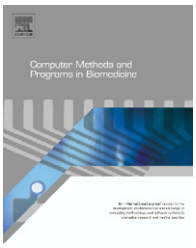
COMPUTER METHODS AND PROGRAMS IN BIOMEDICINE 104 (2011) 50–61

journal homepage: www.intl.elsevierhealth.com/journals/cmpb



Computer simulation of structured treatment interruption for HIV infection

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ARTICLE INFO

Article history:
Received 11 December 2009
Received in revised form
31 March 2011
Accepted 19 July 2011

ABSTRACT

The use of highly active antiretroviral therapy (HAART) for suppression of measurable levels of virus in the body has greatly contributed to restoration and preservation of the immune system in HIV positive patients. However, short and long term problems associated with HAART have led to proposals for alternative treatment strategies for controlling HIV infection. In particular, structured treatment interruptions (STIs) that consist of therapy withdrawal and re-initiation according to specific criteria have been considered. The aim of these STIs was one or both of: (i) to stimulate the immune system to react to HIV, (ii) to allow re-emergence of wild-type virus and thereby reduce problems of drug resistance. However, a number of clinical trials of STIs have shown adverse outcomes for patients under discontinuous therapy, including serious health risks associated with treatment interruptions. In this paper we consider in some detail two of the larger clinical studies, namely, (a) strategies for management of anti-retroviral therapy (SMART); (b) Staccato study. For each of these studies we perform computer simulations of the treatment strategies. These simulations suggest several underlying reasons for the adverse outcomes during treatment interruption. In particular, HIV infection exhibits rapid dynamic load changes, and therefore measurement based treatment regimes need to be carefully designed to avoid large transients in healthy CD4+T cell count. Furthermore, repeated treatment interruptions may accelerate the emergence of resistant mutant virus and may increase the infection of long term reservoirs such as macrophages which will accelerate progression to AIDS.

1. Introduction

According to statistics in a global summary of the AIDS epidemic from The World Health Organization (WHO) [1], by the end of 2007 an estimated 33 million people worldwide were living with HIV. That same year, some 2 million died of AIDS, and the number of people receiving highly active antiretroviral therapy (HAART) was reported as 2,990,000, while an estimated 9,700,000 need HAART.

Since HIV protease inhibitors (PI) were first invented around 1989, ongoing efforts have been made around the world to develop new treatment guidelines to limit or prevent the replication of HIV [2]. The introduction of HAART, a combination of three or more different antiretroviral drugs, has proven to bring benefits for most HIV positive patients. However, despite the benefits of HAART the treatment comes with the risk of significant medication-related toxicity and side effects [2]. In addition, although since 1996 the price of HIV/AIDS treatment has been significantly reduced, the high costs of drugs cause serious difficulties for developing nations to obtain the most recent and effective drugs [1]. For these, and other reasons, STIs have been proposed as one potential mechanism to help reduce the cost and side effects of HAART.

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doi:10.1016/j.cmpb.2011.07.011

This work begins with a brief description of the HIV infection process, and introduces a mathematical model of this problem in Section 2. Then, in Section 3, we review a number of clinical trials involving STIs and their results. The outcomes of two of the larger trials, namely, the SMART and the Staccato trials, based on STIs are discussed in detail in Sections 4 and 5, respectively. In both cases, computer simulation studies using the mathematical model described in Section 2.2 are used to explain the negative outcomes of these trials. From analysis of these results, an alternate STI regimen is discussed in Section 6. The paper is concluded in Section 7.

2. Preliminaries

Pathogens are accessible to antibodies only in the blood and extracellular spaces. However, some bacteria and parasites, and all viruses, replicate inside cells, where they may not be detected by antibodies. The destruction of these invaders is the function of T lymphocytes, which are responsible for the cell-mediated immune responses of adaptive immunity. From the end of their development in the thymus, T lymphocytes include two main classes, one of which carries the cell-surface protein called CD8 on its surface and the other bears a protein called CD4. CD8 Cytotoxic T Lymphocytes (CTL) are involved in the destruction of infected cells and the destruction of intracellular pathogens in macrophages. On the other hand, CD4+T-lymphocytes or simply CD4+T cells have different important functions in the adaptive immune response.

