Abstract

Antiretroviral therapy is able to suppress the viral load below the detection limit, but it is not able to eradicate HIV reservoirs. Thus, there is a critical need for a novel treatment to eradicate the reservoir in order to eliminate the need for a lifelong adherence to antiretroviral therapy, which is expensive and potentially toxic. In this paper, we investigate the possible pharmacological strategies or combinations of strategies that may be beneficial to reduce or possibly eradicate the latent reservoir. We do this via studies with a validated mathematical model, where the parameter values are obtained with newly acquired clinical data for HIV patients. Our findings indicate that the strategy of reactivating the reservoir combined with enhancement of the killing rate of HIV-specific CD8+ T cells is able to eradicate the reservoir. In addition, our analysis shows that a targeted suppression of the the immune system is also a possible strategy to eradicate the reservoir.

Key words: HIV, reservoir cells, immune response, mathematical model, inverse problem, stability analysis.
1 Introduction

Antiretroviral therapy (ART) for HIV infection typically consists of several antiretroviral drugs that each target a specific stage of the HIV life cycle and is effective in suppressing the viral load below the detection limit. However, HIV can persist at undetectable levels in the presence of ART and cessation of ART leads to a viral rebound within three to four weeks (e.g., see [21] and references therein). Thus, the majority of HIV patients must adhere to a lifelong ART regimen in order to control the HIV infection.

It has been found that the persistence of HIV is due to several potential viral reservoirs, including long-living resting CD4+ T cells that contain integrated HIV DNA as well as anatomical sanctuaries that are not reached by the antiretroviral drugs (see [21] for details). We remark that resting CD4+ T cells with replication competent HIV DNA integrated into their host genomes, i.e., latently infected CD4+ T cells, refer to those resting CD4+ T cells that contain integrated HIV DNA and are capable of producing virus only upon activation. Reactivation of latently infected CD4+ T cells serve as a major source of viral rebound upon treatment failure, and hence they are considered to be the most challenging obstacle to HIV eradication (e.g., see [8] and references therein). Thus, there is a critical need for a novel treatment to eradicate this latent reservoir in order to eliminate the need for lifelong adherence to ART, which is not only expensive and toxic [24, 25], but also leaves HIV patients at risk for developing Acquired Immunodeficiency Syndrome (AIDS) [9].

To have a better understanding of latency, we give a brief introduction to the replication and transcription of HIV and refer the interested reader to [6, 10, 20] and references therein for more information. Figure 1 depicts the diagram for HIV replication and transcription. Following binding to the main receptor of CD4+ T cells, HIV fuses with the cell membrane and releases its contents into the cytoplasm. Once inside the cell, reverse transcriptase converts HIV RNA into a double-stranded DNA molecule (linear cDNA) with long terminal repeats (LTRs), which contain upstream regulatory regions that serve as binding sites for transcription factors which can upregulate virus production. The cDNA is then transported into the nucleus and integrated into the host chromosome with an integrase enzyme. The integrated HIV DNA, i.e., provirus, may then either lie dormant or be transcribed into viral RNA. We remark that if the cDNA fails to integrate, then the cDNA circularizes to form a 2-long terminal repeat (2-LTR) circle. Several mechanisms have been proposed that contribute to the establishment of latently infected CD4+ T cells. Two possible sources of latency are the activated CD4+ T cells that transition to a resting state following infection, and direct infection of a resting CD4+ T cell.

Strategies for eradication of HIV have been proposed in the literature (e.g., see [13, 17, 21, 22] and references therein), including gene therapy to make cells resistant to HIV and pharmacological approaches to eliminate the reservoirs. Specifically, efforts to eradicate the latent reservoir to date have focused on reactivating the latently infected CD4+ T cells in the presence of ART. In theory, this reactivation strategy could induce virus production and subsequent cell death in latently infected CD4+ T cells either from direct cytopathic
effects or immune clearance, while, simultaneously, ART could block new infection from the released virus. For example, histone deacetylase (HDAC) inhibitors have emerged as the lead drug candidate for reactivation of the latently infected CD4+ T cells. However, as suggested in [13, 19], simple reactivation of latently infected CD4+ T cells may not be sufficient for eradication of latent reservoir. We propose that a data-driven systems modeling approach can be used to quantitatively estimate the effect of adjuvant therapies, given in addition to ART, on patient viral load and latent reservoir levels. Thereby, the potential for adjuvant drug candidates to eradicate HIV reservoirs can be evaluated in silico based on previously obtained patient data to inform the design of future clinical trials.

In this paper, we use a mathematical model to elucidate immuno-modulatory strategies that could be used in addition to ART to assist in eradicating the latent reservoir by inducing a stable virus-free or virus-undetectable state. The model we used is adopted from [3], and it includes both previously mentioned sources of establishing latently infected cells. It was found in [3] that this model provides reasonable fits to patients enrolled in a clinical trial that tested the efficacy of ART regimens. The model fit the data for all of the 14 patients considered from that trial, and the clinical data in [3] were from patients that all underwent ART and had at least one treatment interruption. The available clinical data analyzed in [3] included the total number of CD4+ T cells and censored viral load. In addition, the model from [3] was found to have impressive predictive capability when comparing model simulations (with parameters estimated using only half of the longitudinal observations) to the corresponding full longitudinal data sets. Recently, we obtained new clinical data from a study performed at Massachusetts General Hospital in which all patients in the study have never gone off ART after ART was initiated. This newly acquired data includes the
amount of integrated HIV DNA, a novel measurement that has not previously been used in mathematical modeling of HIV, in addition to the usual measurements for the total number of CD4+ T cells and the censored viral load. In the following, we use these new data to obtain estimates for the parameters in the model. We then investigate the possible eradication strategies by varying the estimated values of a number of model parameters.

2 Mathematical Model

As we stated earlier, we use exactly the same model as that in [3] with model states and their descriptions given in Table 1. The schematic of this model is depicted in Figure 2. But for generality, we allow the differentiation rate from $T_1$ to $T_2$ to be different from the one from $T_1^*$ to $T_2^*$ and the activation rate of $T_2^*$ to be different from that of $T_2$.

<table>
<thead>
<tr>
<th>states</th>
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<tr>
<td>$T_1$</td>
<td>cells/µl-blood</td>
<td>uninfected activated CD4+ T-cells</td>
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<tr>
<td>$T_1^*$</td>
<td>cells/µl-blood</td>
<td>infected activated CD4+ T-cells</td>
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<td>free infectious virus</td>
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<tr>
<td>$V_{NI}$</td>
<td>RNA copies/ml-plasma</td>
<td>free noninfectious virus</td>
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<tr>
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<td>HIV-specific effector CD8+ T-cells</td>
</tr>
<tr>
<td>$E_2$</td>
<td>cells/µl-blood</td>
<td>HIV-specific memory CD8+ T-cells</td>
</tr>
</tbody>
</table>

Table 1: Model States.

Figure 2: Flow chart of model (2.1) with compartments as described in Table 1. PI and RTI denote protease inhibitor and reverse transcriptase inhibitor, respectively.
We remark that the “infected activated CD4+ T cells” $T_1^*$ are defined as those activated CD4+ T cells that are capable of producing infectious free virus, and that the “uninfected activated CD4+ T cells” denote the rest of the activated CD4+ T cells. In addition, the “infected resting CD4+ T cells” $T_2^*$ are those resting CD4+ T cells that contain integrated HIV-1 DNA and are capable of producing infectious free virus only upon activation (that is, $T_2^*$ denotes the “latently infected CD4+ T cells”), and the “uninfected resting CD4+ T cells” are the rest of the resting CD4+ T cells.

