

Optimal multi-drug approaches for reduction of the latent pool in HIV ^{*}

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Abstract HIV disease is well-controlled by the use of combination antiviral therapy (cART), but lifelong adherence to the prescribed drug regimens is necessary to prevent viral rebound and treatment failure. Populations of quiescently infected cells form a “latent pool” which causes rapid recurrence of viremia whenever antiviral treatment is interrupted. A “cure” for HIV will require a method by which this latent pool may be eradicated. Current efforts are focused on the development of drugs that force the quiescent cells to become active. Previous research has shown that cell-fate decisions leading to latency are heavily influenced by the concentration of the viral protein Tat. While Tat does not cause quiescent cells to become active, in high concentrations it prevents a newly infected cell from becoming quiescent. In this paper, we introduce a model of the effects of two drugs on the latent pool in a patient on background suppressive therapy. The first drug is a quiescent pool stimulator, which acts by causing quiescent cells to become active. The second is a Tat analog, which acts by preventing the creation of new quiescently infected cells. We apply optimal control techniques to explore which combination therapies are optimal for different parameter values of the model.

Keywords: Biomedical system modeling, simulation and visualization, HIV, Cryptic Viremia

1. INTRODUCTION

Human Immunodeficient Virus (HIV) infection is a widespread chronic illness, affecting over 34 million people, with as many as 2.5 million new infections each year (Sidibé et al. (2012)). Untreated infection results in the progressive depletion of the helper T-cell population, and the resulting immunodeficiency leads to death by opportunistic infection (Thompson et al. (2012)).

The advent of multi-drug approaches to treating HIV infection, known as combination antiretroviral therapy (cART), has resulted in HIV infection becoming a chronic, manageable disease. The durability of viral suppression in some patients has led to hopes that eradication of the virus and a “cure” for the disease may be possible (Hamer (2004)).

When cART is interrupted, rapid viral rebound occurs in almost all patients, regardless of the duration of virus suppression (Phillips et al. (2002)). This is most commonly attributed to the ability of HIV to infect cells without entering active replication. If the infected cell then takes on a memory phenotype, the latently infected cell can persist for decades without triggering an immune response, but may re-activate at any time, triggering a rebound of active infection (Chun et al. (1997); Ramratnam et al. (2000); Finzi et al. (1997)).

The decision point where an infected cell either becomes actively infected or remains quiescent is stochastically determined by a feed-forward process in the transcription of HIV RNA from integrated viral DNA (Singh and Weinberger (2009); Singh et al. (2010)). When RNA polymerase binds to the HIV promoter region, transcription usually fails to complete unless the HIV viral protein Tat binds to the emerging complex (Jordan et al. (2003); Lassen et al. (2006); Lin et al. (2003)). Once a single RNA transcript is completed, large numbers of Tat are produced and this binding becomes certain. Conceivably, a drug that acted in a similar manner to Tat could bias this decision and prevent the formation of latently infected cells, as illustrated in Figure 1.

Current approaches to the eradication of the viral reservoir formed by these latent cells have focused on a so-called “shock-and-kill” strategy, where immune-stimulating agents trigger latently infected cells to begin active production of the virus. This allows the cells to become targets of the immune system for killing, or to be killed by the cytopathic effects of the budding virus (Geraert et al. (2008); Hamer (2004); Pomerantz (2003, 2002); Smith et al. (2012); Rong and Perelson (2009c); Dahl et al. (2010)). Commonly discussed agents include interferon-type drugs, which are generalized immune activators (van Praag et al. (2001)).

All of the “shock-and-kill” strategies are predicated on the assumption that successful virus replication is essentially halted by the background regimen of cART, and that the

^{*} AS is supported by the National Science Foundation Grant DMS-1312926

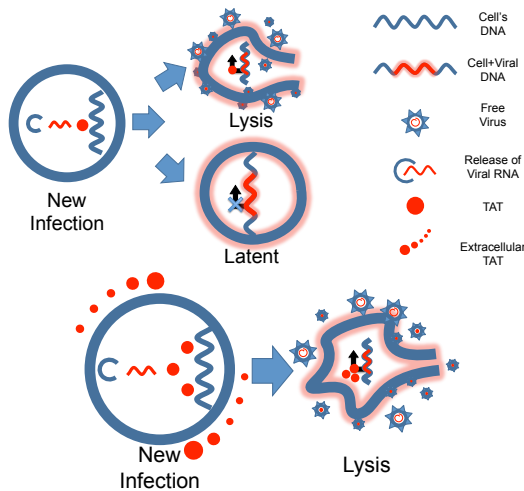


Figure 1. **The effect of additional Tat on viral latency.** Following cell entry, reverse transcription, and integration, an infected cell becomes either latent or actively infected depending on whether intracellular Tat binds the early transcription product. Exogenous sources of Tat bias this toward active infection.