2.1. The HIV life cycle

Like most viruses, HIV does not have the ability to reproduce independently. Therefore, it must rely on a host to aid reproduction. In order to establish infection, HIV uses the protein gp120 to bind to CD4 receptors. Although HIV infects a variety of cells, the main target of HIV are CD4+T cells. To reproduce, HIV uses three enzymes found in the inner core of the virus: reverse transcriptase (RT), integrase (IN) and protease (PR), as depicted in Fig. 1. Once HIV is released into the host cell, RT reverse transcribes the viral RNA into a viral DNA segment.

However, this reverse transcription process is error prone, and mutations in the viral DNA commonly occur at this step. Integrase enzymes promote the insertion of the viral DNA into the host cell. Following transcription the provirus may remain dormant and produce few or no new DNA copies. When infected cell are activated, the provirus uses RNA polymerase to encode itself inside a messenger mRNA and creates new proteins that are broken by protease enzymes to form the core of the new virus. These viral components are assembled, and then released by a budding process to release a mature viral particle to the extracellular space.

2.2. A mathematical model of HIV infection dynamics

We present a differential equation model for the dynamics of HIV infection. This model describes the dynamic evolution in time of several key species including: healthy CD4+T cells (T), infected CD4+T cells (T_i^*), healthy macrophages (M), infected macrophages (M_i^*), viral particles (V_i), and the immune system CTL response to HIV infection (Z). Since it is also known that viral mutation may play a key role in HIV dynamics, see [4], our mathematical model includes m different viral mutants, indexed by the variable i in the following extended model based on [6]:

$$
\begin{aligned}
\dot{T} &= s_T - d_T T - \sum_{i=1}^{m} r_i^T \\
\dot{T}_1^* &= (1-\mu) r_1^T + \mu r_2^T - d_{T^*} T_1^* - \delta_1 T_1^* Z_1 \\
\dot{T}_i^* &= \mu r_{i-1}^T + (1-2\mu) r_i^T + \mu r_{i+1}^T - d_{T^*} T_i^* - \delta_i T_i^* Z_i \\
&\qquad i = 2, \ldots, m-1 \\
\dot{T}_m^* &= \mu r_{m-1}^T + (1-\mu) r_m^T - d_{T^*} T_m^* - \delta_m T_m^* Z_m \\
\dot{M} &= s_M - d_M M - \sum_{i=1}^{m} r_i^M \\
\dot{M}_1^* &= (1-\mu) r_1^M + \mu r_2^M - d_{M^*} M_1^* \\
\dot{M}_i^* &= \mu r_{i-1}^M + (1-2\mu) r_i^M + \mu r_{i+1}^M - d_{M^*} M_i^*, \qquad i = 2, \ldots, m-1 \\
\dot{M}_m^* &= \mu r_{m-1}^M + (1-\mu) r_m^M - d_{M^*} M_m^* \\
\dot{V}_i &= p_i^T T_i^* + p_i^M M_i^* - d_V V_i, \quad i = 1, \ldots, m \\
\dot{Z}_i &= s_Z + \kappa T_1^* Z_i - d_Z Z_i, \quad i = 1, \ldots, m
\end{aligned}
\tag{1}
$$

HIV may infect a number of different cells; activated CD4+T cells, resting CD4+T cells, quiescent CD4+T cells, macrophages and dentritic cells. For simplicity only activated CD4+T cells and macrophages are considered as host of the virus. $r_i^T = \beta_i^T V_i T$, $i = 1, \ldots, m$ is the infection rate of healthy CD4+T cells by viral strain i and $r_i^M = \beta_i^M V_i M$, $i = 1, \ldots, m$ is the infection rate of healthy macrophages by viral strain i.

The terms s_T, s_M and s_Z represent the constant supply of new CD4+T cells, macrophages and CTL cells from the thymus, bone marrow and other cell sources. $\kappa T_i^* Z_i$ is the antigenic stimulation rate of CTL response. CTL mediated clearance of infected CD4+T cells is represented by the term $\delta_i T_i^* Z_i$. Clinical studies of macrophages infected with HIV show that they can form multinucleated cells that may reach large sizes before degeneration and necrosis [5]. Detailed studies of infected macrophages showed that cytoplasmic virus had its origin in the Golgi element. Infected cells with cytoplasmic virus obscured from the immune system have the potential of circulating in the host like "Trojan Horses". For this reason, our mathematical model does not have any reaction between infected macrophages and CTLs.

