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Predicting Differential Responses to Structured Treatment Interruptions During HAART

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Highly active antiretroviral therapy (HAART) has been used clinically in various administration schemes for several years. However, due to the development of drug resistance, evolution of viral strains, serious side effects, and poor patient compliance, the combination of drugs used in HAART fails to effectively contain virus long term in a high proportion of patients. Our group and others have suggested a change to the usual regimen of continuous HAART through structured treatment interruptions (STIs). STIs may provide similar clinical benefits as continuous treatment such as reduced viral loads and reestablishment of $CD4^+$ T cells while allowing patients drug holidays. We explore the use of STIs using a previously published model that accurately represents $CD4^+$ T-cell counts and viral loads during both untreated HIV-1 infection and HAART therapy. We simulate the effects of different STI regimens including weekly and monthly interruptions together with variations in treatment initiation time. We predict that differential responses to STIs as observed in conflicting clinical trial data are impacted by the duration of the interruption, stage of infection at initiation of treatment, strength of the immune system in suppressing virus, or pre-therapy $CD4^+$ T-cell count or virus load. Our results indicate that dynamics occurring below the limit of detection (LOD) are influenced by these factors, and contribute to reemergence or suppression of virus

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during interruptions. Simulations predict that short-term viral suppression with varying interruption strategies does not guarantee long-term clinical benefit.

1. INTRODUCTION

Highly active antiretroviral therapy (HAART) consists of a combined drug regimen that includes three nucleoside agents alone or two nucleoside agents combined with a protease inhibitor (PI) or a nonnucleoside reverse transcriptase inhibitor (NNRTI). It was believed that initiating HAART in early chronic infection could delay the emergence of viral resistant mutants as well as inhibit virus from localizing in non-blood reservoirs (Lillo *et al*., 1999; Oxenius *et al*., 2000). However, very soon after infection, virus establishes in lymphoid as well as blood compartments (Haase, 1999; Crowe and Sonza, 2000). Additionally, in most patients, cessation of treatment results in almost immediate viral rebound and concurrent $CD4^+$ T-cell decline (within days or weeks) similar to the initial acute phase of infection (Chun *et al*., 1999; Kilby *et al*., 2000). One major reason for this phenomenon is likely replication of virus in various physiological compartments (Devereux *et al*., 2002; Pomerantz, 2002) and virus hidden within latently infected host $CD4^+$ T cells (Furtado *et al*., 1999). Due to the long half-life of this infected class, the latent reservoir would fail to be eradicated even with therapy as long as 60 years (Finzi *et al*., 1999). Thus, early and continuous therapy does not confer an indefinite clinical benefit.

The optimal benefit of continuous drug therapy is extended viral suppression and restored immunological function (through enhancement of $CD4^+$ T-cell levels). However, patients undergoing continuous HAART for several years often experience detrimental short- and long-term side effects including nausea, diarrhea, risk of heart disease, acute retroviral syndrome, liver and nutritional problems, and eventual bone disease (Telenti and Rizzardi, 2000; Fellay *et al*., 2001; Powderly, 2002; Fagard *et al*., 2003). Because of these side effects and a host of personal issues, full patient compliance with HAART is rare (Ammassari *et al*., 2001; Bartlett, 2002). Virological suppression [blood virus below the limit of detection (LOD) (Vella and Palmisano, 2000)] requires strict adherence to treatment and compliance is often less than that required for effective control of viral replication (Paterson *et al*., 2001; Bartlett, 2002).

Intermittent therapy (therapy alternated with drug holidays) could provide a means to delay disease progression to AIDS, while simultaneously limiting exposure to the toxic effects of drug treatment through drug holidays. We have suggested that structured treatment interruptions could indeed be a viable therapy option, based on model results indicating there is a lag time before viral rebound occurs after cessation of therapy (Kirschner and Webb, 1996). This method

has been cited as 'Structured' (Dybul *et al*., 2001) or 'Supervised' (Altfeld and Walker, 2001) 'Intermittent Therapy' (SIT), 'Structured Therapeutic Interruptions' (STI) (Carcelain *et al*., 2001) and most commonly the 'Structured Treatment Interruption' (STI) (Lori *et al*., 2000; Garcia *et al*., 2001; Hance *et al*., 2001; Lori and Lisziewicz, 2001; Ortiz *et al*., 2001; Ruiz *et al*., 2001; Frost *et al*., 2002; Gulick, 2002; Havlir, 2002) strategy. Positive outcomes of STIs may include any or all of the following: maximization of drug availability, an increase in the virus-specific T-cell response, and suppression or significant reduction of viral load (Vella and Palmisano, 2000; Altfeld and Walker, 2001; Deeks, 2001; Lori and Lisziewicz, 2001). A subjective assessment of improvement can be indicated by an increase in patient quality of life through less exposure to drugs. Drawbacks of STIs may include emergence of drug resistance, failure to resuppress virus, or loss of immunity (Telenti and Rizzardi, 2000; Abbas and Mellors, 2002). Until a vaccine or more effective treatment method is discovered, various groups have explored STIs as a practical alternative to overcome both clinical and individual limitations of continuous HAART as outlined above. In this work, we review STI studies and their findings, and use a mathematical model to explain observed variations in key clinical trials.

2. Review of Clinical Studies

Various treatment schedules have been explored during clinical trials for STIs. There have been reports of 7 days on treatment alternated with 7 days off treatment (we abbreviate this 7/7) (Dybul *et al*., 2001; Ananworanich *et al*., 2003), 30 days on treatment followed by 30 days off (we abbreviate this 30/30) (Neumann *et al*., 1999; Ortiz *et al*., 2001), and several variations in between, such as stopping treatment indefinitely until viral load increases beyond a certain level. These studies have varied in their conclusions as to the benefits of STIs, exhibiting a range of positive, unfavourable, or inconclusive results (summarized in Table 1).

Most of these studies use rather small patient sample sizes, owing to both limitation in structuring these clinical trials as well as in identifying patients willing to discontinue effective continuous treatment. In some trials, if viral load increases beyond a pre-specified or detectable level, therapy is immediately reinstated. Thus, how well patients both suppress virus during periods of drug interruption and adhere to therapy schedules can greatly influence study protocol and results. Our focus in this work is to identify factors that explain differences in trial outcomes. The major differences we are interested in are those that either lead to viral suppression or reemergence *during* an interruption. These likely include factors such as dynamics of viral load below the LOD and their indication of the strength of the immune system to suppress virus during the interruption.

In recent years, the focus of HIV-1 research has included viral reservoirs other than the blood compartment. By studying the effect of HAART and STI in the

Table 1. Summary of clinical STI studies to date[a].

