cycles and patient-specific differences, there is a great deal of residual variation in the length of the delay. Due to the low numbers of productively infected cells in the periphery during the early stages of therapy interruption in patients with undetectable loads on HAART, at least some of the variation in the delay may be due to stochastic effects. To illustrate this, I consider a simple mathematical model with a constant rate of influx of productively infected cells into the periphery (0.5 cells per day), which have an average lifetime of one day, and an infection rate which is assumed to be zero during therapy, and which gives a viral doubling time of 1.5 days following interruption. Figure 3a shows viral growth curves for five runs of the model, illustrating the highly noisy dynamics at low levels, which results in a variable time

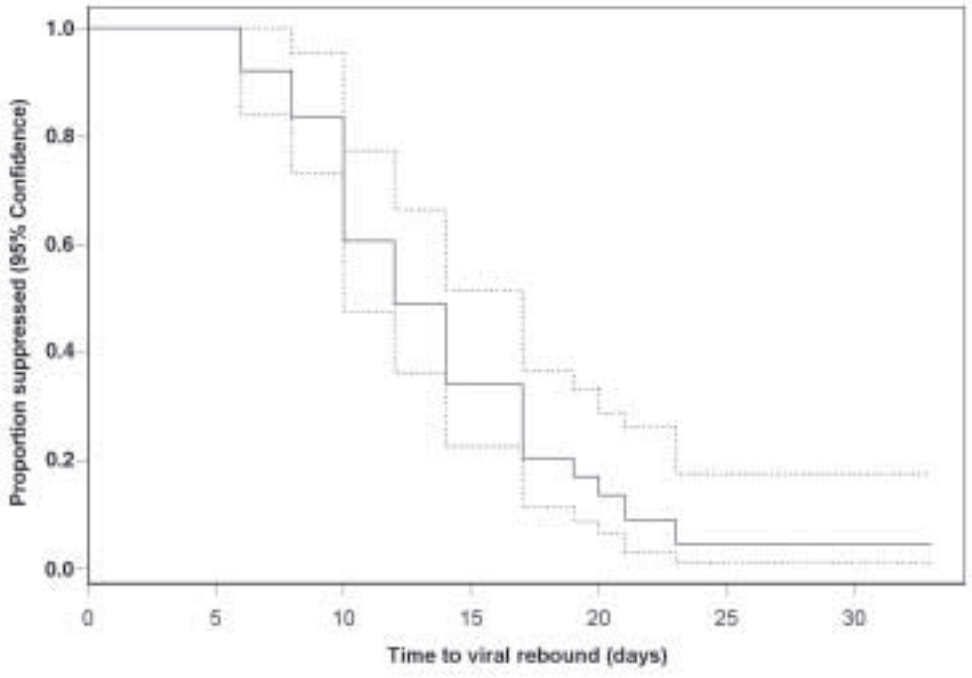


**Figure 2.** *The proportion of individuals with plasma viral loads below 50 copies per ml. following therapy interruption, based on data from 11 individuals suppressed on HAART who underwent four interruptions as part of the Autovac trial[24].*