formation of new quiescently infected cells during the viral burst following activation is essentially impossible. Recent work, however, has shown that anatomical reservoirs with limited antiviral activity may serve as sanctuary sites, and permit significant amounts of efficient viral replication during apparently effective cART treatment (Luo et al. (2013); Cardozo et al. (2012); Buzón et al. (2010); Hatano et al. (2013)). This so-called Cryptic Viremia may create a condition where reservoir flushing can actually increase the size of the latent reservoir.

In Section 2 of this paper, we introduce a new model of HIV infection dynamics that incorporates the activity of two potential drugs targeting the latent reservoir. The first drug is an interferon-like drug which acts by increasing the activation rate of latently infected cells. The second drug is a Tat analog, which acts by decreasing the likelihood of infection events leading to the formation of latently infected cells. We find the bifurcation points for this model where interferon therapy along is not able to clear the latent reservoir. In Section 3, we simulate the behavior of the model using model parameter values derived from the existing literature, and use a simple optimal control formulation to explore the usefulness of a hypothetical second drug, the Tat analog, in the presence and absence of cryptic viremia. We show that, while drugs that increase the activation rate are sufficient to clear the reservoir when cryptic viremia is absent, drugs that inhibit the establishment of latent infection must be used together with the activation rate enhancing drugs to achieve clearance when cryptic viremia is present.

2. HIV MODEL

Our HIV dynamic model is based on the extensively studied model of HIV infection first introduced by Perelson (1993). In this model the behavior of uninfected cells, infected cells and HIV virus is modeled by the equations:

$$\dot{T} = \underbrace{\lambda}_{\text{T-cell Production}} - \underbrace{d_T T}_{\text{T-cell Death}} - \underbrace{\beta TV}_{\text{Free Virus Infection}} \quad (1a)$$

$$\dot{I} = \underbrace{\beta TV}_{\text{Free Virus Infection}} - \underbrace{d_I I}_{\text{Infected Cell Death}} \quad (1b)$$

$$\dot{V} = \underbrace{k I}_{\text{Free Virus Production}} - \underbrace{d_V V}_{\text{Free Virus Death}} \quad (1c)$$

Here $T(t)$, $I(t)$ and $V(t)$ represent uninfected cells, infected cells and virus population at time t , respectively. The rate of production of uninfected cells is represented by λ . The death rates of uninfected cells, infected cells and free virus are d_T , d_I and d_V . The rate of virus production by infected cells is given by k , which can be reduced by the activity of protease inhibitors. The infection rate is given by β , which can be reduced by the activity of reverse-transcriptase inhibitors and integrase inhibitors. For the purpose of simplicity, we will model the activity of the background cART regimen as a reduction in β .

To include latent reservoir, we add latent cell dynamics, as in Rong and Perelson (2009b).

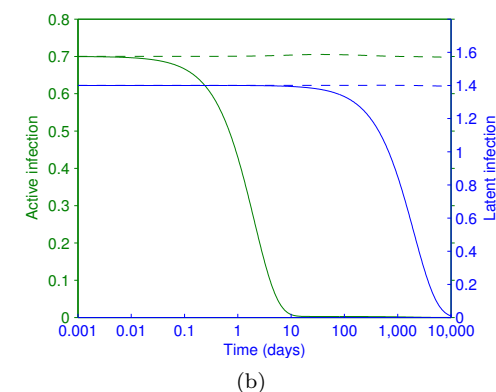
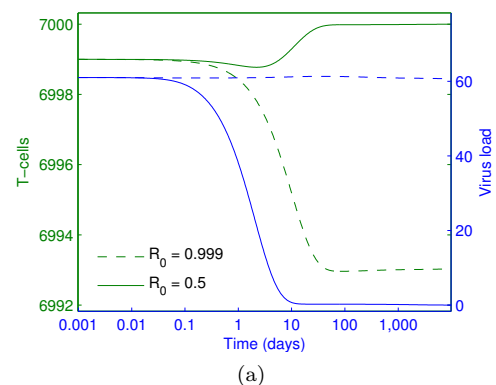