Entry criteria		Number	Treatment schedule	Results	Reference
$CD4^{+}$[b]	VL[c]	Patients			
			Positive outcome		
>300	<50	10	7 days on/7 days off for up to 68 weeks	Viral control maintained ↓ Side effects	Dybul *et al.* (2001)
$\frac{CD4}{CD8} > 1$	<20	12	Off until VL > 3000 copies ml^{-1} or max. 30 days off	Viral control maintained	Ruiz *et al.* (2000)
$\frac{CD4}{CD8} > 1$	<50	12	Off until VL > 3000 copies ml^{-1} or max. 30 days off	Progressive ↓ in viral replication ↑ HIV-1-specific T-cell response	Ruiz *et al.* (2001)
>500	<20	10	1 month off/6 months on or VL > 200 copies ml^{-1}	↓ Viral setpoint ↑ HIV-1-specific T-cell response	Garcia *et al.* (2001)
Varied	Varied	3	3 weeks on/1 week off	No emergence of drug resistance ↓ Viral setpoint	Lori *et al.* (2000)
>500	<50	8	Off until VL > 5000 copies ml^{-1}	↑ HIV-1-specific T-cell response	Rosenberg *et al.* (2000)
Varied	Varied	5	Median 55 days off	↑ HIV-1-specific T-cell response	Papasavvas *et al.* (2000)
<200	>50 000[d]	68	8 weeks off	↑ $CD4^{+}$ T-cell count ↓ Viral load	Katlama *et al.* (2003)
			Neutral outcome		
>400	<400	8	30 days on/30 days off for 7 months	↑ HIV-1-specific T-cell response No viral control	Ortiz *et al.* (2001)
Varied	<500	14	Range 14–196 days off	Viral rebound to pre-HAART levels	Hatano *et al.* (2000)
Varied	Varied	5	Varied	↓ Latently infected cells	Blankson *et al.* (2002b)
>150	≥5000[e]	10	28 days on/28 days off	No ↑ drug resistance	Neumann *et al.* (1999)
>400	<200	3	Median 7 days off for 11 cycles	Transient ↑ in T-cell response Virus rebound in all patients	Carcelain *et al.* (2001)
Varied	<50	11	Varied	Resistance ≠ interruptions	Papasavvas *et al.* (2003)
>350	<50	18	Off until VL > 5000 copies ml^{-1} or CD4 decline 25% from baseline	Viral rebound within 2–3 weeks	Davey *et al.* (1999)
>300	<50	97	2 weeks off/8 weeks on for 4 cycles	No change in setpoint	Oxenius *et al.* (2002)
>300	<50	133	2 weeks off/8 weeks on for 4 cycles	Viral load similar to pre-HAART levels	Fagard *et al.* (2003)

Table 1 (*continued*).

Entry criteria		Number	Treatment schedule	Results	Reference
$CD4^{+}$[b]	VL[c]	Patients			
			Unfavourable outcome		
>300	<50	14	2 weeks off/8 weeks on for 4 cycles	Viral rebound within 8 days	Fisher *et al.* (2003)
>300	<50	52	4 weeks off/8 weeks on	↑ Drug resistance	Dybul *et al.* (2003)
>350	<50	600	7 days on/7 days off or CD4 < 350	Majority of patients exhibit viral rebound >500 copies ml^{-1}	Ananworanich *et al.* (2003)
Varied	Varied	40	Median 214 days off	↑ AIDS events	Poulton *et al.* (2003)
Varied	Varied	2	Varied	↓ HIV-1-specific T-cell response	Blankson *et al.* (2002a)

[a] Those shown have a clearly defined treatment schedule.
[b] $CD4^{+}$ T-cell count is in units of cells μl^{-1}.
[c] Viral load is in units of RNA copies ml^{-1}.
[d] Salvage therapy.
[e] Therapy naive patients.

lymph nodes, Altfeld *et al.*, emphasized the importance of examining the immune response in the lymphoid compartment together with peripheral blood (Altfeld *et al.*, 2002). Because the lymph system contains 98% of total lymphocytes (Rosenberg and Janossy, 1999) and is the site of most viral replication (Haase, 1999), studying the effects of STIs solely in blood may give only partial clues as to complete viral replication dynamics. Analysis of key studies presented in Table 1, however, can be used to design further large-scale STI trials that incorporate data from multiple anatomical compartments at various time points. We take a novel step in this direction by applying a model that encompasses both blood and lymph tissue dynamics to examine the effect of STIs.

3. Model of HIV-1 Infection

We previously constructed a model that captures the acute, asymptomatic, and end-stages of typical HIV-1 disease progression (Bajaria *et al.*, 2002). This model considered $CD4^{+}$ T-cell and virus populations in two physiological compartments of blood and lymph tissues (LT). Fig. 1 describes the infection dynamics included in our model and the mathematical description is given in what follows.

Using nine nonlinear ordinary differential equations, we tracked differing dynamics of naive (N) and memory (M) cells circulating between blood ($\mathcal{B}$) and lymph tissue ($\mathcal{L}$) compartments. During infection, virus levels are monitored in both the blood $V_{\mathcal{B}}$ as well as lymph node $V_{\mathcal{L}}$, together with latently (L), abortively (A), and productively (P) infected cell classes in the lymph tissues

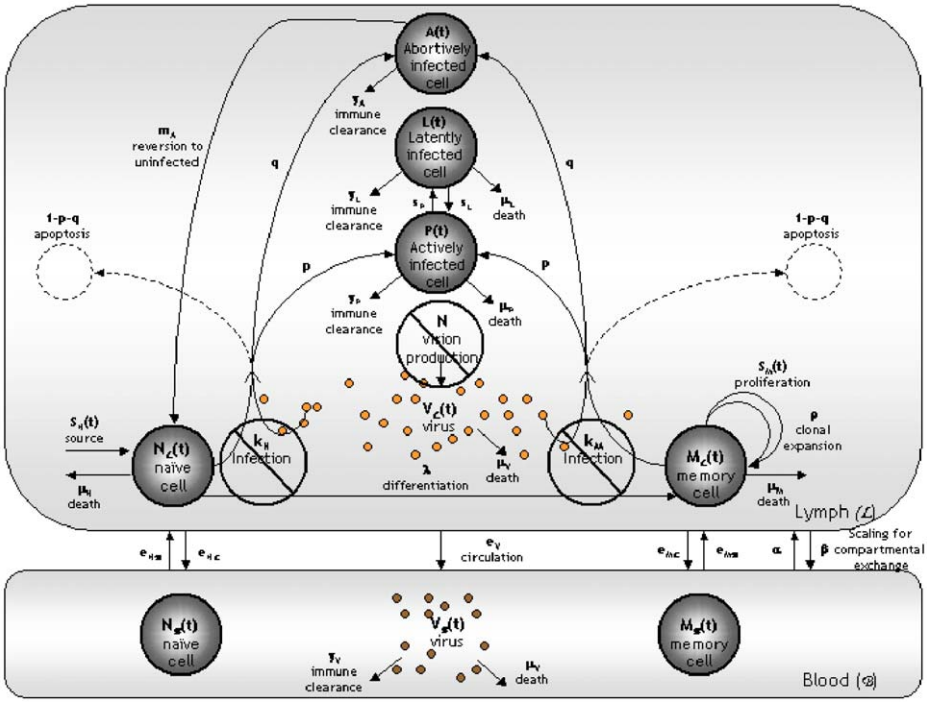

Figure 1. **HIV-1 lymphocyte circulation model.** The model developed here explores mechanisms describing differences in disease progression based on the differential interaction of a single viral strain ($V(t)$) of HIV-1 with naive ($N(t)$) and memory ($M(t)$) subclasses of CD4$^+$ T cells, together with effects to lymphocyte circulation between blood ($\mathcal{B}$) and LT ($\mathcal{L}$). Infection directly produces abortive ($A(t)$) and productively ($P(t)$) infected classes of cells that are assumed to be present predominantly in the LT compartments, owing to the assumption that infected cells in the blood (containing only 2% of all T cells) constitute a relatively small contribution to overall infection, and that most HIV-1 replication occurs in the LT (Schrager and Fauci, 1995; Chun *et al*., 1997a,b; Richman, 2000). Latently infected cells ($L(t)$) are derived only from productively infected cells that have deactivated (Li *et al*., 1996; Cloyd *et al*., 2000). A cell that has become abortively infected (bystander) is increased in its homing capability by upregulation of L-selection (Kirschner *et al*., 2000). Therefore it is believed the majority of these cells either undergo apoptosis (- - -) or revert to the naive class if they have not been signaled through their antigen receptors (Cloyd *et al*., 2000). Any cell that becomes productively infected (whether naive or memory), must have been activated, and thus is counted as a memory cell until its death. Memory cells and productively infected cells have a higher death rate than naive cells (owing to activation-induced cell death) (Muro-Cacho *et al*., 1995). All initial conditions and parameters are shown in Table 2.