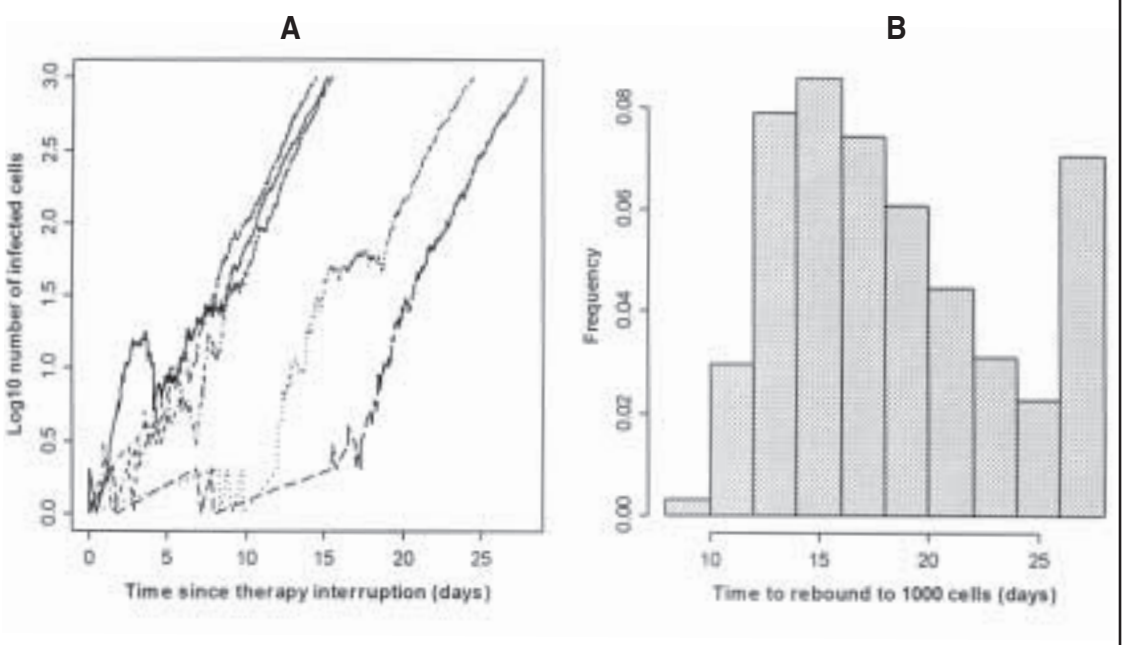


**Figure 3.** *Results of a simple stochastic model of the outgrowth of virus following interruption of therapy. The rate of production of productively infected cells is assumed to be 0.5 day$^{-1}$; the rate of outgrowth of virus is assumed to be 0.2 $log_{10}$ per day; and the average lifetime of an infected cell is assumed to be 1 day. At the beginning of each simulation, there are no infected cells. The simulation is stopped if the number of infected cells reaches 1000, or the time reaches 28 days, whichever is the sooner. a) Representative dynamics for five simulations. $Log_{10}$ (1 + number of infected cells) is plotted against time since interruption. b) Histogram of the time taken to reach 1000 cells (or 28 days) for 1000 simulations. Extensive heterogeneity can be generated by stochastic effects. Code to perform the simulations is available from the author on request.*

to reach a given population size (Fig. 3b). These stochastic effects could be amplified if viral outgrowth from low levels relied upon the inflammatory effects of HIV replication in order to recruit target cells to local sites of infection in solid tissue. A continuous chain of infection may be needed to result in sufficient stimulation to recruit enough target cells to allow viral rebound. In this respect, the dynamics of the emergence of virus during the early stages of HAART may be similar to the initial spread of virus during acute HIV infection[27], although originating from multiple sites rather than the single site of exposure.

STI studies exclude patients on non-nucleoside reverse transcriptase inhibitors (NNRTIs) due to the long half-life of this class of drug. Interrupting therapy in these patients may lead to a prolonged period of suboptimal drug concentrations, during which drug resistance may emerge. Although other classes of drugs have shorter half-lives than NNRTIs, there is still concern that the window of time during which drug levels are below that needed to suppress viral replication is sufficient to select for drug resistance. However, this phenomenon may contribute little to the evolution of drug resistance. Firstly, for drug resistance to emerge, the level of drug has to be low enough to allow the resistant virus to grow, but high enough to give the resistant mutant a competitive advantage. The period of time over which this is the case is short. Secondly, selection is ineffective in small populations. During the time of suboptimal drug concentrations, the number of productively infected cells is very small, and hence evolution during the early stages of interruption will be relatively unaffected by drug selection pressure.

## Dynamics and evolution during the growth stage

After the virus has reached detectable levels in the blood, it undergoes exponential growth, reflecting the relative lack of growth-limiting factors. Although immune responses may wane on highly active therapy[28], they do not totally disappear. Nevertheless, it is clear that these immune responses are insufficient to control viral replication, as virus generally rebounds, and that immune responses are well below their maximum, as numbers of HIV-specific cytotoxic T-cells are boosted following interruption in acutely-infected[10,11] and chronically-infected patients[12-14,26,29].

It is difficult to reliably estimate the growth rate of virus in individuals during interruptions when their viral loads are undetectable prior to interruption. The short length of the growth stage and the highly variable time to viral rebound result in relatively few detectable viral loads sampled during the growth stage. Protocols involving intensive