Viral proliferation is modeled as occurring in both activated infected CD4+T cells and infected macrophages, as represented by $p_i^T T_i^*$ and $p_i^M M_i^*$. Cells and virus have a finite life span, with natural death terms expressed by $d_T T$ for non-infected CD4+T cells, $d_{T^*} T^*$ for infected CD4+T cells, $d_M M$ for non-infected macrophages, $d_{M^*} M^*$ for infected macrophages, $d_Z Z_i$ for CTL and $d_V V_i$ for the virus.

Nominal parameter values for the various reaction constants are based on [4,7], and are presented in Table 1. For simulation purposes, we propose a linear mutation tree as is shown in Fig. 2, where the "wild type" is represented by V_1, and

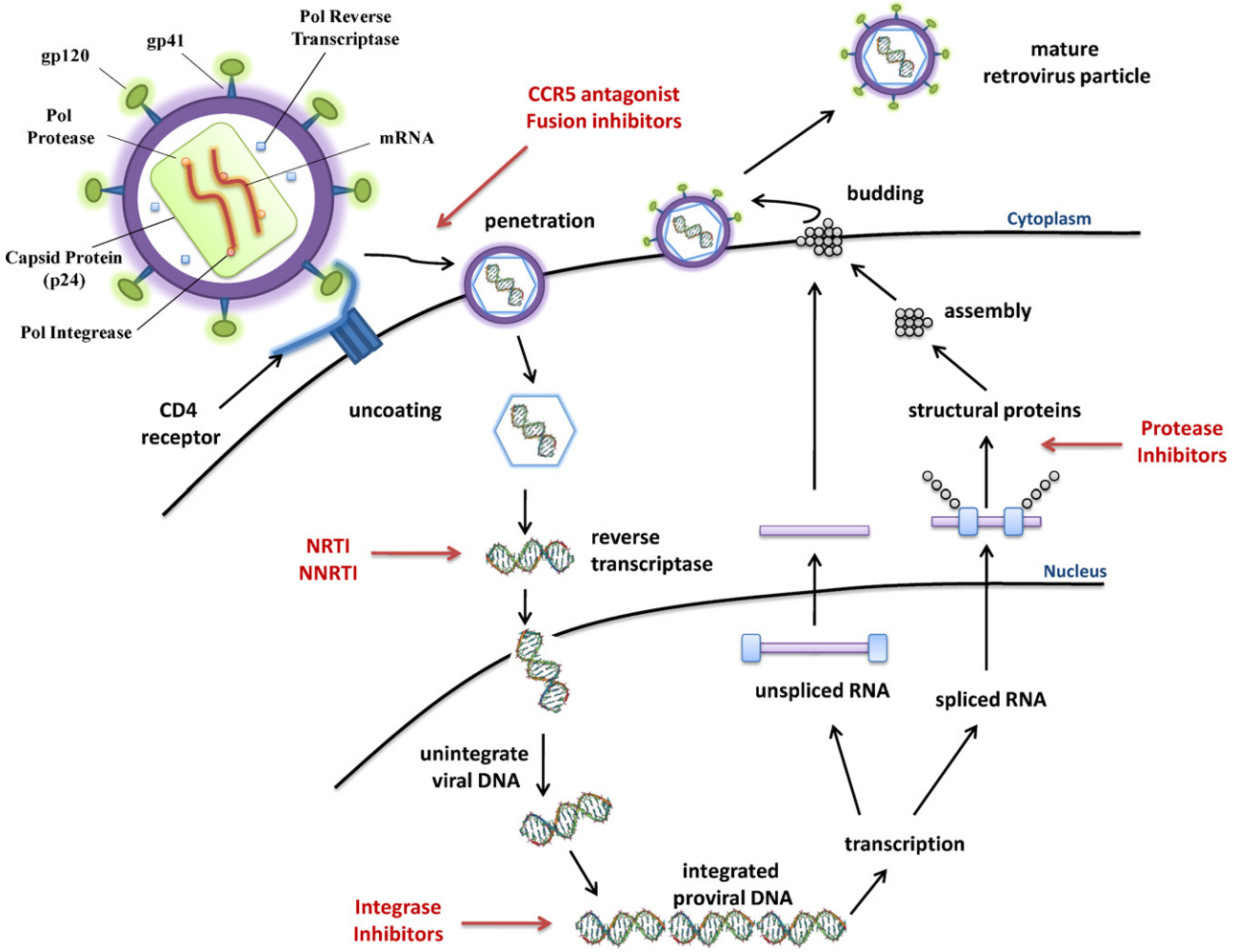

Fig. 1 – Interaction between free HIV and CD4+T cells during viral replication.

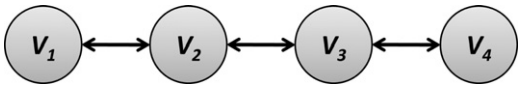

Fig. 2 – Linear mutation tree used for the computer simulation studies. This mutation tree shows a wild type genotype v_1, which may mutate linearly and escape drug treatment effects, then we can consider it as a high resistant genotype v_4.

V_4 is the highly resistant genotype. Note that a range of additional factors could be considered in a more detailed model, for instance [8] and [9].

2.3. *Drug treatments for HIV*

For some decades now, several drug treatments have been developed that interfere with the HIV replication cycle described in Section 2.1. Indeed, drugs are now available that target each of the key steps in the replication cycle. HIV entry to a target cell may be impaired by either 'fusion inhibitors' (that bind to the viral fusion protein gp120) or by CCR5 antagonists (that bind to the CCR5 co-receptor and thereby interfere with membrane fusion). For many years now, a range of RTIs (Reverse Transcriptase Inhibitors) have been developed, in many cases promoting premature termination of the reverse transcription process. Another drug class is the Integrase Inhibitors that work by impairing integration of viral DNA into the host genome. Protease Inhibitors (PIs) were one of the first drug classes to be developed to combat HIV which work by binding to the HIV protease and preventing the required protein cleavages. More recently, there have also been tests of maturation inhibitors which target the final viral maturation of the HIV life cycle.