The corresponding compartmental ordinary differential equation (ODE) model is given by

\[
\begin{align*}
\dot{T}_1 &= -d_1T_1 - (1 - \xi_1)k_1V_IT_1 - \gamma_T T_1 + p_T \left( \frac{\alpha_T V_I}{V_I + K_V} + a_A \right) T_2, \\
\dot{T}_1^* &= (1 - \xi_1)k_1V_IT_1 - \delta T_1^* - mE_1T_1^* - \gamma_{T_s} T_1^* + \gamma_T T_1 - \frac{1}{2} T_2^* - \left( \frac{\alpha_T V_I}{V_I + K_V} + a_A \right) T_2, \\
\dot{T}_2 &= \lambda_T \frac{K_T}{V_I + K_T} + \gamma_T T_1 - d_2T_2 - \frac{1}{2} T_2^* - \left( \frac{\alpha_T V_I}{V_I + K_V} + a_A \right) T_2, \\
\dot{T}_2^* &= \gamma_{T_s} T_1^* + (1 - f\xi_1)k_2V_IT_2 - \frac{1}{2} T_2^* - \left( \frac{\alpha_T V_I}{V_I + K_V} + a_A \right) T_2, \\
\dot{V}_I &= (1 - \xi_2)10^3N_T\delta T_1^* - cV_I - 10^3[(1 - \xi_1)\rho_1 k_1 T_1 + (1 - f\xi_1)\rho_2 k_2 T_2] V_I, \\
\dot{V}_{NI} &= \xi_210^3N_T\delta T_1^* - cV_{NI}, \\
\dot{E}_1 &= \lambda_E + \frac{b_E T_1^*}{T_1 + K_M} E_1 - \frac{d_E T_1^*}{T_1 + K_d} E_1 - \delta E_1 E_1 - \gamma_E \frac{T_1 + T_1^*}{T_1 + T_1^* + K} E_1 + \frac{p_E a_E V_I}{V_I + K_V} E_2, \\
\dot{E}_2 &= \gamma_E \frac{T_1 + T_1^*}{T_1 + T_1^* + K} E_1 + \frac{b_E K_M}{E_2 + K_M} E_2 - \delta E_2 E_2 - \frac{a_E V_I}{V_I + K_V} E_2, \\
\end{align*}
\]

with initial condition

\[
(T_1(0), T_1^*(0), T_2(0), T_2^*(0), V_I(0), V_{NI}(0), E_1(0), E_2(0))^T = (T_1^0, T_1^{\rho_0}, T_2^0, T_2^{\rho_0}, V_I^0, V_{NI}^0, E_1^0, E_2^0)^T.
\]

Next we give a brief description of the above model, and refer interested readers to [3] for details on the rational behind this model. In the $T_1$ compartment, $d_1T_1$ denotes the natural death of $T_1$, and $(1 - \xi_1)k_1V_IT_1$ is used to represent the infection process that results from encounters between the uninfected activated CD4+ T-cells $T_1$ and free virus $V_I$, where $\xi_1$ is the relative efficacy of the reverse transcriptase inhibitor (RTI) with $0 \leq \xi_1 < 1$. The term $\gamma_T T_1$ is used to account for the phenomenon of differentiation of uninfected activated CD4+ T-cells into uninfected resting CD4+ T-cells $T_2$. In the $T_1^*$ compartment, $\delta T_1^*$ denotes the loss of infected activated CD4+ T-cells due to the cytopathic effect of HIV, and the corresponding gain term for $V_I$ include a multiplicative factor $N_T$ to account for the number of RNA copies produced during this process and a factor $(1 - \xi_2)$ to account for the protease inhibitor (PI) treatment, where $\xi_2$ denotes the relative efficacy of PI with $0 \leq \xi_2 < 1$. The term $mE_1T_1^*$ is used to account for the elimination of the infected activated CD4+ T-cells by the HIV-specific effector CD8+ T-cells, and $\gamma_{T_s} T_1^*$ is used to account for the phenomenon of differentiation of infected activated CD4+ T-cells into latently infected CD4+ T-cells $T_2^*$ at rate $\gamma_{T_s}$.

In the $T_2$ compartment, $\lambda_T \frac{K_T}{V_I + K_T}$ is used to account for the source rate of naive CD4+ T-cells, and $d_2T_2$ denotes the natural death of $T_2$. The term $(1 - f\xi_1)k_2V_IT_2$ is used to represent the
infection process that results from encounters between the uninfected resting CD4+ T-cells $T_2$ and free virus $V_I$, where the parameter $f$ (0 $\leq f \leq 1$) is used to account for the fact that treatment is potentially less effective in $T_2$ than in $T_1$. The term $\left(\frac{a_T V_I}{V_I + K_V} + a_A\right) T_2$ denotes the activation of the uninfected resting CD4+ T cells, and the corresponding gain term for $T_1$ include a multiplicative factor $p_T$ to account for the net proliferation due to clonal expansion and programmed contraction. Similarly, the term $\left(\frac{a_T V_I}{V_I + K_V} + a_A\right) T_2^*$ in the $T_2^*$ compartment is used to account for the activation of latently infected CD4+ T cells, and the corresponding gain term for $T_1^*$ also includes a multiplicative factor $p_T$. The term $d_2 T_2^*$ represents the natural death of $T_2^*$.

In the $V_I$ and $V_{NI}$ compartments, $cV_I$ and $cV_{NI}$ respectively denote the clearance of free infectious virus $V_I$ and free noninfectious virus $V_{NI}$, and the factor $10^3$ is introduced to convert between microliter and milliliter scales. The term $10^3[(1-\xi_1(t))\rho_1 k_1 T_1 + (1-f_1(t))\rho_2 k_2 T_2] V_I$ in the free virus compartment $V_I$ is used to account for the removal of free virus that takes place when free virus infects $T_1$ and $T_2$.

The first four terms in the $E_1$ compartment denotes the source, nonlinear infected cell-dependent birth, nonlinear infected cell-dependent death, and constant death, respectively. The term $\gamma_E \frac{T_1 + T_2^*}{T_1 + T_2^* + K_E} E_1$ is used to include the essential role that activated CD4+ T-cells play in the generation of memory CD8+ T-cells, where the parameter $K_E$ is a half-saturation constant and $\gamma_E$ is the maximum rate at which $E_1$ differentiates into $E_2$. In the $E_2$ compartment, $\frac{b_{E_2} K_{E_2}}{E_2 + K_{E_2}} E_2 - \delta_{E_2} E_2$ is used to denote the homeostatic regulation of $E_2$ with $b_{E_2}$ being the maximum proliferation rate and $\delta_{E_2}$ being the death rate. The term $\frac{a_{E_2} V_I}{V_I + K_V} E_2$ denotes reactivation of HIV-specific memory CD8+ T-cells, and the corresponding gain terms for $E_1$ include a multiplicative factor $p_E$ to account for the net proliferation due to clonal expansion and programmed contraction.

### 3 Inverse Problems

The data for our investigations come from HIV patients who received ART treatment at Massachusetts General Hospital and have not gone off ART once the treatment began. Specifically, there are six patients whose treatment began during the acute phase (the clinical ID numbers for these patients are prefixed with “AC”), and there are six patients whose treatment began during the chronic phase.