sampling of small numbers of chronically-infected patients have shown that the viral growth rate, when averaged across individuals, decreases over successive therapy interruptions[12,13,26]. Ortiz, et al.[14] did not find that viral growth rates decreased over successive interruptions; this may be due to the low frequency of viral load measurements and high limit of detection (400 copies/ml) in their study. Given that CD4+ counts do not progressively decrease over multiple STI cycles, decreased target-cell availability is unlikely to be the cause of decreased viral replication rates. In addition, highly frequent sampling of viral loads has revealed that there are apparently stochastic fluctuations in viral loads after multiple STIs[25]. These fluctuations are too large to be explained in terms of measurement error, and they may reflect a highly dynamic relationship between viral load and HIV-specific immune responses. In individuals who are failing therapy, their viral load is much closer to the setpoint, and hence the rate of increase of viral load following interruption is much lower. Deeks, et al.[16] showed a median increase of viral load of 0.84 (range 0.27 to 1.07) $\log_{10}$ copies per ml over a three month period in a population of highly experienced patients with high viral loads on therapy who interrupted therapy, compared to a median increase of 0.31 (range –0.09 to 0.65) $\log_{10}$ copies per ml in patients who continued therapy.

A common way to summarize the ability of the virus to grow *in vivo* is to calculate the basic reproductive rate, $R_0$, defined as the total number of productively infected cells produced by a single productively infected cell in the absence of limiting factors on viral growth i.e. when there is a high availability of target cells and a low level of HIV-specific immune responses. $R_0$ has been used as a measure of the impact of STIs on reducing viral replication[25,26]. Mathematical models can be used to estimate $R_0$ from the rate of exponential growth. However, a number of assumptions regarding the life cycle of HIV have to be made, in particular, the distribution of the time over which the infected cell produces virus particles[30]. Under the "standard" model of viral dynamics[31], virus is assumed to be produced as soon as a target cell is infected. $R_0$ can be calculated under this model from the rate of viral rebound, r (in $\log_e$ units), using the expression $R_0 = 1 + rD$, where D is the average lifetime of an infected cell. In contrast, assuming a fixed delay between infection of a cell and production of virus of length $D_1$, followed by an exponentially distributed period of viral production of average length $D_2$, $R_0$ is given by the expression $R_0 = (1 + rD_2)\exp(rD_1)$[32]. For a given rate of viral rebound, r, $R_0$ may be much higher under the more realistic fixed-delay model than under the standard model of viral dynamics. Due to the sensitivity of estimates of $R_0$ on the underlying model, it may be more appropriate to simply use estimates of the viral growth rate to compare the dynamics during the growth phase in different studies.

The genotype of the virus that emerges during the growth stage is dependent on the founding virus. If the reservoir is genetically homogeneous, then the virus emerging from the periphery will also be homogeneous. However, if the reservoir comprises several genetically different viral variants, then the pattern of evolution in the periphery depends upon the rate of seeding from the reservoir. A low seeding rate will result in the rebounding viral population being founded by a small number of viruses, resulting in a homogenous virus emerging during each STI cycle, with different variants emerging over successive interruptions. A high seeding rate will result in less founder effects, and will result in the emergence of a heterogeneous viral population, the genetic composition of which will remain relatively stable over successive STI cycles. As both the genetic diversity in the reservoir and the seeding rate from the reservoir are likely to vary between individuals, the pattern of viral evolution during STIs is likely to be highly variable between individuals and over time, even in the absence of any immune selection pressure driving viral evolution. This prediction is consistent with preliminary data on evolution of the *env* gene during STIs[33].

It may take several rounds of viral replication to generate further genetic diversity by mutation, and hence the risk of evolving new resistance mutations during short interruptions is low, although it rises steeply as the duration of interruption increases and the viral load approaches setpoint[34,35]. However, pre-existing resistant virus may emerge under certain conditions. Martinez-Picado, et al.[36] have demonstrated the emergence of the M184V mutation in reverse transcriptase in two patients from low levels to high levels over the course of three STI cycles. Both patients had evolved this mutation during prior suboptimal AZT + 3TC therapy. The emergence of the M184V mutation despite its high cost in terms of lowered viral replication[37] may be compensated for by a selective advantage during therapy. To illustrate this, I consider a simple model, which considers two populations of viruses: "wild-type" and "resistant". Let $B_W(t)$ and $B_M(t)$ be the *per capita* growth rates of wild-type and resistant virus at time t during therapy interruption. Due to high levels of target cells and/or insufficient HIV-specific immune responses, both wild-type and resistant virus increase in absolute numbers, although wild-type virus grows faster (i.e. $B_W(t) > B_M(t) > 0$). I denote the length of the therapy interruption by $t_1$. Let $D_W(t)$ and $D_M(t)$ be the *per capita* rates of decay of virus at time t during potent therapy. I assume that both wild-type and resistant virus responds to therapy, but resistant virus decays slower (i.e. $D_W(t) > D_M(t) > 0$). I denote the length of the therapy period by $t_2$. Assuming exponential growth of virus during interruption, and exponential rates of viral decay during therapy, resistant virus will increase when $(B_W - B_M)t_1 < (D_M - D_W)t_2$ i.e. when the interruption is short relative to the length of the treatment period ($t_1 < t_2$), and when

the lower growth rate of the resistant virus during interruption is compensated for the lower decay rate during therapy. Clearly, this model is grossly oversimplified; in particular, it does not include a reservoir of virus, and so may be insufficient to account for observed increases in the frequency of drug resistance mutations over successive interruptions. However, it illustrates the importance of the duration of the off- and on-therapy periods in the evolution of resistance.