[see Bajaria *et al*. (2002) for a complete discussion of these cell types]. Equations for the model system are as follows:

$$\frac{dN_{\mathcal{B}}(t)}{dt} = \beta e_{N\mathcal{L}} N_{\mathcal{L}}(t) - e_{N\mathcal{B}} N_{\mathcal{B}}(t) \tag{1}$$

$$\frac{dM_{\mathcal{B}}(t)}{dt} = \beta e_{M\mathcal{L}} M_{\mathcal{L}}(t) - e_{M\mathcal{B}} M_{\mathcal{B}}(t) \tag{2}$$

$$\frac{dN_{\mathcal{L}}(t)}{dt} = S_N(t) + \alpha e_{N\mathcal{B}} N_{\mathcal{B}}(t) - e_{N\mathcal{L}} N_{\mathcal{L}}(t) - k_N V_{\mathcal{L}}(t) N_{\mathcal{L}}(t) - \mu_N N_{\mathcal{L}}(t) + m_A A(t) \quad (3)$$

$$\frac{dM_{\mathcal{L}}(t)}{dt} = \lambda\mu_N N_{\mathcal{L}}(t) + S_M(t) + \frac{\rho M_{\mathcal{L}}(t) V_{\mathcal{L}}(t)}{V_{\mathcal{L}}(t) + K_2} + \alpha e_{M\mathcal{B}} M_{\mathcal{B}}(t) - e_{M\mathcal{L}} M_{\mathcal{L}}(t) - k_M V_{\mathcal{L}}(t) M_{\mathcal{L}}(t) - \mu_M M_{\mathcal{L}}(t) \quad (4)$$

$$\frac{dL(t)}{dt} = s_P P(t) - s_L L(t) - \gamma_L (N_{\mathcal{L}}(t) + M_{\mathcal{L}}(t)) * L(t) - \mu_L L(t) \quad (5)$$

$$\frac{dP(t)}{dt} = p * (k_N V_{\mathcal{L}}(t) N_{\mathcal{L}}(t) + k_M V_{\mathcal{L}}(t) M_{\mathcal{L}}(t)) - \gamma_P (N_{\mathcal{L}}(t) + M_{\mathcal{L}}(t)) * P(t) - \mu_P P(t) + s_L L(t) - s_P P(t) \quad (6)$$

$$\frac{dA(t)}{dt} = q * (k_N V_{\mathcal{L}}(t) N_{\mathcal{L}}(t) + k_M V_{\mathcal{L}}(t) M_{\mathcal{L}}(t)) - \gamma_A (N_{\mathcal{L}}(t) + M_{\mathcal{L}}(t)) * A(t) - m_A A(t) \quad (7)$$

$$\frac{dV_{\mathcal{L}}(t)}{dt} = N\mu_P P(t) - e_V V_{\mathcal{L}}(t) - \mu_V V_{\mathcal{L}}(t) \quad (8)$$

$$\frac{dV_{\mathcal{B}}(t)}{dt} = \beta e_V V_{\mathcal{B}}(t) - \gamma_V (N_{\mathcal{B}}(t) + M_{\mathcal{B}}(t)) * V_{\mathcal{B}}(t) - \mu_V V_{\mathcal{B}}(t). \quad (9)$$

Equations (1) and (2) describe uninfected naive and memory cells in the blood. Their populations depend on influx from the LT at rates $e_{N\mathcal{L}}$ and $e_{M\mathcal{L}}$ respectively (scaled by β for compartmental exchange) and loss to emigration at rates $e_{N\mathcal{B}}$ and $e_{M\mathcal{B}}$. Equations (3) and (4) have similar circulation terms (scaled by α for entry into the LT). The source of naive cells represents input from the thymus, which decreases as a function of age, as the following relation: $S_N(t) = \mu_N * N_L(0) * 0.97^{\frac{t}{365}}$. The memory cell source is dependent on differentiation from naive cells at rate λ, HIV-1 antigen-induced proliferation (ρ), and proliferation to maintain homeostasis of total CD4$^+$ T-cell number (based on carrying capacity) described by $S_M(t) = \frac{R}{N_{\mathcal{L}}(t)+K_1}$. Uninfected naive and memory cells are lost due to either death (μ_N and μ_M) or infection (k_N and k_M). Productively infected cells arise from a proportion (p) of naive and memory cells that become infected, and are also lost to death (μ_P) or clearance by the immune response (γ_P). Similarly, abortively infected cells are a proportion (q) of all infected cells, that are cleared by the immune response at rate γ_A. Abortively infected cells either undergo apoptosis or revert back to naive cells (m_A). Productively infected cells can shut down at rate s_P becoming latently infected cells, or arise from latently infected cells that become activated at rate s_L and have not been cleared by the immune response (γ_L). Virus ($V_{\mathcal{L}}$) is produced in the LT from productively infected cells (at rate N) and has a short half-life (μ_V). Virus in the blood flows in from the LT (e_V), and is cleared by the immune response (γ_V) or lost to natural death (μ_V).

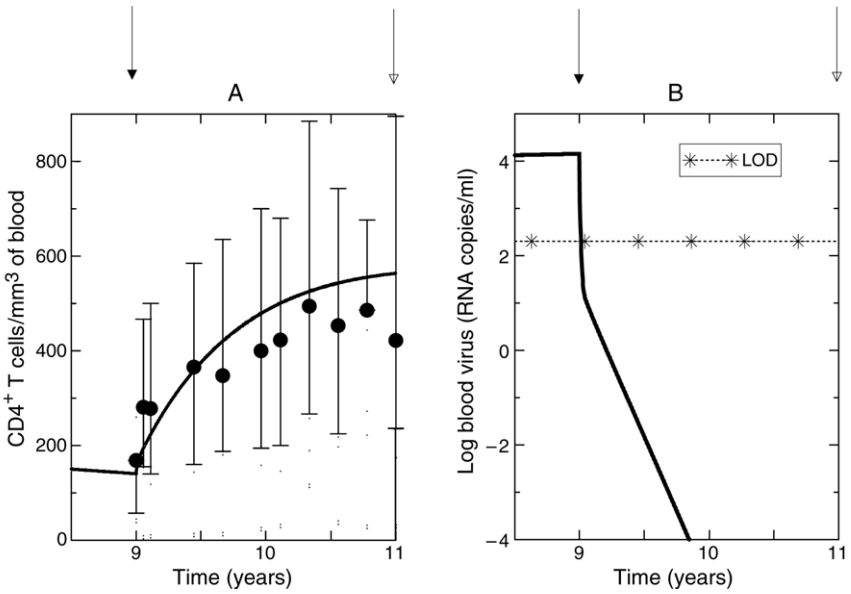

Figure 2. **Simulation of the effect of HAART on CD4$^+$ T cells and virus in the blood compared with data.** In the model simulation, continuous therapy was administered at 9 years (filled arrow) after infection and subsequently removed at 11 years (open arrow), with 90% efficacy throughout [infection (k_N and k_M) and virion production (N) are reduced by 90% at the onset of therapy]. Panel (a) shows comparison with data (•) from Notermans *et al.* (1999). In this study, viral loads were decreased below the 200 copies ml^{-1} LOD (2.31 log) [indicated by $*$ in panel (b)] within 12 weeks of the start of therapy. All initial conditions and parameter values are as in Table 2.