The observables are the total number of CD4+ T cells per $\mu l$-blood (where for the model (2.1) this is given by $z_1(t; q) = T_1^*(t; q) + T_2^*(t; q) + T_1(t; q) + T_2(t; q)$ with $q$ being a column vector for those model parameters and initial conditions that need to be estimated), the viral load per $ml$-plasma (where for the model (2.1) this is represented by $V_I(t; q) + V_{NI}(t; q)$), and integrated HIV-1 DNA CCR5.
To obtain the number of integrated HIV-1 DNA per µl-blood, we use the following formula

\[
\text{integrated HIV-1 DNA} = \frac{1}{2} \frac{\text{integrated HIV-1 DNA}}{\text{CCR5}} \times \left( T_1^* + T_2^* + T_1 + T_2 \right),
\] (3.1)

which is obtained using the assumption that each cell has two copies of the CCR5 gene. If we further assume that each infected CD4+ T cell contains only one copy of integrated HIV-1 DNA, then (3.1) gives us the measurement of the latently infected CD4+ T cells (for the model (2.1) this is given by \( \tilde{z}_3(t; q) = T_2^*(t; q) \)).

**Remark:** Based on [17] and the references therein, measurements of integrated HIV-1 DNA may be a useful surrogate marker of latently infected cells (even though a large fraction of integrated HIV-1 DNA are defective). In addition, it was shown in [12] that for infected patients about 85% of infected CD4+ T cells in blood contain only one copy of HIV-1 DNA.

With regard to the viral load measurements, it is worth noting that if the measurements of RNA copies are below the limit of quantification for the assay used (400 copies/ml-plasma, 200 copies/ml-plasma, 80 copies/ml-plasma, 50 copies/ml-plasma, 48 copies/ml-plasma, 25 copies/ml-plasma or 20 copies/ml-plasma), then the observed viral load value is censored to be at its detection limit; that is, in these cases the observed values do not represent the true data values anymore. Furthermore, observations of viral load and CD4+ T cells may not be at the same time points and the observation times and intervals vary substantially among patients. So, in general, for patient number \( j \) we have CD4+ T-cell data pairs \( (t_{ij}, \bar{y}_{ij}^1) \), \( i = 1, \cdots, N_j^1 \), potentially different time point viral RNA data pairs \( (t_{ij}^j, \bar{y}_{ij}^2) \), \( i = 1, \cdots, N_j^2 \), and latently infected T-cell data pairs \( (t_{ij}^j, \bar{y}_{ij}^3) \), \( i = 1, \cdots, N_j^3 \). (We remark that \( \{t_{ij}^j\}_{i=1}^{N_j^3} \) is a subset of \( \{t_{ij}^j\}_{i=1}^{N_j^1} \) with \( N_j^3 \) far less than \( N_j^1 \) for all the patients investigated.) Hence, the clinical data for carrying out the inverse problem involves partial observations, measurements from combined compartments, and highly censored viral load measurements.

### 3.1 Parameter Estimation

To obtain patient specific-parameter estimates for the model, we use individual patient data and carry out an inverse problem for each. The technique we use in this paper is adapted from the one in [1, 3], where a statistically-based censored data method (an expectation maximization algorithm) was used. We note that the variance \( \sigma_1^2 \) in CD4 measurements, the variance \( \sigma_2^2 \) in viral load measurements and the variance \( \sigma_3^2 \) in latently infected CD4+ T cells are likely to be different due to assay differences. Hence, we use the expectation maximization algorithm based on the maximum likelihood estimation technique for \( (q, \sigma_1^2, \sigma_2^2, \sigma_3^2) \) which, under normality assumptions on the measurement errors, results in a weighted least-squares technique (see [4, 5, 18]) with solution given by

\[
\hat{q}^j = \arg \min_{q \in Q} J^j(q)
\] (3.2)
for the log$_{10}$-transformed system of model (2.1) for patient $j$. In the above equation,

$$
J^j(q) = \frac{1}{N_1} \sum_{i=1}^{N_1} |y_1^{ij} - z_1(t_i^j; q)|^2 + \frac{1}{N_2} \sum_{i=1}^{N_2} |y_2^{ij} - z_2(t_i^j; q)|^2 + \frac{1}{N_3} \sum_{i=1}^{N_3} |y_3^{ij} - z_3(t_i^j; q)|^2,
$$

where

$$
\hat{\sigma}_k^2 = \frac{1}{N_k} \sum_{i=1}^{N_k} |y_k^{ij} - z_k(t_i^j; \hat{q})|^2, \quad k = 1, 2, 3
$$

with $y_k^{ij} = \log_{10} \tilde{y}_k^{ij}$ and $z_k(t_i^j; q) = \log_{10}(\tilde{z}_k(t_i^j; q)), i = 1, \ldots, N_k, k = 1, 2, 3$. As we noted earlier, $\{t_i^j\}_{i=1}^{N_k}$ is a subset of $\{q_i^{1j}\}_{i=1}^{N_1}$. Hence, at these time points $t_i^j$, $i = 1, \ldots, N_k$, we are able to observe $T_1 + T_1^* + T_2$, but at other time points we can only observe the total number of CD4+ T cells. The number of latently infected CD4+ T cells is negligible compared to the total number of CD4+ T cells (less than one/µl-blood compared to hundreds/µl-blood) and $N_3$ is much smaller than $N_1$. Hence, for simplicity we use (3.3) as our cost function.

As noted in [1, 3], by using a log-transformed system one can resolve a problem of states becoming unrealistically negative due to round-off error: nonnegative solutions of this model should stay so throughout numerical simulation. This approach also enables efficient handling of unrealistic cases where states become infinitesimally small during integration due to parameters selected by optimization algorithms. From a statistical point of view, log transformation is a standard technique to render the observations more nearly normally distributed, which also supports use of the weighted least squares criterion as an equivalent to maximum likelihood estimation.

The expectation maximization (EM) algorithm is outlined below. To simplify the notation, we drop the patient index $j$ in this algorithm description. The following notation will be used in the algorithm: the relevant censoring point at time $t_i^j$ is represented by $L^i$ and $\chi^i$ is the indicator function for the set $\{y_2^i > L^i\}$, $\phi$ denotes the standard normal probability density function and $\Phi$ is the corresponding cumulative distribution function. For each patient, we carried out the following parameter estimation algorithm:

- (Step 1) Create adjusted data $\tilde{y}_i^j$ by replacing censored $y_2^i$ values by $L^i/2$, and use ordinary least squares to estimate $\hat{q}^{(0)}$ using both CD4+ data $y_1^i$ and adjusted viral RNA data $\tilde{y}_i^j$ (which now includes replaced censored values).

$$
\hat{q}^{(0)} = \arg \min_{q \in Q} \left[ \frac{1}{N_1} \sum_{i=1}^{N_1} |y_1^i - z_1(t_i^j; q)|^2 + \frac{1}{N_2} \sum_{i=1}^{N_2} |\tilde{y}_i^j - z_2(t_i^j; q)|^2 + \frac{1}{N_3} \sum_{i=1}^{N_3} |y_3^i - z_3(t_i^j; q)|^2 \right].
$$
Obtain an initial estimate for $\sigma_1^2$, $\sigma_2^2$ and $\sigma_3^2$ from

$$\left(\hat{\sigma}_1^{(0)}\right)^2 = \frac{1}{N_1} \sum_{i=1}^{N_1} |y_i^1 - z_1(t_i^1; \hat{q}^{(0)})|^2,$$

$$\left(\hat{\sigma}_2^{(0)}\right)^2 = \frac{1}{N_2} \sum_{i=1}^{N_2} |\tilde{y}_i^2 - z_2(t_i^2; \hat{q}^{(0)})|^2,$$

$$\left(\hat{\sigma}_3^{(0)}\right)^2 = \frac{1}{N_3} \sum_{i=1}^{N_3} |y_i^3 - z_3(t_i^3; \hat{q}^{(0)})|^2$$

respectively. Set $k = 0$.