Larger studies of STIs suggest that mutations at codon 184 in reverse transcriptase are the most likely to emerge during STIs. In a study of Swiss patients partaking in the Swiss-Spanish Intermittent Treatment Trial, Perrin, et al.[38] found that STIs were not associated with the frequent selection of drug resistant mutants, except M184I/V, especially in patients with high viral loads during the first viral rebound. In previous studies of patients undergoing lamivudine monotherapy, initially the M184I mutation emerges in the plasma virus population, but is later replaced by virus harbouring the M184V mutation; this order arises as the M184I mutant is produced at a higher rate by mutation than M184V, but the M184V mutant is associated with a higher replication rate[8]. The presence of M184I in patients undergoing STIs suggests that, in at least a fraction of patients, *de novo* resistance is emerging, rather than the re-emergence of M184V that evolved previously during suboptimal therapy. It is not clear whether lamivudine resistance emerges preferentially due to the small number of mutations required to confer resistance, or whether the long intracellular half-life of this drug may contribute to resistance, by resulting in a prolonged period of suboptimal drug levels. Further circumstantial evidence that evolution of resistance during STIs may be restricted to lamivudine comes from the RIGHT 901 trial[39], which found no evidence of development of resistance during treatment with hydroxyurea/didanosine/stavudine or didanosine/stavudine/indinavir combination therapy.

## Dynamics and evolution during the setpoint stage

The viral load in untreated infected individuals remains relatively constant over the asymptomatic period. Much attention has focused on the factors that determine viral "setpoint" due to its clinical relevance; individuals with high viral setpoints progress more rapidly to AIDS[40]. The initial interest in therapy interruptions was sparked by the case of the "Berlin patient", who began HAART soon after infection and interrupted for a week due to epididimitis 15 days into therapy[41]. Following a further interruption four months into therapy (during which the virus did not rebound), therapy was discontinued, and the patient's viral load remained undetectable in the blood. Studies of structured therapy interruptions in selected acutely-infected patients have repeated this result[10,11].

The way in which therapy interruption could result in a decline in viral setpoint was described using mathematical models by Dominik Wodarz and co-workers[42-45], who argued that there are two potential viral setpoints: a low viral setpoint maintained by strong, CD4+ dependent cytotoxic T-cell (CTL) responses, and a high viral setpoint maintained by weak CD4+ independent CTL responses. The key element in these models is the way in which CTL precursors (denoted w) proliferate in response to the number of infected CD4+ cells (denoted y) and by the amount of CD4+ help (denoted x). In the simplest of these models[42], proliferation of the CTL precursor pool is given by cxyw, where c is a constant reflecting CTL activation. On the one hand, infected cells, y, result in proliferation of CTL precursors, w, with concomitant increases in immune control; on the other hand, infected cells result in loss of uninfected CD4+ T-cells, x, and hence reduction of CTL precursor proliferation. This phenomenon results in the two different steady states. Low viral loads are attained when the activation rate of CTL precursors is high and the viral growth rate is low; conversely, high viral loads are attained when the activation rate of CTL precursors is low and the viral growth rate is high. By allowing limited viral replication through therapy interruptions, CD8+ T-cells are stimulated, but damage to the CD4+ compartment is limited, and hence the system can switch from a state of weak CD4+ independent CTL responses to strong CD8+ CTL responses.

One potential application of the models of Wodarz, et al. is to investigate how the number of interruptions, the length of each interruption and the intervals between interruptions determine whether a switch from low to high control of viral replication occurs. The models can then be used to find interruption protocols that give the highest chance of success (defined, for example, as sustained low viral loads) given that data on viral load and clinical markers may be incomplete and unreliable[46,47]. This kind of approach (known as "control theoretic" in the modeling literature) has been adopted in previous studies of HIV by Larry Wein and co-workers[48-50], where models of viral dynamics and evolution were used to find regimens that minimize viral load on therapy and the probability that drug resistance will emerge. Álvarez-Ramírez, et al.[51] have used a similar approach to investigate the effect of treatment during early infection on viral production.