Table 2 presents initial conditions for the nine system variables as well as parameter values used in our simulations. These were estimated previously (Bajaria *et al.*, 2002).

In previous work (Bajaria *et al.*, 2002), model simulations were compared with clinical data on disease progression in untreated patients and shown to be an accurate prediction of HIV-1 disease progression in many long-term non-progressors (LTNP) and typical progressors (Pantaleo *et al.*, 1995; Fauci *et al.*, 1996; Greenough *et al.*, 1999; Sabin *et al.*, 2000). In that work (Bajaria *et al.*, 2002), we also simulated the effect of continuous HAART therapy on disease progression. In Fig. 1, we indicate how HAART therapy plays an inhibiting role (i.e., reduced infection and viral production rates). A 90% reduction in these viral virulence parameters (viral infection (k_N and k_M) and production (N) rates) was applied during a 2 year continuous treatment window, over years 9 to 11 post-infection. Model results for the dynamics of blood CD4$^+$ T cells and blood virus during HAART are shown in Fig. 2 [LT results can be seen in Bajaria *et al.* (2002)]. Simulations using this 90% level of drug effectiveness fit well with data from (Notermans *et al.*, 1999), indicating that during continuous therapy patients are able to suppress virus rather effectively. For the typical progressor, the model predicts that

Table 2. Initial conditions and parameter values for HIV-1 model.

Variable	Definition	Initial value	Units	Reference
$N_{\mathcal{B}}(0)$	Uninfected naive $CD4^+$ T cells (blood)	450	Cells mm^{-3}	Haase (1999)
$M_{\mathcal{B}}(0)$	Uninfected memory $CD4^+$ T cells (blood)	550	Cells mm^{-3}	Haase (1999)
$N_{\mathcal{L}}(0)$	Uninfected naive $CD4^+$ T cells (LT)	9×10^{10}	Cells	Haase (1999)
$M_{\mathcal{L}}(0)$	Uninfected memory $CD4^+$ T cells (LT)	1.1×10^{11}	Cells	Haase (1999)
$L(0)$	Latently infected $CD4^+$ T cells (LT)	0	Cells	
$P(0)$	Productively infected $CD4^+$ T cells (LT)	0	Cells	
$A(0)$	Abortively infected $CD4^+$ T cells (LT)	0	Cells	
$V_{\mathcal{B}}(0)$	Virus concentration in blood	0	HIV-1 RNA ml^{-1}	
$V_{\mathcal{L}}(0)$	Total virus in LT	10 (can vary)	HIV-1 RNA	
α	Scaling term (blood to LT)	5×10^6	mm^3or μl	
β	Scaling term (LT to blood)	2×10^{-7}	mm^3or μl	
$e_{N\mathcal{L}}$	Circulation of naive cells (LT to blood)	1.0	/Day	Sprent (1973), Sprent and Basten (1973)
$e_{N\mathcal{B}}$	Circulation of naive cells (blood to LT)	40.0	/Day	Sprent (1973), Sprent and Basten (1973)
$e_{M\mathcal{L}}$	Circulation of memory cells (LT to blood)	0.25	/Day	Sprent (1973), Sprent and Basten (1973)
$e_{M\mathcal{B}}$	Circulation of memory cells (blood to LT)	10.0	/Day	Sprent (1973), Sprent and Basten (1973)
e_V	Circulation of virus (LT to blood)	0.5	/Day	Estimated
μ_N	Death rate of uninfected naive cells (LT)	0.002	/Day	Richman (2000)
μ_M	Death rate of uninfected memory cells (LT)	0.003	/Day	Richman (2000)
μ_L	Death rate of latently infected T cells	0.000525	/Day	Saag and Kilby (1999), Chun *et al*. (1999)
μ_P	Activation-induced cell death	0.5	/Day	Cavert *et al*. (1997)
μ_V	Death rate of free virus	3.0	/Day	Stafford *et al*. (2000), Perelson *et al*. (1993)
λ	Direct differentiation from naive to memory cells	0.1	Scalar	Estimated
k_N	Infection rate of naive cells by virus	8.0×10^{-12}	/Day-virion	Estimated

Table 2 (*continued*).

Variable	Definition	Initial value	Units	Reference
k_M	Infection rate of memory cells by virus	1.0×10^{-12}	/Day-virion	Estimated
p	Proportion of productively infected cells	0.0187	Scalar	Estimated
q	Proportion of abortively infected cells that survive	0.675	Scalar	Wang *et al*. (1999)
m_A	Reversion rate of abortively infected cells to naive cells	0.2	/Day	Chen and Cloyd (1999)
s_L	Rate latently infected cells activate	0.03	/Day	Estimated
s_P	Rate productively infected cells deactivate	0.01	/Day	Estimated
N	Average number of virions produced per productively infected cell	500	Virions/cell	Chen and Cloyd (1999)
γ_V	Clearance rate of free virus (blood)	0.95	/Day-cell	Estimated
γ_L	Clearance rate of latently infected cells (LT)	2.0×10^{-13}	/Day-cell	Estimated
γ_P	Clearance rate of productively infected cells (LT)	2.0×10^{-13}	/Day-cell	Estimated
γ_A	Clearance rate of abortively infected cells (LT)	8.0×10^{-14}	/Day-cell	Estimated

during HAART there is a characteristic biphasic return of $CD4^+$ T cells [Fig. 2(a)] and decline in viral load [Fig. 2(b)] as documented in several clinical studies [e.g., Pakker *et al*. (1998), Zhang *et al*. (1998)] as well as theoretical analyses [e.g., Hlavacek *et al*. (1999), Pakker *et al*. (1998), Blankson *et al*. (2000)].

Here, we use our mathematical model described above to conduct virtual STI trials and identify factors that may contribute to differences in clinical outcomes, while accounting for both blood and LT dynamics. Based on our comparison with clinical trial data, we hypothesize that virus likely remains variably suppressed during an interruption. We show results based on our assumption of differing levels of a host to suppress virus. Parameters and initial conditions in our model simulations of STIs are the same as in Table 2 for a typical progressor under the influence of HIV-1 infection [see Fig. 4 of Bajaria *et al*. (2002)].

4. VIRTUAL STI TRIALS

Mathematical modeling has been used to describe dynamics of viral rebound during treatment interruptions (Neumann *et al*., 1999; Bonhoeffer *et al*., 2000; Dorman *et al*., 2000; Frost *et al*., 2002). Our goal here is to understand what factors

contribute to differences between clinical trials of STIs that resulted in success or failure (Table 1), offer insights into dynamics occurring below the viral detection level (which cannot be observed clinically), and explore how treatment administered at various disease stages can influence a patient's response to STIs. Success in an STI trial could include suppression of virus below detection, enhancement of T-cell mediated immunity, or reduction of drug toxicity, while failure may be indicated by emergence of drug resistance, reduction in $CD4^+$ T-cell count, or greater possibility of HIV-1 transmission (Telenti and Rizzardi, 2000; Vella and Palmisano, 2000; Altfeld and Walker, 2001; Deeks, 2001; Abbas and Mellors, 2002).