Figure 2. **Reservoir Behavior without Treatment.** Cryptic viremia ($R_0 = 0.999$) provides a mechanism for the maintenance of a steady-state latent reservoir. Without cryptic viremia ($R_0 = 0.5$), the reservoir decays without intervention. Results are shown for uninfected T-Cells (a), Actively Infected T-Cells (b), Free virus (c), and latently infected T-Cells (d).

$$\dot{T} = \underbrace{\lambda}_{\text{T-cell Production}} - \underbrace{d_T T}_{\text{T-cell Death}} - \underbrace{\beta TV}_{\text{Free Virus Infection}} \quad (2a)$$

$$\dot{I} = \underbrace{(1 - \rho)\beta TV}_{\text{Active Infection}} - \underbrace{d_I I}_{\text{Infected Cell Death}} + \underbrace{\alpha L}_{\text{Reactivated Cells}} \quad (2b)$$

$$\dot{L} = \underbrace{\rho\beta TV}_{\text{Latent Infection}} - \underbrace{\frac{1}{2}\alpha L}_{\text{Net Clearance}} \quad (2c)$$

$$\dot{V} = \underbrace{k I}_{\text{Free Virus Production}} - \underbrace{d_V V}_{\text{Free Virus Death}}, \quad (2d)$$

with $L(t)$ as the latent cell population, ρ as probability that a new infected cell becomes latent, and α as the rate of reactivation of an latent cell. Table 1 shows all parameters used in the model and experimentally-derived values for them obtained from Luo et al. (2012).

Parameter	Value	Units	Biological meaning
λ	7×10^2	$\frac{\text{cells}}{\mu\text{L} \times \text{day}}$	Uninfected birth rate
d_T	0.1	$\frac{1}{\text{day}}$	Uninfected death rate
d_I	1	$\frac{1}{\text{day}}$	Infected death rate
d_V	23	$\frac{1}{\text{day}}$	Virus decay rate
k	2×10^3	$\frac{\text{copies}}{\text{cell} \times \text{day}}$	Virus copies per cell
β	2×10^{-6}	$\frac{1}{\text{copies} \times \text{day}}$	Infection Rate
α	0.001	$\frac{1}{\text{day}}$	Reactivation rate
ρ	0.001	-	Latency Probability

Table 1. All parameters values were taken from Luo et al. (2012).

In this new model, we assume that the net clearance of the latent pool is half of the reactivation rate of a latent cell. The actual ratio is unknown, as the term $\frac{1}{2}\alpha L$ in Equation 2c is the net effect of reservoir cell activation, reservoir cell division, and reservoir cell death, while αL in Equation 2b represents activation only. The assumption that the net loss of reservoir cells to an activation process is less than the net production of actively infected cells is consistent with the experimental observation that the latently infected reservoir is remarkably stable despite relatively high levels of ongoing activation (Rong and Perelson (2009a)).

The steady state value for the latent pool is

$$L = \frac{2\rho((1 + \rho)k\beta\lambda - d_I d_T d_V)}{(1 + \rho)\alpha k\beta} \quad (3)$$

Note that the latent pool decays to zero if and only if

$$R_0 < \frac{1}{(1 + \rho)}, \quad (4)$$

where

$$R_0 = \frac{k\beta\lambda}{d_I d_T d_V} \quad (5)$$

is the infectivity ratio of the virus during cART therapy. The baseline probability of a cell becoming latently infected ρ has been experimentally estimated at about 0.001

(Rong and Perelson (2009a)), so a stable steady-state value of the latent pool can only occur when R_0 is very close to 1. We have shown, however, that sanctuary site dynamics can enforce exactly this condition in the presence of cryptic viremia (Luo et al. (2013)), the presence of which has been experimentally verified (Buzón et al. (2010)). In this