Despite the promising results of therapy interruption in patients with very recent infection, the impact of therapy interruptions on viral load is much less in patients who interrupt later into infection. Of the 133 chronically infected patients in the Swiss-Spanish Intermittent Therapy Trial (SSITT), where patients interrupted therapy four times prior to cessation of therapy, 18% had a plasma viral load below 5,000 copies 52 weeks after stopping therapy, although this dropped to 11% 96 weeks after stopping therapy[15]. The sim-

ple models of Wodarz, et al. predict that the response to therapy interruptions should be greater in individuals who exhibit greater HIV-specific immune responses, and suggest that the poorer response of chronically infected individuals to STIs may be due to the level of damage sustained by the CD4+ compartment in chronic infection. However, although several studies have shown that, on average, viral replication decreases and HIV-specific CTL responses increase, at the individual level there is surprisingly little correlation between the extent to which viral replication is reduced and the increase in CTL responses[13,26]. One possible explanation for this discrepancy is that analysis of CTL responses is confounded by viral evolution; different antigenic variants may arise during each therapy interruption. The exposure of the immune system to different antigenic variants may result in weaker HIV-specific immune responses than if the immune system was exposed to a homogenous viral population.

In some patients who interrupt therapy, the viral loads "overshoot" the equilibrium. Figure 4 shows two individuals from the Autovac trial[24] who ceased therapy following four STI cycles. Although the peak viral loads are similar in these two patients, one patient exhibits a dramatic drop in viremia. This transient stage poses a difficulty in designing STI protocols, which include a criterion for re-initiating therapy if the viral load becomes too high to minimize the damage done to the immune system by the outgrowth of the virus. Some patients may actually respond well to STIs, but are placed back on therapy before this becomes apparent. The role of viral factors, such as replication rate, and host factors, such as the strength of HIV-specific immune responses, in determining whether an individual overshoots their setpoint or not is yet to be established.

The pattern of evolution of resistant virus during the setpoint stage differs from that during the growth stage due to the presence of limiting factors on the viral population, which result in competition between viruses. Viruses that emerge during the growth stage, when competition between viruses is low, may be outcompeted to low frequencies following attainment of viral setpoint when competition between viruses is high. In patients with high levels of resistant virus on HAART, therapy interruption results in reversion of drug resistance to wild-type over a period of several months[16,17]. The rate at which this reversion occurs reflects the lower replication rate of resistant virus relative to wild-type virus. In order to estimate the relative fitness of resistant virus, changes in population dynamics over the interruption need to be controlled for. If we assume a constant death rate, $\delta$, over the interruption, the fitness of wild-type virus relative to resistant virus can be calculated from the ratio of wild-type virus to mutant virus W/M (which can be obtained by clonal sequencing or real-time PCR, for example) and from the number of mutant viruses, M, at time t following interruption using the following expression:

$$\log\left(\frac{W_t}{M_t}\right) = \log\left(\frac{W_o}{M_o}\right) + s[\log(Mt) - \log(Mo) + \delta t]$$

This model was originally derived by Maree, et al.[52] in the context of estimating fitness from *in vitro* virus competition experiments and has been applied to data on the reversion of drug resistance virus during STIs by Bonhoeffer and De Boer[53]. From equation, the selective advantage of wild-type virus over mutant virus, s, is given by the slope of the plot of $\log(W_t/M_t)$ against $\log(M_t)$