- (Step 2) Define $\hat{y}_i^{(k)} = z_2(t_i^2; \hat{q}^{(k)})$ and $\hat{\zeta}_i^{(k)} = \frac{L_i - \hat{y}_i^{(k)}}{\hat{\sigma}_2^{(k)}}$. Update the data and residuals by

$$\tilde{y}_i^{(k)} = \chi_i^1 y_i^1 + (1 - \chi_i^1) \left[\hat{y}_i^{(k)} - \hat{\sigma}_2^{(k)} \phi(\hat{\zeta}_i^{(k)})\right],$$

$$\tilde{r}_i^{(k)} = \chi_i^1 (y_i^1 - \hat{y}_i^{(k)})^2 + (1 - \chi_i^1) (\hat{\sigma}_2^{(k)})^2 \left[1 - \hat{\zeta}_i^{(k)} \phi(\hat{\zeta}_i^{(k)})\right].$$

- (Step 3) Update the estimate of $q$ to $\hat{q}^{(k+1)}$ by performing the weighted least squares minimization in the parameters $q$

$$\hat{q}^{(k+1)} = \arg \min_{q \in Q} \left[\frac{1}{N_1(\hat{\sigma}_1^{(k)})^2} \sum_{i=1}^{N_1} |y_i^1 - z_1(t_i^1; q)|^2 + \frac{1}{N_2(\hat{\sigma}_2^{(k)})^2} \sum_{i=1}^{N_2} |\tilde{y}_i^2 - z_2(t_i^2; q)|^2 + \frac{1}{N_3(\hat{\sigma}_3^{(k)})^2} \sum_{i=1}^{N_3} |y_i^3 - z_3(t_i^3; q)|^2\right],$$

and computing

$$\left(\hat{\sigma}_1^{(k+1)}\right)^2 = \frac{1}{N_1} \sum_{i=1}^{N_1} |y_i^1 - z_1(t_i^1; \hat{q}^{(k+1)})|^2,$$

$$\left(\hat{\sigma}_2^{(k+1)}\right)^2 = \frac{1}{N_2} \sum_{i=1}^{N_2} \tilde{r}_i^{(k)},$$

$$\left(\hat{\sigma}_3^{(k+1)}\right)^2 = \frac{1}{N_3} \sum_{i=1}^{N_3} |y_i^3 - z_3(t_i^3; \hat{q}^{(k+1)})|^2.$$

If relative changes in $\hat{q}$, $\hat{\sigma}_1$, $\hat{\sigma}_2$ and $\hat{\sigma}_3$ are small, terminate. Otherwise set $k = k + 1$ and then go to Step 2.

Although the EM algorithm may seem unnecessarily complicated, it should be noted that previous attempts to fit censored clinical data without the EM algorithm produced extremely poor results. For more details about the EM algorithm and how to carry it out, interested readers are referred to [1, 7, 15].

### 3.2 Numerical Results

For all the simulation results shown below, we fixed the values of the parameters $d_2$, $f$, $\delta_{E1}$, $\delta_{E2}$, $K_s$, $K_{b1}$, $K_{b2}$, $K_d$, $K_{\gamma}$, $V_{NI}^0$, $E_{1}^0$ and $E_{2}^0$ as those obtained in [3], and estimated the
rest of model parameters and initial conditions (30 out of 42). We then fixed another 10 parameters at the population averages across the 12 patients investigated, and re-estimated the remaining 20 parameters for each patient. Table 2 specifies all the fixed parameters (19 model parameters and 3 initial conditions) and their corresponding values.

<table>
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<th>Value</th>
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<td>$K_V$</td>
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</tr>
<tr>
<td>$K_s$</td>
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<tr>
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<td>3.571e+03</td>
</tr>
<tr>
<td>$E_1^0$</td>
<td>6.821e-02</td>
</tr>
<tr>
<td>$E_2^0$</td>
<td>6.909e-01</td>
</tr>
</tbody>
</table>

Table 2: The values of parameters $d_2$, $f$, $\delta_{E1}$, $\delta_{E2}$, $K_{\gamma}$, $K_{b1}$, $K_d$, $K_s$, $V^0_{NI}$, $E_1^0$ and $E_2^0$ are adopted from Table 5 of [3], and the values of the remaining parameters are fixed as the population averages across the 12 patients investigated.

### 3.2.1 Modeling Fitting

Figures 3 – 5 depict model fitting results for those patients whose treatment started during the acute stage, and Figures 6 – 8 present similar results for those patients whose treatment started during the chronic stage, where time zero is the time point where we have the first observation. From these figures, we see that we obtain reasonable fits for all the 12 patients investigated. In addition, we found that the predicted number of HIV-specific CD8+ T cells ($E_1 + E_2$) is within the normal range (the maximum value is less than 300 copies/µl).
Figure 3: Clinical data ('x') for the total number of CD4+ T-cells, latently infected CD4+ T-cells and viral load are shown in the upper, middle and lower rows, respectively. Dark circles denote the predicted censored data values. A solid line along the x-axis in the upper figure indicates periods when the patient is on ART treatment, while a dashed line indicates off-treatment periods.
Figure 4: Clinical data (‘x’) for the total number of CD4+ T-cells, latently infected CD4+ T-cells and viral load are shown in the upper, middle and lower rows, respectively. Dark circles denote the predicted censored data values. A solid line along the x-axis in the upper figure indicates periods when the patient is on ART treatment, while a dashed line indicates off-treatment periods.
Figure 5: Clinical data (’x’) for the total number of CD4+ T-cells, latently infected CD4+ T-cells and viral load are shown in the upper, middle and lower rows, respectively. Dark circles denote the predicted censored data values. A solid line along the x-axis in the upper figure indicates periods when the patient is on ART treatment, while a dashed line indicates off-treatment periods.
Figure 6: Clinical data (‘x’) for the total number of CD4+ T-cells, latently infected CD4+ T-cells and viral load are shown in the upper, middle and lower rows, respectively. Dark circles denote the predicted censored data values. A solid line along the x-axis in the upper figure indicates periods when the patient is on ART treatment, while a dashed line indicates off-treatment periods.
Figure 7: Clinical data (‘x’) for the total number of CD4+ T-cells, latently infected CD4+ T-cells and viral load are shown in the upper, middle and lower rows, respectively. Dark circles denote the predicted censored data values. A solid line along the x-axis in the upper figure indicates periods when the patient is on ART treatment, while a dashed line indicates off-treatment periods.
Figure 8: Clinical data (‘x’) for the total number of CD4+ T-cells, latently infected CD4+ T-cells and viral load are shown in the upper, middle and lower rows, respectively. Dark circles denote the predicted censored data values. A solid line along the x-axis in the upper figure indicates periods when the patient is on ART treatment, while a dashed line indicates off-treatment periods.
3.2.2 Stability Analysis

In this section, we obtain the off-treatment (i.e., $\xi_1 = \xi_2 = 0$) equilibrium for model (2.1) with parameter values obtained for each patient. Results show that none of these patients has a locally asymptotically stable (a.s.) uninfected equilibrium, which is characterized by zero infected CD4+ T cells and zero viral load level (i.e., $T^*_1 = 0$, $T^*_2 = 0$, $V_I = 0$ and $V_{NI} = 0$).