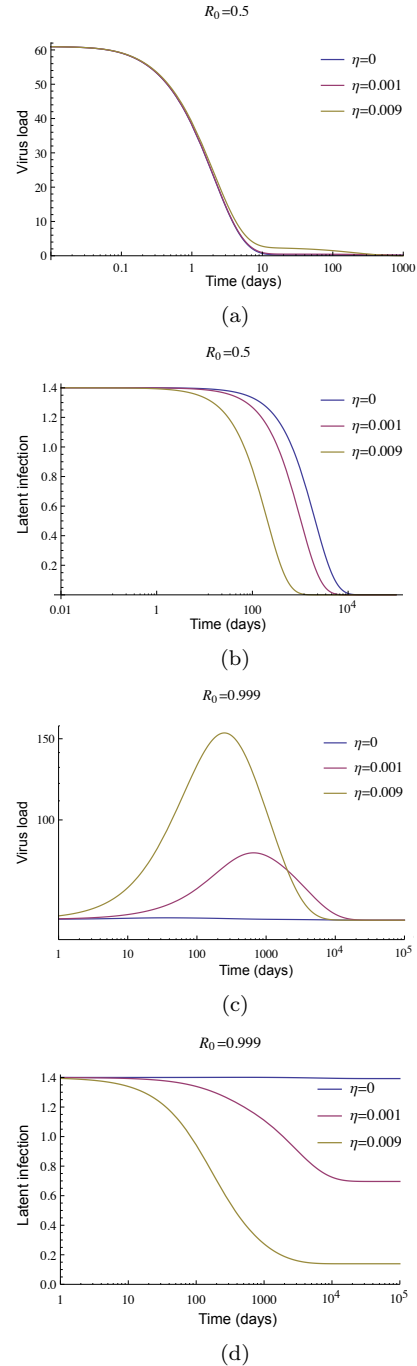


Figure 3. **Effect of Interferon Therapy Alone** Interferon therapy accelerates the decay of the reservoir in the absence of cryptic viremia (a) and (b), but does not change the outcome. In the presence of cryptic viremia (c) and (d), interferon therapy reduces the steady-state reservoir level, but does not eradicate it. The application of interferon also creates a significant transient burst of viremia in the presence of cryptic viremia.

paper, therefore, we will consider both the condition where cryptic viremia is not present (Inequality 4 holds), or where cryptic viremia is present (Inequality 4 is violated). In all cases, we will assume that suppressive therapy is present, and that $R_0 < 1$.

We also want to model the activity of our two reservoir-targeting drugs. The interferon-like drugs enhance the activation rate of quiescently infected cells. While it is possible that the drugs will asymmetrically affect the various factors that play into the meaning of α in Equations 2b and 2c, the simplest assumption is that the drug affects them equally, and this effect can be modeled by replacing α in Equations 2b and 2c with $(\alpha + \eta(t))$, where $\eta(t)$ is the administration of the interferon-like drug.

Modeling the effect of a Tat-analog drug on the probability of latency is slightly more complex. If we assume that the likelihood of any given Tat molecule present in the cell is an independent binary random process, and that one Tat molecule is transmitted with each successful infection event, then the effect of adding a Tat analog at a concentration that increases the average number of Tat molecules per cell by $\lambda(t)$ can be written as:

$$\rho(t) = 1 - \sum_{n=0}^{\infty} (1 - \rho^{1+n}) P_{\lambda(t)}(n) \quad (6)$$

where $P_{\lambda}(n)$ is a Poisson distribution with mean $\lambda(t)$. As a practical matter, $\lambda(t) = 1$ is sufficient to render new latent infections vanishingly improbable.

The dynamics of this model in the absence of any reservoir targeted therapy ($\lambda(t) = \eta(t) = 0$) is shown in Figure 2, using the parameter values shown in Table 1, with β chosen such that R_0 is either equal to 0.5 (no cryptic viremia) or 0.999 (cryptic viremia). It is clear that when $R_0 = 0.999$, the latent reservoir reaches a steady-state of approximately 1.4 latently infected cells per μL of whole blood, consistent with levels observed in patients. This also enables the maintenance of a steady-state viral load, which is also observed in patient on long-term cART therapy. Conversely, when $R_0 = 0.5$, the latent reservoir continues to decay slowly toward zero, with a half-life of approximately 5 years. When we consider the effect of Interferon-like therapy alone, the behavior of the model is shown in Figure 3. When $R_0 = 0.5$, the administration of interferon has no measurable effect on the virus load, which decays uniformly in all conditions. The addition of the drug does have a measurable effect on the decay rate of the latently infected population, though not on the outcome; the latent pool decays toward zero regardless of the value of $\eta(t)$.