4.1. ***The STI candidate profile.*** The most significant issue that arises when considering patients as potential candidates for STI studies is whether or not they are treatment responders during periods *off* therapy. Inside a treatment window, patients ideally exhibit complete suppression of virus. Continued viral suppression, a function of both virus as well as host immunity, is also the most desirable patient response during a treatment interruption. This assessment can only be made during an interruption, and this study does not attempt to correlate during HAART responsiveness to performance in that period. Clinical studies have similarly determined that extended viral suppression during continuous therapy does not correlate with the ability to suppress virus during an interruption (Davey *et al.*, 1999). We hypothesize that measures of viral load and $CD4^+$ T-cell counts during the tail end of the first and subsequent interruptions can serve as an estimate of patient immune profile, indicating the likelihood of achieving clinical and individual success with STIs. We define immunological responsiveness quantitatively through monitoring $CD4^+$ T-cell levels, the most common cell marker of HIV-1 disease progression. We define virological responsiveness by the maintenance of blood viral levels below the LOD during treatment interruptions, and focus on correlating an overall patient profile with sustained suppression of virus (below the LOD) or viral reemergence during interruptions in clinical trials.

As described above, we reduce viral infection and production rates during HAART to 10% of their values at the onset of therapy, corresponding to a 90% efficacy of treatment. Using this level of drug effectiveness in our previous work (Bajaria *et al.*, 2002) gave results that fit well with data from (Notermans *et al.*, 1999), indicating that drug efficacy is likely high during HAART (Fig. 2). This level of viral suppression, however, is characteristic of periods *on* therapy. Here, we examine viral suppression strengths in patients while *off* therapy, or during an interruption. Clinical trial data on viral rebound at cessation of therapy is variable; some patients are able to maintain viral levels below the LOD while others experience minimal or even significant rebounds in their viral loads (see Table 1). This suggests that patients who have been successful on continuous HAART have differing abilities to continue to suppress virus after therapy is stopped; variations that can be attributed to both host and viral factors. We hypothesize that because virus remains variably suppressed among patients during interruptions, the ability to

suppress virus during an interruption is an important indicator of an overall patient immune profile. For our purposes, we define the *immune strength to suppress virus* during interruption to be 0% (or no suppressive ability) if viral infection and production rates rebound to 100% of their pre-therapy values, 10% suppressive ability if viral parameters rebound to 90%, etc. This measure of suppressive ability provides an estimation of the interplay between patient-specific host and viral dynamics after therapy is interrupted. For simplicity in our simulations, periods on and off therapy are modeled by a reduction or increase, respectively, in viral infectivity and production. Changing several other parameters, such as the rate of infected cell clearance by the immune response, would result in similar dynamics [these parameters were all shown to be bifurcation parameters in our previous study (Bajaria *et al*., 2002)]. Although suppression is difficult to measure *in vivo*, particularly below the LOD, an estimation of suppressive strength supplies a framework upon which we can understand differing responses to STIs, and from which we can estimate dynamics of future larger-scale clinical STI efforts. In each of our simulations to follow, we compare results for different STI scenarios by imposing variable strengths of suppression (as reported in the legends). We acknowledge the possibility that reasons other than altered immune ability could lead to variable viral suppression for the following sets of data (elaborated in the Discussion), but focus on this as a key hypothesis for the differences in clinical trial findings.

To validate our model as predictive of STI dynamics, we simulate two STI clinical studies with differing schedules: one by Ortiz (UCSF group) (Ortiz *et al*., 2001) and the other from Dybul (NIH) (Dybul *et al*., 2001) (see Table 1). To this end, we initiate our virtual STI trials at similar $CD4^+$ T-cell levels and viral loads as that of the pre-STI criteria in the two studies. We then examine the underlying host and viral dynamics for the clinical data that occur below the LOD that are unobservable without the aid of a mathematical model. Throughout our virtual trials, we present viral loads and $CD4^+$ T-cell counts as indicators of immune strength for each patient that provide an estimate of the patient profile, the role of which we explore in the following sections. This work offers insight into which patient parameters lead to sustained or limited viral suppression during an interruption, and suggests patient characteristics that may be necessary to achieve benefit from an STI strategy.

4.1.1. *Revisiting Ortiz* et al. *(2001).* Similar to the Ortiz work (see Table 1), we initiate our virtual study after continuous treatment has been administered for approximately 2 years. We then conduct a cycle of 1 month STI, 1 month HAART, 1 month STI, 1 month HAART, a final 3 month STI, and then resumption of HAART for comparative analysis (abbreviated 30/30 in subsequent sections). In Fig. 3, we present results of simulating the Ortiz study for differing levels of viral suppression ability during interruptions. We show in Fig. 3(a) and 3(b) the dynamics of $CD4^+$ T cells and virus since initial infection (at time 0), to the beginning of continuous HAART (at 4 years), to the initiation of STIs at 6 years.