To have a clear idea of the locally a.s. equilibrium possessed by each patient, we plotted the viral load level versus the concentration of latently infected CD4+ T cells for those locally a.s. equilibrium in Figure 9. We observe from this figure that all the patients have at least one locally a.s. infected equilibrium: patients AC34, AC161 and PCJ have two while all the other patients have only one. In addition, we note that patients AC31, AC34, AC81 AC161, CDM, GMM, PCJ and SBD have a locally a.s. infected equilibrium with viral load level below the detection limit of 20 copies/ml. For these eight patients, we found that it takes 16, 36, 9 and 8 months of treatment to eventually converge to this undetectable steady state for patients AC31, AC34, AC161 and PCJ, respectively. With the estimated initial conditions it will eventually converge to the undetectable steady state for AC81, CDM, GMM and SBD without any treatment. From Figure 9, we also see that if the concentration of the latently infected CD4+ T cells is greater than 1 cells/$\mu$l, then the viral load is above the detection limit of 20 copies/ml.
Figure 9: Plots of viral load level versus the concentration of latently infected CD4+ T cells for those locally asymptotically stable equilibrium obtained for each patient. The dashed black line indicates the detection limit of 20 copies/ml.
4 Immuno-modulatory Strategies for Eradication of the Latent Reservoir

In this section we use our validated mathematical model together with patient-specific parameter estimates to investigate which pharmacological strategy or combination of strategies that affect the immune system may help eradicate the latent reservoir. We first present results from an off-treatment stability analysis that was used to investigate the effects of altering (increasing or decreasing) several sets of immune system parameters for each patient. These stability analysis results are then corroborated by a simulation analysis in which we investigated all possible combinations of immune system parameter changes. We note that the calculation for equilibrium stability for one choice of parameter changes for each patient requires approximately 8 hours on a single core desktop computer. In contrast, simulating the dynamics, e.g., for 6 patient years, for all possible parameter combinations we consider requires approximately 100 hours per patient. We note that although we did not calculate stability for all possible immune system parameter changes, the changes which we did investigate are among those we feel are clinically relevant.

Specifically, our analyses considered changes to the following parameters:

- $a_A$: activation rates of uninfected resting CD4+ T cells,
- $a_{As}$: activation rates of latently infected CD4+ T cells,
- $a_E$: activation rates of HIV-specific memory CD8+ T cells,
- $p_T$: proliferation rate of CD4+ T cells,
- $p_E$: proliferation rate of HIV-specific CD8+ T cells,
- $m$: elimination rate of infected activated CD4+ T cells by HIV-specific effector CD8+ T cells.

Stability analysis of these parameters was motivated by existing drugs that might be effective as adjuvant therapies to ART. For example, interferon therapy has several effects, including stimulating CD4+ and CD8+ T cell proliferation and increasing the efficacy of CD8+ T cells. The activation of T cells could be increased with HDAC inhibitor therapy, and could be decreased with immuno-suppression therapy.

4.1 Stability Analysis: Modulation of Immune System Parameters

In our stability analysis, a strategy is deemed to be useful to eradicate the reservoir only if it can make all the patients have a locally a.s. uninfected equilibrium. The rational is as
follows. A given combination of therapies aimed at eradication of the reservoir is given to the patient while he/she remains on ART. When the viral load is close to zero, ART can be withdrawn as the existence of a locally a.s. uninfected equilibrium ensures that the patient’s state will eventually converge to this equilibrium. Once the patient is totally cured, then the given combination of therapies can be withdrawn.

If a strategy is found to be unable to make the patient have a locally a.s. uninfected equilibrium, we compare its results with the ones obtained for the case where the estimated values for all the parameters are not changed, i.e., the baseline, to determine whether or not it is beneficial to the patient. We remark that if a proposed strategy and/or the baseline case have more than one locally a.s. equilibrium, then we only compare the results for their corresponding equilibrium with the lowest viral load level. We examined whether any of the following strategies is useful in eradication the reservoir: increasing/decreasing the activation rates, increasing/decreasing the activation and proliferation rates, increasing the activation rates and the efficacy of CD8+ T cells, and, lastly, increasing the activation and proliferation rates and the efficacy of CD8+ T cells.

### 4.1.1 Stability Analysis: Varying the Activation Rates

In this section, we consider the strategies for varying only the activation rates. Specifically, we determine which strategy, activation versus deactivation, is useful in eradicating the reservoir. The deactivation strategy is motivated by the results in [23], where the immunosuppressant therapy “sirolimus” is used to treat HIV-infected kidney transplant recipients and it was found that a longer duration of immunosuppressant therapy was associated with a lower HIV DNA levels.

### 4.1.2 Results for Increasing the Activation Rates

Since different types of drugs may have different effects on the activation rates of different types of T cells, we investigate the following four cases:

- (IA1): increase $a_{As}$ by 900%,
- (IA2): increase $a_A$ and $a_{As}$ by 900%,
- (IA3): increase $a_{As}$ and $a_E$ by 900%,
- (IA4): increase $a_A$, $a_{As}$ and $a_E$ by 900%.

Stability analysis shows that for all the four cases none of these 12 patients has a locally a.s. uninfected equilibrium. To have a clear idea of the effect of each case on these patients, we plotted the viral load level versus the concentration of latently infected CD4+ T cells.
for those locally a.s. equilibrium obtained for each case, and compare them with the ones obtained for the baseline case. The results are shown in Figure 10. We observe from this figure that for strategy (IA3) all the patients have a lower level of latently infected CD4+ T cells for those locally asymptotically stable equilibrium obtained for each case, and compare them with the ones obtained for the baseline case. The results are shown in Figure 10. We observe from this
T cells than their corresponding ones obtained for the baseline case. This is also true for most of the patients in the other three cases. Thus, increasing the activation rates in general makes most of the patients have a lower level of latently infected CD4+ T cells. However, each strategy has a different effect on the viral load level. Specifically, the viral load level obtained for case (IA1) is either similar to or larger than that obtained for the baseline case, the one obtained for case (IA2) is bigger than that for the baseline case, and the one obtained for case (IA3) is smaller than the one for the baseline case. The results obtained for case (IA4) are mixed: half of the patients have a lower viral load level than that for the baseline case, and the rest of them have a larger viral load level.

Based on these results, we see that strategy (IA3) is beneficial to the patients while all the other three strategies have a mixed effect on the patients. Thus, if possible, one should focus on only increasing the activation rates for the latently infected CD4+ T cells and HIV-specific memory CD8+ T cells. Otherwise, it may have a detrimental effect on the patients.