HIV promotes high mutation rates, and therefore, treatment with any single drug is often ineffective. For this reason, most treatment regimes involve a number of different antiretroviral drugs, carefully selected to minimise adverse drug interactions and the possibility of cross resistant mutations. These combinations of therapy are often referred to as HAART, which can be included in the mathematical model (1), by adjusting several of the key parameters, depending on the drug dosage given to a patient.

The action of HAART in CD4+T cells is modeled by replacing β_i^T with $(1-\eta_i^T u_{RT})\beta_i^T$ and p_i^T with $(1-\eta_i^T u_{PI})p_i^T$, where

Table 1 – Nominal values of reaction constants used for computer simulation studies.

Constant	Value	Description
s_T	10	$CD4+T$ supply rate
d_T	0.01	$CD4+T$ death rate
β_1^T	3.28×10^{-5}	V_1 infection rate in $CD4+T$
β_2^T	3.1×10^{-5}	V_2 infection rate in $CD4+T$
β_3^T	3.1×10^{-5}	V_3 infection rate in $CD4+T$
β_4^T	2.7×10^{-5}	V_4 infection rate in $CD4+T$
p_1^T	50	V_1 replication rate in $CD4+T$
p_2^T	45	V_2 replication rate in $CD4+T$
p_3^T	45	V_3 replication rate in $CD4+T$
p_4^T	40	V_4 replication rate in $CD4+T$
β_1^M	2.44×10^{-7}	V_1 infection rate in M
β_2^M	2.3×10^{-7}	V_2 infection rate in M
β_3^M	2.3×10^{-7}	V_3 infection rate in M
β_4^M	1.5×10^{-7}	V_4 infection rate in M
p_1^M	29	V_1 replication rate in M
p_2^M	27	V_2 replication rate in M
p_3^M	27	V_3 replication rate in M
p_4^M	19	V_4 replication rate in M
d_{T^*}	0.4	$CD4+T$ death rate
d_V	2.4	Virus death rate
κ	0.045	CTL proliferation rate constant
d_Z	0.05	CTL death rate
δ_1	1.5×10^{-3}	CTL action to V_1
δ_2	1×10^{-4}	CTL action to V_2
δ_3	1×10^{-5}	CTL action to V_3
δ_4	1×10^{-6}	CTL action to V_4
μ	1.0×10^{-4}	Mutation rate

Table 2 – Drug efficiencies used for computer simulation studies.