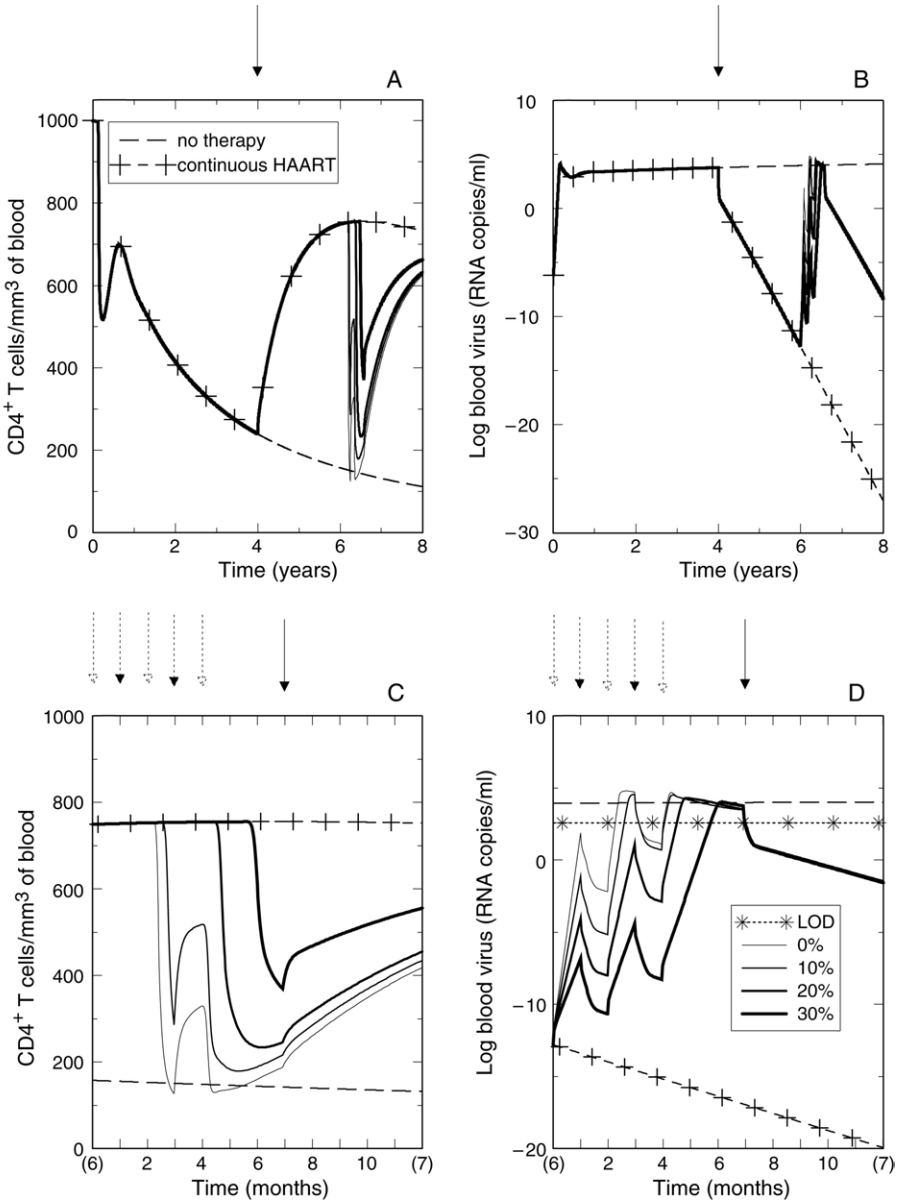

Figure 3. **Simulations of monthly STIs comparing with Ortiz clinical trials in Ortiz *et al.* (2001)**. Panels (a) and (b) depict the whole time course for both $CD4^+$ T-cell and viral dynamics, whereas panels (c) and (d) enlarge the STI treatment period between years 6 and 7. In all panels, thicker lines correspond to greater suppressive ability (indicated in the legend). Strong responders (greater than 30%) exhibit the same behavior as individuals undergoing continuous therapy (+ control, data not shown). Even though viral rebound may occur to a fraction of the pre-treatment levels, we allow viral infectivity and production to remain as slowly increasing functions over time as in Bajaria *et al*. (2002). Positive (continuous therapy) and negative (no therapy) controls are indicated by the + and − lines, while ∗ signifies the LOD of 400 copies ml^{-1} (2.6 log). Solid filled arrows indicate the initiation of continuous treatment, dashed filled arrows periods 'on' therapy during the trial, and open arrows periods 'off' therapy during the trial.

Fig. 3(c) and 3(d) magnify a period of 1 year after initiation of the STI trial such that dynamics during interruptions can be clearly examined.

The Ortiz study compared results after the last STI with patient pre-treatment baseline levels, and found that $CD4^+$ T-cell count and viral load usually fluctuated during the interruptions [see Fig. 2 of Ortiz *et al*. (2001)]. In our virtual trial repeating the Ortiz study, virus rebounded above the LOD during each interruption in the lowest suppressors (0%–30%) (Fig. 3). In our model simulation, stronger viral suppressors (40%–100%) exhibited the same dynamics as continuous therapy patients (i.e., complete viral suppression below the LOD) during interruptions. Therefore, we predict that patients in the Ortiz study were low off-HAART viral suppressors (0%–30%) and thus had viral infection and production rates rebounding to 70%–100% of values prior to therapy during the treatment interruptions. We predict that it is necessary to have at least 40% suppression during interruptions if virus is to remain below the LOD during the full 30-day interruption. At the study conclusion, several Ortiz study patients had steady state levels of virus that were lower than at the onset. When we view results of our simulations at 7 years, we find that viral loads are lower than if no therapy had been administered (negative control, $-$ line in Fig. 3), however not as low as continuous therapy (positive control, $+$ line in Fig. 3), even though both regimens yield viral loads below the LOD of 400 copies ml^{-1} (2.6 log). These extremely low viral levels are achieved by extended continuous therapy in the model simulations and should be interpreted as effective elimination of virus in the blood compartment (but likely not in viral reservoirs or sanctuaries outside the blood).

4.1.2. *Revisiting Dybul* et al. *(2001).* We also perform a virtual trial similar to that in the Dybul study (Dybul *et al*., 2001) with a weekly STI schedule (abbreviated 7/7 in subsequent sections). We begin with undetectable viral loads and $CD4^+$ T-cell counts around 600 cells mm^{-3}, resulting from a previous 1 year duration of continuous HAART [median time spent on HAART prior to STI initiation was 13.5 months in the study (Dybul *et al*., 2001)]. We conduct 26 cycles of 7/7 for a period of 1 year, followed by continuous HAART (Fig. 4).

In this trial (Dybul *et al*., 2001), patients compliant with study protocol had viral loads that remained below the LOD of 50 copies ml^{-1}; those that were not compliant experienced viral growth above the LOD relatively quickly [see Fig. 1 of Dybul *et al*. (2001)]. In our virtual trial, only the non-viral-suppressor (0%) and the 10% viral suppressors have discernable oscillations in $CD4^+$ T-cell count (Fig. 4). These patients with low profiles also exhibit viral load oscillations occurring above the LOD during interruptions. Therefore, patients in this study also had a low viral suppressive ability, but it was only the weakest patient profiles that exhibited virus above the LOD within the interruption period of 7 days.

4.1.3. *The influence of interruption length.* A low suppression profile (0%–30%) in our virtual trial (Fig. 3) resulted in dynamics most similar to patients in the Ortiz clinical trials, indicating that viral infection and production rates

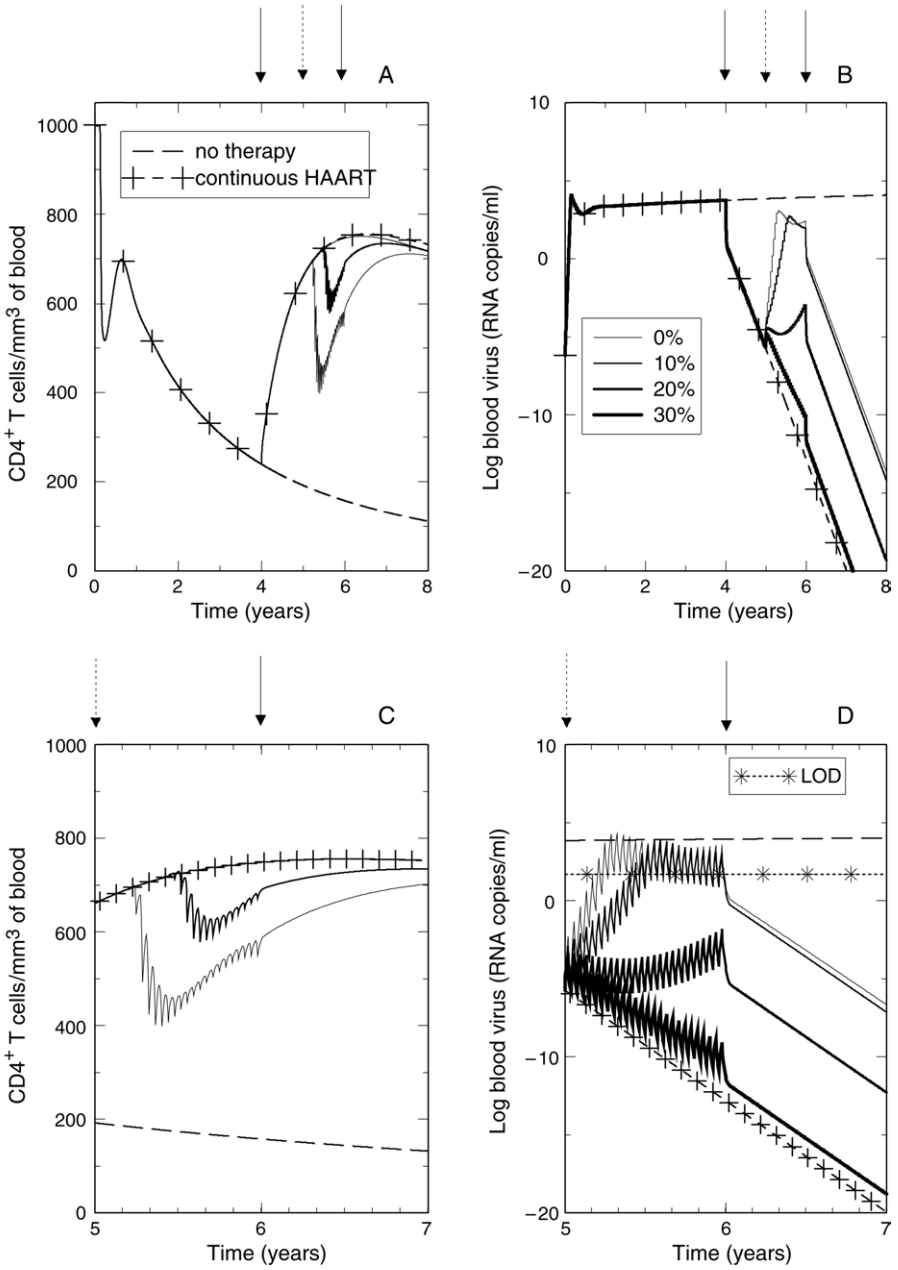

Figure 4. **Simulations of weekly STIs comparing with Dybul clinical trials in Dybul *et al.* (2001)**. The shading of lines is consistent with that in Fig. 3. Again panels (a) and (b) are representative of the entire time course, while panels (c) and (d) enlarge the STI period between years 5 and 7. In panel (d) we see oscillations in viral load throughout the STI period for the 0%–30% suppressors, even below the LOD ($*$) of 50 copies ml^{-1} (1.7 log). All others exhibit similar behavior to the continuous therapy + control (data not shown). The average values of the oscillations were plotted for simplicity in panel (b). Solid filled arrows indicate the initiation of continuous treatment and dashed filled arrows initiation of the STI trial.