The behavior is significantly different when $R_0 = 0.999$. Under these conditions, the administration of the interferon-like drug results in a measurable transient increase in the viral load. The latent cell population decreases following that addition of η , but reaches a new steady-state level rather than decaying toward zero. Even when $\eta = 0.009$ (equivalent to a 10-times increase over the baseline activation rate), the new steady-state latent cell population is still approximately 20% of the baseline latent cell population. This implies that interferon-type therapies along

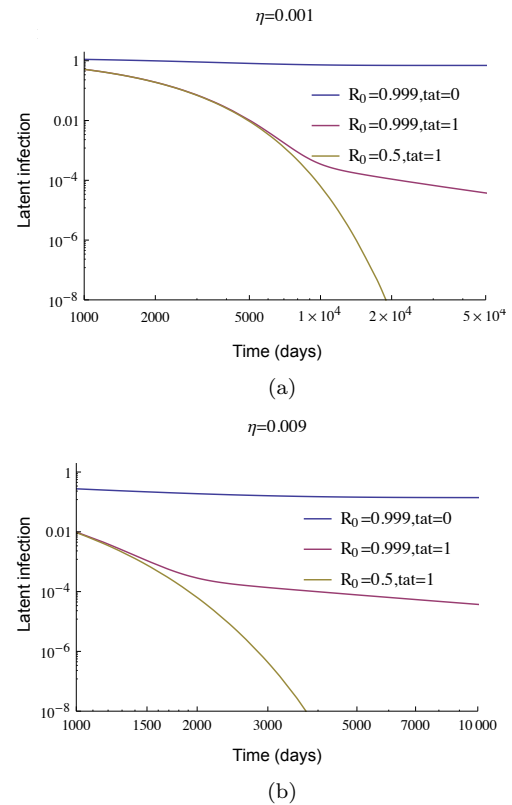


Figure 4. **Effect of Interferon Therapy with additional Tat** The addition of a background Tat analog drug dramatically changes the outcome when interferon is applied. In the presence of cryptic viremia, interferon therapy is now able to eradicate the viral reservoir. Application of a relatively low dose of interferon (a) results in a slower decline in the reservoir when compared to a higher dose of interferon (b).

may be unsuccessful in clearing the latent reservoir when cryptic viremia is present.

In order to address this limitation of interferon-like therapies, we consider the addition of a Tat-like drug together with the interferon-like drug. As we mentioned previously, the effect of additional Tat on the likelihood of latency formation is so dramatic that an administration of $\lambda(t) = 1$ is sufficient to reduce the likelihood of latency by several orders of magnitude. Simulations of the model behavior with $\lambda(t) = 1$ are shown in Figure 4. The addition of Tat eliminates the steady-state behavior of the latent reservoir, which now converges exponentially to zero, at a rate that depends weakly on the level of applied interferon-like drug.

3. OPTIMAL CONTROL AND SIMULATIONS

In order to further explore the synergy between interferon-like and tat-like drugs in reservoir clearance, we explored a simple constrained optimal control formulation of reservoir clearance in the presence of cryptic viremia ($R_0 = 0.999$). While it is true that only the administration of Tat allows for the exponential decay of the latent reservoir in this case, stochastic extinction may eliminate the reservoir if it is reduced to a sufficiently low concentration for an extended period of time.