At this point, a natural question to ask is whether or not one can obtain a locally a.s. uninfected equilibrium for case (IA3) by further increasing the value of $a_E$. To answer this question, we increased $a_{As}$ by 900% but increased $a_E$ by 99900%, and still found that none of the patients has a locally a.s. uninfected equilibrium. In addition, we found that it has detrimental effect on patients AC34 and FB0 as for this case both patients have a much higher viral load level than that for the baseline case (see Figure 11). However, it is beneficial to all the other 10 patients as for this case they all have a much lower viral load level and concentration of latently infected CD4+ T cells than their corresponding ones obtained for case (IA3) (again see Figure 11). Overall, these results suggest that increasing the activation rate for the HIV-specific CD8+ T cells may not be sufficient to eradicate the reservoir and may have detrimental effects on patients if it is increased too much.
Figure 11: Plots of viral load level versus the concentration of latently infected CD4+ T cells for those locally asymptotically stable equilibrium obtained for the baseline case (shown in the legend by “base”), case (IA3) (shown in the legend by “a\textsubscript{As}, a\textsubscript{E}: 900%”), and the case for increasing $a\textsubscript{As}$ by 900% and $a\textsubscript{E}$ by 99900% (shown in the legend by “$a\textsubscript{As}: 900\%, a\textsubscript{E}: 99900\%$”). The dashed black line indicates the detection limit of 20 copies/ml.
4.1.3 Results for Decreasing the Activation Rates

In this section, we consider the strategy for decreasing the activation rates of T cells. Specifically, we consider the following three cases:

- (DA1): decrease $a_A$ by 90%,
- (DA2): decrease $a_A$ and $a_{As}$ by 90%,
- (DA3): decrease $a_A$, $a_{As}$ and $a_E$ by 90%.

For case (DA1), we found that all of the patients except patient FBO have a locally a.s. uninfected equilibrium. If we further decrease the estimated $a_A$ by 99%, then patient FBO also has a locally a.s. uninfected equilibrium. We obtained exactly the same results for case (DA2). This suggests that decreasing the activation rates for CD4+ T cells can create stability in the uninfected equilibrium. In addition, decreasing the activation rates for the uninfected resting CD4+ T cells ($T_2$) plays a major role in creating this stability. This is expected as decreasing the activation rates for $T_2$ decreases the number of activated CD4+ T cells, which are the most permissive target for HIV infection.

For case (DA3), we found that all the patients except AC31, AC34, AC81 and FBO have a locally a.s. uninfected equilibrium. Specifically, patients AC31, AC34, AC81 do not have a physical equilibrium in this case, and patient FBO still has one locally a.s. infected equilibrium. If we further decrease $a_A$, $a_{As}$ and $a_E$ by 99%, then all the patients have a locally a.s. uninfected equilibrium. This suggests that global suppression of activation is also possible to create the stability in the uninfected equilibrium.

4.1.4 Summary Remarks on Strategies for Varying Activation Rates

Our analysis suggests that increasing the activation rates in general makes most of the patients have a lower level of latently infected CD4+ T cells. However, it may dramatically increase the viral load level, depending on the intensity and the types of T cells that were activated. This conforms with the results observed in [16], where their results suggest that eradication strategies such as these aimed mainly at the reactivation of latently infected resting CD4+ T cells may face a difficult task because they observed high levels of proliferation in the memory cells and yet they saw no substantial decay in integrated HIV DNA.

Our results also suggest that decreasing the activation rates can create the stability in uninfected equilibrium and thus it can be used as a strategy to eradicate the reservoir. In addition, the intensity of suppression of the activation of uninfected resting CD4+ T cells is essential to the stability of the uninfected equilibrium. Based on the results in [23], this finding is not unexpected from the clinical point of view.
4.1.5 Stability Analysis: Varying Both Activation and Proliferation Rates

In this section, we consider the strategies for varying both the activation rates and the proliferation rates. Specifically, we determine which strategy, increasing versus decreasing of the activation and proliferation rates, is useful in eradicating the reservoir. The strategy for decreasing the activation and proliferation rates (i.e., suppression of the immune response) is again motivated by the study in [23] for using immunosuppressive therapy to treat HIV-infected kidney transplant recipients.

4.1.6 Results for Increasing Both Activation and Proliferation Rates

In this section, we consider the strategy for increasing both the activation rates and the proliferation rates. Specifically, we consider the following cases:

- (IAP1): increase $a_A$, $a_{As}$ and $a_E$ by 900%, and increase $p_E$ by 900%,
- (IAP2): increase $a_A$, $a_{As}$ and $a_E$ by 900%, and increase $p_T$ and $p_E$ by 900%,
- (IAP3): increase $a_A$, $a_{As}$ and $a_E$ by 900%, and increase $p_E$ by 900%.
- (IAP4): increase $a_A$, $a_{As}$ and $a_E$ by 900%, and increase $p_T$ and $p_E$ by 900%.

Results show that none of these patients has a locally a.s. uninfected equilibrium for these four cases. To have a clear idea of the effect of each case on these patients, we plot the viral load level versus the concentration of latently infected CD4+ T cells for those locally a.s. equilibrium obtained for each case, and compare them with the ones obtained for the baseline case. The results are shown in Figure 12. We observe from this figure that for the first three cases both the viral load level and the concentration of the latently infected CD4+ T cells are smaller than their corresponding ones obtained for the baseline case. This is also true for most of the patients using the strategy (IAP4). In addition, we see that the strategy (IAP1) is the best one in terms of viral load level. This means that increasing the proliferation rates for the CD4+ T cells and increasing the activation rates for the uninfected resting CD4+ T cells both have a negative effect on the patients. Thus, if possible, one should focus on only increasing the activation rates for the latently infected CD4+ T cells and HIV-specific CD8+ T cells and only increasing the proliferation rates for the HIV-specific CD8+ T cells.

At this point, a natural question to ask is whether or not one can obtain a locally a.s. uninfected equilibrium for case (IAP1) by further increasing the value of $p_E$. To answer this question, we increased $a_A$, $a_{As}$ and $a_E$ by 900% but increased $p_E$ by 99900%, and still found that none of these patients has a locally a.s. uninfected equilibrium. However, we found that for this case all the patients have a lower viral load level and smaller concentration of latently infected CD4+ T cells than their corresponding ones obtained for case (IAP1) (see Figure 13). This results suggest that increasing the proliferation rates for the HIV-specific CD8+ T cells may not be sufficient to eradicate the reservoir but it is beneficial to patients.
Figure 12: Plots of viral load level versus the concentration of latently infected CD4+ T cells for those locally asymptotically stable equilibrium obtained for the baseline case (shown in the legend by “base”), case (IAP1) (shown in the legend by “$a_{As}, a_E, p_E$: 900%”), case (IAP2) (shown in the legend by “$a_{As}, a_E, p_T, p_E$: 900%”), and case (IAP3) (shown in the legend by “$a_A, a_{As}, a_E, p_E$: 900%”) and case (IAP4) (shown in the legend by “$a_A, a_{As}, a_E, p_T, p_E$: 900%”). The dashed black line indicates the detection limit of 20 copies/ml.
Figure 13: Plots of viral load level versus the concentration of latently infected CD4+ T cells for those locally asymptotically stable equilibrium obtained for the baseline case (shown in the legend by “base”), case (IAP1) (shown in the legend by “$a_{As}, a_E, p_E: 900\%$”) and the case for increasing $a_{As}$, $a_E$ by 900% and $p_E$ by 99900% (shown in the legend by “$a_{As}, a_E : 900\%, p_E: 99900\%$”). The dashed black line indicates the detection limit of 20 copies/ml.
4.1.7 Results for Decreasing Both Activation and Proliferation Rates

In this section, we consider the case for decreasing both the activation rates and the proliferation rates. Specifically, we decreased $a_A$, $a_{As}$, $a_E$, $p_T$ and $p_E$ by 90% and found that all the patients have a locally a.s. uninfected equilibrium. In addition, all these patients only have this one physical equilibrium. This suggests that decreasing both the activation rates and proliferation rates can create the stability in the uninfected equilibrium, and hence a proper suppression of the immune system can be used as a strategy to eradicate the reservoir. Recall that it was found in [23] that a longer duration of immunosuppressant therapy was associated with a lower HIV DNA levels. Hence, our finding is not unexpected from the clinical point of view.