rebound to at least 70% of levels present before onset of therapy. This can be attributed to both host and viral factors, such as the strength of the immune system and viral virulence parameters. Stronger suppression profiles (40%–100%) during interruptions produced the same dynamics as under continuous HAART; uninterrupted $CD4^+$ T-cell reconstitution and biphasic decay of viral load. The Dybul trial exhibited suppression in those patients that remained compliant with the study protocol. Those that were not adherent experienced viral rebound. In our virtual trial (Fig. 4), only very low suppression profiles (0%–10%) exhibited rebound, while profiles of 20%–100% did not exhibit viral reemergence above the LOD during any interruptions. Therefore, viral suppressive ability plays a larger role when interruption length is increased. Thus, we can conclude that because there is a generally weak ability to suppress virus during interruptions, a shorter interruption is the 'safer' approach. However, other studies of weekly schedules have found the emergence of virus above the LOD during similar short interruptions (Fisher *et al.*, 2003). This could be attributed to several different factors such as differences in drug regimen, sensitivity of viral load assay, and treatment history prior to initiating the study, each indicating the need for further study of short interruption periods.

4.2. ***Treatment initiation.*** Clinical trials have been conducted at different disease stages. Some groups have studied the benefits of administering an STI schedule early in infection (Rosenberg *et al.*, 2000; Oxenius *et al.*, 2000; Tremblay *et al.*, 2003). However, continuous therapy was reinstituted immediately after STI trial completion and thus it is not possible to assess whether an overall immunological and/or virological benefit was achieved. STI trials have also been conducted with patients in the chronic phase (Ortiz *et al.*, 1999; Haslett *et al.*, 2000; Papasavvas *et al.*, 2000; Ruiz *et al.*, 2000, 2001; Carcelain *et al.*, 2001; Garcia *et al.*, 2001; Fagard *et al.*, 2003), or those with AIDS, as a salvage therapy (Lori *et al.*, 2000; Gulick, 2002; Katlama *et al.*, 2003). We now use this model to explore a variable that has yet to be tested extensively or exclusively in the clinic: timing of STI initiation. Our virtual model provides a method to assess the response to interruptions (without the influence of prior or later continuous therapy) even a significant time period after the trial conclusion.

We apply or virtual model to study therapy-naive patients (Fig. 5). In our previous simulations (Figs. 3 and 4), HAART is initiated at four years post-infection, a time chosen so that the initial state of virtual patients approximated that of the clinical trial patients, and also so the dynamics of the acute stage would not influence the results. Most notably in Figs. 3 and 4 are significant differences in dynamics in the lower ranges of response (0%–30%), that contributed to individual clinical trial observations (Table 1). Therefore, we choose a virtual patient profile with 20% suppressive ability as an average approximation of clinical trial patient profiles [from Dybul *et al.* (2001) and Ortiz *et al.* (2001)] and conduct STI virtual trials for a 1 year duration at different stages of disease progression. The two schedules chosen are again the 7/7 and 30/30, as both schedules provide an overall of 6

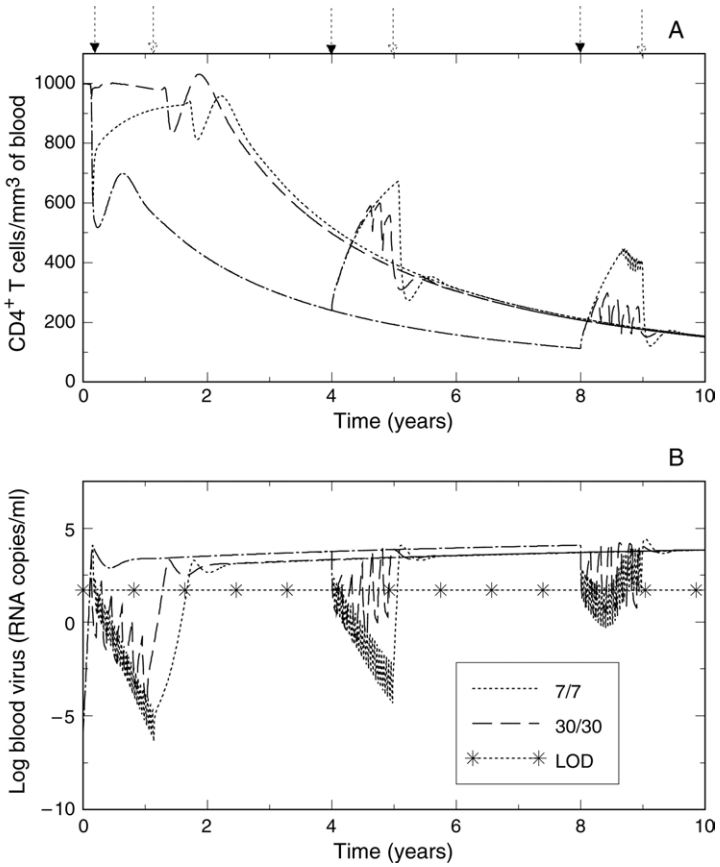

Figure 5. **Optimal initiation of STIs**. $CD4^+$ T-cell counts (a) and viral load (b) are presented for 50 day, 4 year, and 8 year STI initiation times (after infection). Patients are assumed to have a 20% ability to suppress virus during an interruption. Two schedules are explored within this framework: 7/7 (dotted) and 30/30 (dashed) schedules are each simulated over a period of 1 year. The viral LOD ($*$) of 50 copies ml^{-1} (1.7 log) is indicated in panel (b). Dashed filled arrows above the graphs indicate the start time of 1 year of STIs (either 7/7 or 30/30) while open arrows indicate end time. The dot–dashed line in both panels indicates the positive control (no therapy) until STI is initiated.

months on treatment, and 6 months off. Three initiation times chosen are 50 days, 4 years, and 8 years after infection corresponding to the acute stage, chronic stage, and end-stage disease of a typical disease progressor. This will aid in determining the influence of STI initiation for those weakly suppressing virus during interruptions, similar to clinical trial patients.