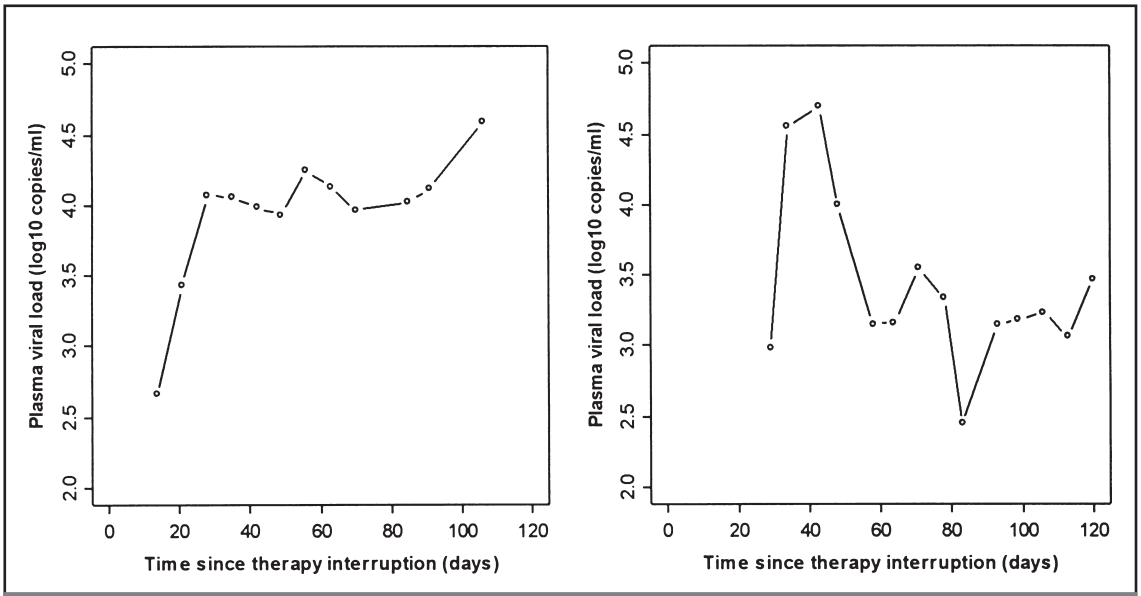


**Figure 4.** *Nonlinear rebound of viral loads in two representative patients following therapy interruption. In some patients, viral load reaches setpoint in a monotonic fashion, whilst others show a dramatic drop following a peak of viremia.*

– $\log(M_0) + \delta t$. In order to calculate the number of mutant viruses, we need to multiply the viral load measurement by the frequency of mutant virus; obtaining error bounds for the fitness is complicated by that fact that there are errors in both the y and x axis[53,54]. The attractive feature of this model is that the viral dynamics do not have to be modeled explicitly; all that is needed is the viral loads at each time point. This neat mathematical result stems from the assumption of a constant death rate of infected cells over the period of interruption. However, this may not be the case. By comparing the initial rate of growth of virus, the viral setpoint attained, and estimating the death rate of infected cells at the setpoint by the rate of decline of virus on therapy, Oxenius, et al.[26] have suggested that the death rate of infected cells at setpoint is higher than that when viral loads are at the limit of detection. Changing death rates will result in the model fitting poorly to the data.

## Conclusions

Mathematical models have been very useful in exploring the response of HIV to antiviral therapy. In this review, I have aimed to show how such models can guide the design and analysis of trials of structured therapy interruptions. There are a number of questions that have been raised by STI studies, which offer possibilities for future research. These include the following:

- In patients with suppressed viral load, where does the virus that emerges following interruption come from?
  - Possible reservoirs include virus attached to follicular dendritic cells in lymphoid tissues, which can persist even in the absence of ongoing replication, or in the central nervous system or other "drug sanctuaries".
- What are the viral dynamics in compartments other than plasma following interruption?
  - Most studies have concentrated on the dynamics in the blood; however, the majority of infected cells are in lymphoid tissues. Viral rebound in the central nervous system may result in neurological pathology, and viral rebound in genital secretions may increase infectiousness.
- Why are measures of HIV-specific immune responses unrelated to virological response to STIs?
  - While it is clear that some patients exhibit a viral setpoint following STI that is lower than their pre-therapy setpoint, other patients exhibit very little virological response. Surprisingly, this variation is not simply correlated to measures of the strength of HIV-specific cytotoxic T-cell responses. It is not clear whether this reflects an inability of CTL responses to clear the virus (for example, due to viral evolution), or our inability to measure the strength of these responses.
- Why does lamivudine resistance preferentially emerge during therapy interruptions?
  - The emergence of mutations at residue 184 in reverse transcriptase may reflect virological factors such as the low mutational barrier to the generation of these mutations, or pharmacological factors such as the long intra-cellular half-life of lamivudine. The clinical impact of the emergence of this mutation in the context of STIs has also yet to be addressed.
- How do the details of the interruption protocol affect the dynamics and evolution of HIV during STIs?
  - There is no general agreement about what is the "best" STI protocol for a given aim in terms of length of on- and off-therapy cycles, the number of cycles, the viral load above which therapy should be re-initiated, and so on. A meta-analysis of different STI protocols may guide the design of future protocols.

My personal view is that each of these possible avenues of research may benefit from a modeling component, and that as well as the clinical importance of studies of STIs, there is a lot we can learn about the basic biology of HIV from the way that the virus grows and evolves following interruption of therapy.

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