4.1.8 Stability Analysis: Increasing Both Activation Rates and the Efficacy of HIV-Specific Effector CD8+ T Cells

In this section, we consider the strategy for increasing both the activation rates and the efficacy of the HIV-specific effector CD8+ T cells. Specifically, we consider the following two cases:

- (IAM1): increase $a_{As}$, $a_E$ and $m$,
- (IAM2): increase $a_A$, $a_{As}$, $a_E$ and $m$,

where the value of $m$, the efficiency of killing infected cells, is used here to characterize the efficacy of HIV-specific effector CD8+ T cells.

For case (IAM1), we increased $a_{As}$ and $a_E$ by 900% and $m$ by 9900% and found that all the patients except patients FBO, PCJ and SCM have a locally a.s. uninfected equilibrium. However, it does have a beneficial effect on these 3 patients too, as both patients FBO and SCM now have a locally a.s. infected equilibrium with viral load below the detection limit, and patient PCJ now only has an infected equilibrium with viral load $2.997 \times 10^{-3}$ copies/ml, less than the one obtained for the baseline case. If we further increase $m$ by 49900%, then patients FBO, PCJ and SCM also have a locally a.s. uninfected equilibrium, which is the only physical equilibrium obtained for these patients in this case.

For case (IAM2), we increased $a_A$, $a_{As}$ and $a_E$ by 900% and $m$ by 9900% and found that none of the patients has a locally a.s. uninfected equilibrium. Based on this result and the one obtained for case (IAM1), we see that increasing the activation rate for the uninfected resting CD4+ T cells can destroy the stability of the uninfected equilibrium. If we further increase $m$ by 99900%, then all the patients have a locally a.s. uninfected equilibrium. Thus, if we can only globally activate all the T cells, then we need to increase the efficacy of HIV-specific CD8+ T cells significantly high in order to achieve the stability of the uninfected equilibrium.
Overall, the above results and the ones obtained in Section 4.1.2 for simply increasing the activation rates suggest that the efficacy of HIV-specific CD8+ T cells is essential to the stability of the uninfected equilibrium, and thus it is essential for successful eradication of the reservoir. This is in agreement with the conclusion obtained in [19].

4.1.9 Stability Analysis: Increasing Activation Rates, Proliferation Rates and the Efficacy of HIV-Specific Effector CD8+ T Cells

In this section, we consider the strategy for increasing the activation and proliferation rates as well as the efficacy of the HIV-specific effector CD8+ T cells. Specifically, we consider the following two cases:

- (IAM1): increase $a_{As}$, $a_E$, $p_T$ and $p_E$ and $m$,
- (IAM2): increase $a_A$, $a_{As}$, $p_T$ and $p_E$, $a_E$ and $m$.

For case (IAM1), we increased $a_{As}$, $a_E$, $p_T$ and $p_E$ by 900% and $m$ by 9900% and found that none of these patients has a locally a.s. uninfected equilibrium. Based on this result and the results presented in Section 4.1.8, we again see that increasing the proliferation rates for the CD4+ T cells has a negative effect on the patients as it can destroy the stability of the uninfected equilibrium. If we further increase $m$ by 49900%, then all the patients except patient FBO have a locally a.s. uninfected equilibrium. If we increase $m$ by 99900%, then patient FBO also has a locally a.s. uninfected equilibrium.

For case (IAM2), we increased $a_A$, $a_{As}$, $a_E$, $p_T$ and $p_E$ by 900% and $m$ by 99900% and found that all the patients have a locally a.s. uninfected equilibrium. In addition, all these patients only have this one physical equilibrium.

Based on the above results and the ones obtained in Section 4.1.6, we again see that the efficacy of HIV-specific effector CD8+ T cells is essential to the stability of the uninfected equilibrium.

4.2 Simulation Analysis: Modulation of Immune System Parameters

To verify and extend the analysis of equilibria stability, we also performed simulations for all possible immune system parameter changes for each patient. The parameter changes we considered were chosen from the set \{${a_A, a_{As}, a_E, p_T, p_E, m}$\}. Parameters were altered by decreasing 100-fold or 10-fold, left unchanged, or increased by 10-fold or 100-fold, i.e., \{-99\%, -90\%, 0\%, 900\%, 9900\%\}, for a total of $5^6$ possible combinations per patient. Estimated values, from clinical data fitting, of the six chosen immune system parameters were
altered and the rest of the model parameters were kept the same as their estimated values. We ran simulations for each patient for 3 years on ART, followed by 3 years off ART.

Simulation endpoints, at 6 years, were analyzed to determine which parameter changes could be beneficial towards inducing a stable virus-free or virus-undetectable state while also lowering the concentration of latently infected cells. For our simulation analysis, parameter changes are deemed beneficial if their effect is to lower or keep the viral load below the detection limit after 3 years on ART followed by 3 years off ART. Since simulations can only inform about the short-term dynamics, we also required the viral load and latently infected cell levels to be non-increasing at the 6 year time point (3 years post-ART), e.g., less than or equal to the viral load at 5 years (2 years post-ART). This requirement is used to qualitatively assess the stable convergence of post-ART viral load to levels below the detection limit.

4.2.1 Simulation Analysis: Modulation of Single Immune System Parameters

We first analyzed the effect of changing one of the immune system parameters at a time for each patient, assuming they underwent 3 years of ART. We found that lowering the activation rate of non-infected CD4+ T cells, $a_A$, lowered the viral load and latently infected cell count for all patients; increasing $a_A$ had the opposite effect (Figure 14, top right). In contrast, the majority of patients were insensitive to changes in the activation rate of latently infected cells, $a_{As}$ (Figure 14, top left). Moreover, some patients showed increases in either the viral load and/or the latently infected cell count when the parameter $a_{As}$ was increased. Increases in the parameter $a_E$, the CD8+ T cell activation rate, both lowered the viral load and latently infected cell count for most patients (Figure 14, middle left). However, we found a non-linear relationship between $a_E$ and the viral load and latently infected cell levels for some patients. For example, a 10-fold (900%) increase in $a_E$ was beneficial for patient CDM, but a 100-fold (9900%) had the opposite effect and increased the viral load and latently infected cell levels. Similar non-linear effects due to changes in $a_E$ were found for both chronically and acutely treated patients (SBD, GMM, FB0, AC81, AC65, and AC31).

Decreasing the proliferation rate of CD4+ T cells, $p_T$, lowered the viral load and latently infected cell levels to below the detection limit for all patients, whereas increasing $p_T$ had the opposite effect (Figure 14, middle right). Conversely, increases in the proliferation rate of CD8+ T cells, $p_E$, led to a decrease in viral load and latently infected cell levels, and decreasing $p_E$ had the opposite effect (Figure 14, bottom left).

Lastly, increasing the rate of CD4+ T cell death due to effector CD8+ T cells pushed the viral load to below the detection limit for all patients, while also lowering the latently infected cell levels. For most patients, a 10-fold increase (900%) in $m$ was enough to drive the viral load below the detection limit, however, for patient FBO a 100-fold increase 9900% was necessary (Figure 14, bottom right). For all patients, a 100-fold increase in $m$ was drastically more beneficial than a 10-fold increase, i.e., the viral load was several orders of magnitude lower. Overall, these results suggest that altering kinetic parameters that effect the host immune
Figure 14: The effect of decreasing a parameter 100-fold (−99%, large squares) or 10-fold (−90%, small squares), or increasing a parameter 10-fold (+900%, small triangles) or 100-fold (+9900%, large triangles) on post-treatment levels of viral load and latently infected CD4+ T cells. Data points show the viral load level versus the concentration of latently infected CD4+ T cells after 3 years of ART followed by 3 years off ART. The baseline for each patient with no parameter change is plotted as a circle. The dashed black line indicates the detection limit of 20 copies/ml.
system, especially decreasing $a_A$ and $p_T$, may have beneficial effects for all patients.