As seen in Fig. 5(a), the 7/7 protocol is the more reliable approach for initiating STI in the chronic and AIDS stages of disease, based on maximizing total T-cell count (as measured by area under the curve). If measurements of $CD4^+$ T-cell count are made early in disease progression, the 30/30 schedule appears desirable as it yields higher $CD4^+$ T-cell counts and equal suppression of virus as compared

with the 7/7 schedule. However, as seen in Fig. 5(b), our simulation indicates that dynamics below the LOD during a 30/30 schedule likely contribute to more rapid emergence of virus than when compared to 7/7 [Fig. 5(b)], a result that is not easy to predict clinically. A 7/7 schedule results in delayed reemergence of virus as compared to the 30/30 schedule during both acute and chronic stages of disease. Among possible explanations is that extended interruptions may not effectively prevent active viral replication occurring in the lymph compartment (Orenstein *et al.*, 2000), which cannot be inferred from blood data. The latently infected compartment might also harbor provirus that could potentially become activated during an extended treatment interruption (Blankson *et al.*, 2002b). Enhanced immune responses occurring during treatment of patients who are in the earlier stages of infection is consistent with clinical STI studies (Altfeld and Walker, 2001; Rosenberg *et al.*, 2000), as well as with continuous therapy regimens (Lillo *et al.*, 1999; Oxenius *et al.*, 2000; Ortiz *et al.*, 2002). We also see in Fig. 5(b) that virus is more effectively contained with weekly interruptions, although neither monthly nor weekly interruptions have much of a positive effect late in disease progression. We see in the virtual trial beginning at 8 years that virus does not remain below the LOD at any point during STI treatment. $CD4^+$ T-cell levels increase for 7/7 patients in the AIDS stage to some degree, whereas the 30/30 regimen shows minimal benefit. Some groups have suggested STI as a salvage therapy mechanism (Harrington and Carpenter, 2000; Katlama *et al.*, 2003) and our model shows a 7/7 schedule may have some immunological benefit late in infection. However, clinical data (Poulton *et al.*, 2003) as well as our simulations of acute-stage treatment (Fig. 5) suggest that immune control is more likely to occur with interruption strategies imposed earlier in disease progression. Both approaches administer an overall total of 6 months of treatment and 6 months off. Therefore, considering only maximal viral suppression and $CD4^+$ T-cell levels, the weekly interruption schedule (7/7) would be the desired approach, administered as early as possible.

5. DISCUSSION

We have presented simulations of STIs based on a virtual two-compartmental model of $CD4^+$ T-cell and HIV-1 dynamics during typical disease progression. The virtual trial model was developed to mimic HIV-1 pathogenesis in long-term nonprogressors and typical progressors and model simulations compare well with clinical data (Bajaria *et al.*, 2002). In typical progressors, continuous HAART was simulated by reducing viral production and infection rates, and results were again comparable to clinical findings. Our model was then applied in this study to explore STIs. Mathematical modeling is an important tool for understanding how phenomena occurring below the level of detection contribute to the observed clinical reemergence or suppression of virus, and provides the ability to test novel

hypotheses about STI therapy techniques while taking into consideration dynamics of HIV-1 replication occurring in the lymph tissue compartment as well as blood.

A recurring result in our experiments is the presence of viral oscillations occurring below the LOD in low suppressors that lead to reemergence of virus, at a speed dependent on the strength of viral suppression. Oscillations that continually increase in slope below the LOD predict eventual reemergence while oscillations that remain in constant or decreasing slope imply viral control [see viral load in acute vs. chronic stage in Fig. 5, as well as 20% vs. 30% suppressors in Fig. 4, panel (d)]. We find that the length of an interruption is a key variable in the detection or suppression of oscillations, and suggest that complicated dynamics of virus infection and production in the lymphoid system can become more deleterious during an extended interruption, and should be explored in conjunction with clinical observations from blood.

Even when STIs are terminated and continuous therapy reinstated, oscillations still occur for a short period after the interruptions. In Fig. 5, viral loads fluctuate around the setpoint for a period of a few months before settling into a steady state. Therefore the timing of post-interruption analyses could prove to be a major factor in determining success or failure of a given regimen. It would be beneficial to sample T-cell levels and viral loads as frequently as possible and/or at a later stage to capture steady-state levels during the post-interruption period. Without more sensitive assays, it is impossible to study the *in vivo* effects of treatment below the LOD. We show that timing and frequency of data collection in clinical studies is essential to understanding the effect of antiviral therapy on immune and viral dynamics even after reinstatement of continuous therapy. We use our simulations as a tool to predict the effectiveness of therapy both during and off therapy such that treatment initiation and schedules can be optimized.

In treatment studies, transient relapses of viremia (blips) have been observed in patients undergoing continuous therapy (Hermankova *et al*., 2001; Kimura *et al*., 2002). In STI clinical trials, the cause of the intermittent rise of virus above the LOD during interruptions has yet to be elucidated. Two prevalent hypotheses to account for these intermittent appearances of virus are (1) failure of the immune system to suppress viral growth and (2) the emergence of drug resistant viral strains. Some studies show no correlation of viral reemergence with drug resistance *in vivo* (Havlir *et al*., 2001; Papasavvas *et al*., 2003) while others establish a link (Cohen Stuart *et al*., 2001). Additionally, adherence to continuous therapy does not necessarily correlate to prevention of resistance (Bangsberg *et al*., 2003; Harder, 2003). In Table 1, we see variability in preservation or loss of drug sensitivity in a variety of treatment settings. STI approaches can track these blips in a controlled fashion, and potentially distinguish those that are a result of low viral suppression vs. those that are indicative of drug resistance. If viral samples are taken during interruptions and do not exhibit drug resistance, the blips can be attributed to a lack of viral suppression ability.

If a regimen of STIs were found to be successful, it may have implications in the global arena. Despite the worldwide burden of the AIDS pandemic, most research and development occurs in areas of greatest resources, rather than of greatest need. An optimal treatment strategy would emphasize cost reduction over the long term for patients living with HIV-1 as a chronic treatable condition. Even though the approach seems appealing, further studies would have to be conducted to assess the effects of emergence of drug resistant mutants during repeated interruptions (Martinez-Picado *et al*., 2002; Schweighardt *et al*., 2002), as well as significance of rebounding virus during interruption periods (Davey *et al*., 1999) shown to have a high likelihood of occurrence in trials such as the Swiss HIV Cohort Study (Oxenius *et al*., 2002).

Benefits of STIs include a substantial reduction of side effects, longer total duration of drug use, improved quality of life, greater likelihood of compliance, and reduced economic costs. These advantages must be weighed against the potential risks of irreversible immune function decline and development of drug resistance. A mathematical model can highlight dynamics not easily observable in the clinic, such as viral behavior below the limit of detection, and also explore novel possibilities for STI therapies based on an overall impact of interruptions to disease progression. The virtual HAART simulations in this work were designed to compare clinical STI trials and use mathematical modeling to provide insight into differences between them. Similar to the above observations, the differences in ability to suppress virus during an interruption makes a profound impact in both immediate and long-term performance during HAART and STIs. Although some analyses support enhanced HIV-1-specific T-cell activity when STI is administered in early infection (Rosenberg *et al*., 1997; Papasavvas *et al*., 2000; Ortiz *et al*., 2002; Yu *et al*., 2002), more recent comprehensive studies do not (Fagard *et al*., 2003). Our modeling efforts suggest the possibility of clinical benefits of STIs in stronger treatment responders; reduced drug exposure can still achieve the goals of continuous HAART therapy (increased $CD4^+$ T-cell levels and suppressed viral loads). However, over the long term, virus recrudesces in weaker responders, who constitute the significant majority of patients. If an effective schedule cannot be found for intermittent treatment to successfully reduce virus infectivity and production processes, a combination of STIs together with exogenous IL-2 administration (Kirschner and Webb, 1998; Hengge *et al*., 2002), hydroxyurea (Garcia *et al*., 2003), or other immunotherapy techniques may have some benefit (Chun *et al*., 1999; Telenti and Rizzardi, 2000). There is still need for controlled, randomized studies to evaluate the full impact of STI trials on a large cohort of patients.

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