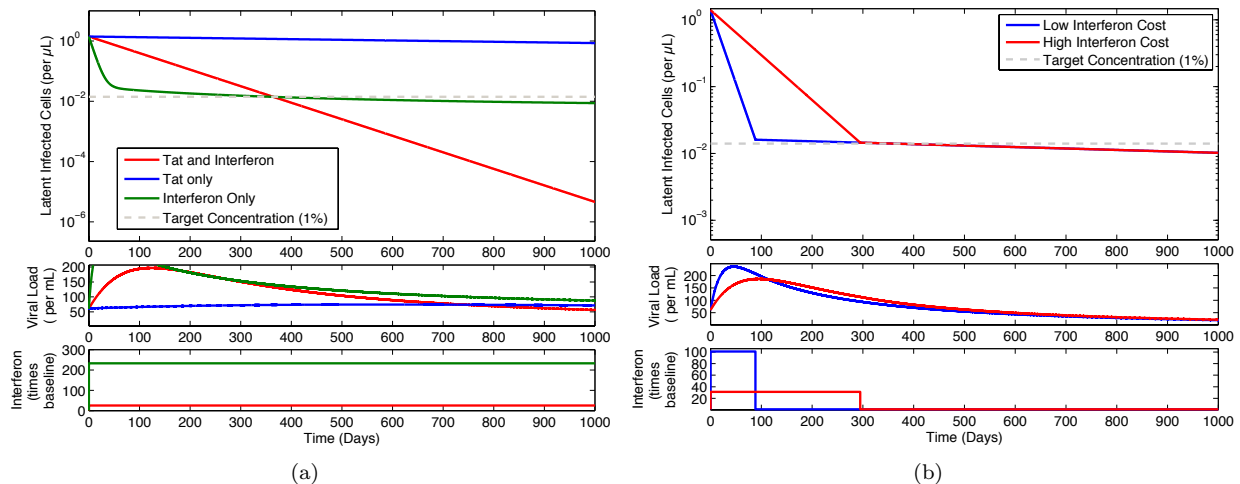


Figure 5. **Optimal Reduction of Latent Reservoir** When constant-dosage schedules are considered (a), the constrained optimization problem is infeasible when only Tat analog is administered. Application of Tat together with interferon allows the constraint to be achieved with less than 1/10 the dose of interferon when compared to interferon alone. When pulsed administration of interferon is considered (b), Tat administration enables short-dose schedules of the interferon to achieve the constraint.

To simulate the goal of stochastic extinction, we formulated an optimization problem where latent cell trajectories were constrained to drop below 1% of their initial concentration by the end of the first year of therapy, and to remain below the 1% level between year 1 and year 2 of therapy. We consider fixed-dose schedules of Tat and interferon, and minimize the application of interferon.

Figure 5(a) shows the results of this optimization for three conditions. When Tat is applied alone ($\eta(t) = 0$), the problem was infeasible no matter how large $\lambda(t)$ was allowed to grow. While the administration of Tat causes the latent reservoir to decay in this case, the half-life is measured in decades. When interferon is applied alone ($\lambda(t) = 0$), the constraint is achievable with a minimum administration of $\eta(t) = 0.238$, or an effect 240 times the baseline. If Tat is administered $\lambda(t) = 1$, the constraint is achievable with an interferon dose of only $\eta(t) = 0.025$. Furthermore, the achieved trajectory continues to decay exponentially toward zero throughout year 2.

The administration of interferon causes a migration of latently infected cells into the active infection compartment, and the administration of Tat prevents the migration of actively infected cells back into the latent pool. This asymmetry of action enables dynamic treatments, with short-term applications of interferon followed by long-term administration of Tat. This may be desirable due to the poorly tolerated side-effects of interferon therapy. To explore this possibility, we modified the optimal control problem to minimize a weighted average of the pulse height and pulse width of a single applied interferon pulse during a constant application of Tat. The trajectories for two different weighting values are shown in Figure 5(b). Both a short, high intensity application and a longer, lower intensity application of interferon are able to meet the desired treatment constraints. This implies that dynamic therapeutic schedules may be of value, and should be explored further in future work.

4. CONCLUSIONS

Recent findings suggest that cryptic viremia in sanctuary sites may be common in treated HIV patients. These findings have significant implications for reservoir-flushing approaches to “cure” HIV infection. In this paper, we have demonstrated that reservoir-flushing approaches using interferon-like treatments may be incapable of clearing the reservoir in the presence of cryptic viremia. We have shown that, in the presence of cryptic viremia, additional drugs that reduce the probability of latent infection may be necessary to successfully clear the pool of latently infected cells. We further demonstrated through simulation that a significant synergism between these two drugs exists when applied in the presence of cryptic viremia.

The results in this paper are preliminary, and many known effects have been neglected for the sake of simplicity. It would be premature to engage in a more complete exploration of the optimal control problem, as no Tat analog drug with the properties described in this paper yet exists. This is primarily because the phenomenon of cryptic viremia has only recently been recognized, and in the absence of cryptic viremia, such a drug would have no measurable effect. Indeed, the fastest decay of the latent reservoir was always observed in the case where cryptic viremia was absent, indicating that cryptic viremia, rather than latency formation, may be the better target for therapy.

We have modeled the HIV infection dynamics as a single, well-mixed compartment with a uniform R_0 throughout. In fact, cryptic viremia is inherently a spatially heterogeneous phenomenon, with local regions where $R_0 > 1$, and larger regions where $R_0 < 1$, resulting in average behavior with R_0 appearing to be very slightly less than one (Cardozo et al. (2012)). In future work, we will explore a spatially compartmentalized version of the model to determine how such spatial heterogeneity would change the effect of cryptic viremia on the reservoir flushing approaches.

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