Constant	CD4+T	Macrophages
η_1	0.80	0.70
η_2	0.60	0.50
η_3	0.40	0.30
η_4	0	0

u_{RT} denotes the effect of RT inhibitors which act to block new infections, u_{PI} denotes the effect of PI which causes the infected CD4+T cells to produce non-infectious virus and $\eta_i^T < 1$ is the maximum drug efficacy. In addition, various parameters in the model, and the drug efficacy, will be dependent on the mutant considered. Table 2 shows how drugs affect strongly the wild type genotype, while the high resistant genotype is not affected by any treatment.

Despite the promising results of HAART, currently available combinations of antiretroviral therapy fail to eliminate HIV-1 from infected patients, indicating the existence of refractory reservoirs of virus, which current therapies are not able to attack. One of these reservoirs could be macrophages [5], which would play an important role in the last stages of HIV infection.

The action of HAART in macrophages is modeled by replacing β_i^M with $(1-\eta_i^M u_{RT_i})\beta_i^M$ and p_i^M with $(1-\eta_i^M u_{PI_i})p_i^M$, where u_{RT_i} denotes the effect of RT inhibitors which act to block new infections, u_{PI_i} denotes the effect of PI which causes the infected macrophages to produce non-infectious virus. Because macrophages may form multinucleated cells, our model has the maximum drug efficacy for macrophages lower than that for CD4+T cells, see Table 2.

3. An overview of clinical trials of structured treatment interruptions

According to the recommendations of WHO [1], the optimum time to commence HAART is before patients become unwell or present with their first opportunistic infections (OIs). CD4+T cell counts are used to monitor HIV infection, and they are used to guide the decision of when to initiate HAART. For instance, The International Acquired Immune Deficiency Syndrome (AIDS) Society of USA recommends therapy for all symptomatic patients and for asymptomatic patients with CD4+T cell count below 200 cells/μl. Further, therapy should be considered and discussed with patients whose CD4+T cell count is in the range 200–350 cells/μl.

Therapy should typically be deferred for asymptomatic patients with CD4+T cell counts larger than 350 cells/μl [10]. Several studies have been suggested to determine the appropriate moment to commence therapy [11]. Infected patients are in most cases advised to commence HAART even before they develop symptoms of AIDS. The fact that HAART is an aggressive treatment has caused concerns related to consequences of its long term use. This motivated alternative treatment strategies, such as STIs which we now consider in more detail.

STIs were motivated in part by the clinical success of a patient in Germany, who was treated soon after diagnosis of acute HIV infection [12]. Before initiation of treatment in this patient, HIV RNA levels exceeded 80,000 copies/ml on two separate occasions, suggesting that a steady state of viremia had already been reached. After viral suppression on HAART, the therapy was temporarily discontinued, which was associated with recurrence of viremia. However, after a second discontinuation of treatment due to concurrent hepatitis A infection, viral rebound was not observed in that patient, who elected to stop therapy completely and showed continued viral suppression for the next 19 months. Since the patient's immune response progressively improved despite the absence of treatment, it was hypothesized that, intermittent exposure to HIV antigens may have boosted the HIV-specific immune response in this patient via autoimmunization.

For these and other reasons, several trials of STIs based on CD4+T cell count-guided therapy, or other STI regimens, have been made or are currently under way. Here, we briefly mention several of the main trials, and we later focus on two of the largest trials, namely (i) and (ii).

(i) *SMART study* [13], was a large international study designed to examine the effects of intermittent treatment in HIV-positive patients. The study's investigators set out to recruit 6000 patients, all with healthy CD4+T cell counts of 350 cells/μl or greater. They were randomized to receive continuous HAART (the *viral suppression* arm), or to receive intermittent treatment (the *drug conservation* arm) where treatment decisions were based on the patient's CD4+T cell count.