4.2.2 Simulation Analysis: Combinatorial Modulation of Immune System Parameters

Next, we calculated which combination of parameter changes, i.e., immune system modulation strategies, could lower both the viral load and latently infected cell levels for all patients after 3 years of ART. For each set of $5^6$ combinations of parameter changes, we calculated which combinations pushed the viral load below the detection limit after 3 years on ART followed by 3 years off ART. We calculated both patient-specific strategies as well as strategies that were beneficial for all 12 patients considered in this study.

We note that only essential changes were kept, and redundant or ineffective parameter changes were removed. For example, for some patients, lowering $a_A$ induced a viral load below the detection limit, whether $p_E$ was decreased or increased by 100-fold, and we deduced that $a_A$ and not $p_E$ was the essential parameter to change. We also removed any strategies that had a non-linear effect on viral load. For example, if increasing $a_E$ and decreasing $a_A$, both by 10-fold, decreased the viral load, but increasing $a_E$ and decreasing $a_A$, both by 100-fold, increased the viral load, then this strategy was not included. In addition, we also required both 10-fold and 100-fold changes to chosen parameter combinations to reduce the viral load to below the detection limit.

Our patient-specific strategy results highlight the possibility for personalized medicine treatments, where only a small number of parameter changes in immune system kinetics may be necessary for a cure in some patients, whereas other patients may require a diverse set of changes, i.e., combination therapy (Figures 15 and 16). Notably, the only single parameter change strategy that decreases viral load and latently infected cell levels for all 12 patients is decreasing $p_T$.

In total, we found 21 strategies that were able to induce a viral load below the detection limit for all patients (Table 3, Figures 17, 18). In theory, combination therapies that correspond to these strategies, i.e., by inducing the respective parameters changes, may lead to a stable virus-free state for all patients who undergo at least 3 years of ART.
Figure 15: Results for those patients whose treatment began during the acute phase: Plots of viral load level versus the concentration of latently infected CD4+ T cells for patient specific treatment strategies. Increases and decreases in kinetic parameters are denoted by up and down arrows, respectively. Large colored circles = 100-fold changes, Small colored circles = 10-fold changes, Baseline levels with no parameter changes = open black/white circles. The dashed black line indicates the detection limit of 20 copies/ml.
Figure 16: Results for those patients whose treatment began during the chronic phase: Plots of viral load level versus the concentration of latently infected CD4+ T cells for patient specific treatment strategies. Increases and decreases in kinetic parameters are denoted by up and down arrows, respectively. Large colored circles = 100-fold changes, Small colored circles = 10-fold changes, Baseline levels with no parameter changes = open black/white circles. The dashed black line indicates the detection limit of 20 copies/ml. Note: a 100-fold change for the last strategy for patient FBO induced a viral load $< 10^{-200}$. 

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Table 3: Strategies that induce a viral load below the detection limit after 3 years of ART treatment. Increases and decreases in kinetic parameters are denoted by up and down arrows, respectively.
Figure 17: The effect of combination treatment strategies (see Table 3) on viral load and latently infected cell levels for all patients. Baseline levels when no parameters are changed are plotted as triangles; 10-fold and 100-fold parameter changes are shown as small and large circles, respectively. Patient ID colors: AC31 (Yellow), AC32 (Purple), AC34 (Light Blue), AC65 (Red), AC81 (Green), AC161 (Blue), CDM (Black), FBO (Black/Yellow), GMM (Black/Purple), PCJ (Black/Light Blue), SBD (Black/Red), SCM (Black/Green). The dashed black line indicates the detection limit of 20 copies/ml.
Figure 18: A continuation of figure 17. The effect of combination treatment strategies (see Table 3) on viral load and latently infected cell levels for all patients. Baseline levels when no parameters are changed are plotted as triangles; 10-fold and 100-fold parameter changes are shown as small and large circles, respectively. Patient ID colors: AC31 (Yellow), AC32 (Purple), AC34 (Light Blue), AC65 (Red), AC81 (Green), AC161 (Blue), CDM (Black), FBO (Black/Yellow), GMM (Black/Purple), PCJ (Black/Light Blue), SBD (Black/Red), SCM (Black/Green). The dashed black line indicates the detection limit of 20 copies/ml.
4.2.3 Summary Remarks on Simulation Analysis

Although there were no significant differences in the immuno-modulatory strategies revealed to be beneficial between acutely treated and chronically treated patients, there were obvious differences in the personalized strategies chosen between patients. For some patients, changes to a single specific CD4+ or CD8+ T cell related parameter was enough to push the viral load below the detection limit, while other patients required multi-parameter alterations to their immune system kinetics (Figures 15 and 16). A key finding from our simulation results, which is in concordance with our stability analysis, was that the two most beneficial changes for all patients is lowering the activation and proliferation of uninfected CD4+ T cells. This finding was visible in our analysis of single parameter changes and changes to patient-specific parameter combinations (Figures 14, 15 and 16). Moreover, this strategy was present in the list of treatments that were predicted to be beneficial for all patients (Table 3, Figure 17).

Our simulation results also suggest that increasing the activation, proliferation, and efficacy of CD8+ T cells can increase the effectiveness of ART. In general, we found that the most beneficial immuno-modulatory strategy for all patients is to decrease the activation and proliferation of CD4+ T cells, while simultaneously increasing the activation, proliferation, and efficacy of CD8+ T cells.

4.3 Summary Remarks

A concordant finding from both our simulation and stability analyses was that decreasing the activation rates of CD4+ T cells, , may help lower the viral load and eradicate the reservoir of latently infected cells. We only found one strategy for a single patient, SCM, that included increasing , and that strategy also required a decrease in . To confirm this finding, we revisited the patient data set previously analyzed in [3] and found that the two patients with stable virus-free equilibria had significantly lower CD4+ T cell activation rates than the other twelve patients (Mann-Whitney U Test, P = 0.0219, data not shown). Moreover, we found that we could induce stable virus-free equilibria for all fourteen patients from the previous study [3] by decreasing the CD4+ T cell activation rates by 95%, and that this virus-free state could be reached with only 1 year of ART. We note that this finding may be of importance to clinical trials that are currently in progress, e.g., [26], that treat patients with HDAC inhibitors as an adjuvant therapy to ART. Our model predicts that if HDAC inhibitor therapy increases , then it may have the unintended effect of actually increasing the viral load and concentration of latently infected cells when used as the only adjuvant therapy to ART. Instead, we hypothesize that a combination of drugs that can target CD4+ and CD8+ T cell phenotypes separately by decreasing CD4+ T cell activation and proliferation rates while also increasing CD8+ T cell activation, proliferation, and efficacy will be of most benefit to the patient.

Overall, our analysis suggests that simple reactivation of latently infected CD4+ T cells is not sufficient to eradicate the reservoir and that enhancement of the efficacy of the HIV-
specific effector CD8+ T cells is essential to make this strategy to be successful. In addition, our analysis suggests that a proper suppression of the immune system may assist to eradicate the reservoir.

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