(ii) *Staccato trial* [14], was intended to study whether it would be better to stop treatment when the immune system has recovered and start again only when damage reappears. The patients were recruited for this trial from 2002 to

2004 and randomized into three arms. Individuals should either: (a) continue their existing HAART regimen; (b) interrupt treatment in a Week On Week Off (WOWO) manner; or (c) interrupt treatment according to their CD4+T cell count (resuming treatment when the healthy CD4+T cell count falls below 350 cells/μl).

(iii) *BASTA trial* [15] from Italy, whose primary objective was to compare efficacy and safety of continuing a conventional HAART in 114 chronically infected HIV patients with a therapeutic strategy based on long term, immunologically driven treatment interruptions. Patients were randomized in one of the two treatment arms: *Control group* with ongoing HAART, and *STI group* performing long term CD4+T cell count guided STIs. In the STI arm patients stayed off therapy until their healthy CD4+T cell count dropped to below 400 cells/μl. Individuals then commenced a HAART regimen and continued treatment until both their healthy CD4+T cell count exceeded 800 cells/μl and their HIV-RNA dropped below the detection limit of 50 copies/ml.

(iv) *Trivacan study* [16], was a trial conducted in West Africa aimed at assessing the benefits and risks of two different STI strategies compared with continuous HAART. The trial was designed in two phases. In the first phase, 840 HIV-infected adults started continuous HAART regimen. After at least six months on continuous HAART in this pre-randomisation phase, patients were selected for the next phase if they satisfied three criteria: (Healthy CD4+T cell count over 350 cells/μl; undetectable viral load and, absence of current OIs). Patients that satisfied these criteria were then randomized into three arms: (1) continuous HAART; (2) a fixed periodic STI strategy: 2 months on HAART followed by 4 months off HAART; or (3) healthy CD4+T cell count guided STI strategy. This latter arm was discontinued in October 2005. The trial is continuing for patients in arms 1 and 2.

A brief summary of these trials, and a number of other smaller trials, are presented in Table 3. In many cases, these smaller trials involved a number of patients considered too small to allow for the reliable assessment of effects of treatment interruption on clinical outcomes. Next section, we turn to review and analyze in more detail the outcomes from the SMART trial.

4. The SMART trial

4.1. *SMART trial methods*

Patients were randomly assigned to either the *Drug Conservation* group (DC) to receive episodic therapy or *Viral Suppression* (VS) group to receive continuous drug therapy. All patients recruited for this study had healthy CD4+T cell count ≥350 cells/μl, were enrolled over a 3-year period and were followed for an average of 7.5 years. The DC group used a STI therapy whereby HAART was withheld until the patient's CD4+T cell count declined to below 250 cells/μl. Once HAART was reinstated, the treatment was continued until the healthy CD4+T cell count was observed to exceed 350 cells/μl, at which point, treatment was again withdrawn. The VS group used HAART to maintain viral load as low as possible, irrespective of CD4+T cell count. Follow-up visits were scheduled at 1 month and 2 months, every 2 months thereafter for the first year, and every 4 months in the second and subsequent years for data collection visits [13].

4.2. *Clinical outcomes of the SMART trial*

In total, the SMART study enrolled 5472 patients (2720 assigned to DC and 2752 to VS). The patients had been followed up for a median of 14 months, during which there had been 164 recorded instances of disease progression, defined as death, or the development of a serious AIDS-related condition or a serious complication. Table 4 shows the relationship between adverse outcomes, proximal CD4+T cells and viral load levels, see [17]. The primary finding revealed a 2.5 fold increased risk of disease progression or death in DC group as compared with the VS group.

Ref. [18] reveals an analysis to determine why patients in the DC group, who appeared to spend very little time below 200 cells/μl showed worse outcomes. OIs and death occurred more among patients with lower healthy CD4+T cell counts and higher viral loads. It was found that combining both CD4+T cell count and viral load has better predictive power than taking either marker alone. However, it should be noted that CD4+T cell count and viral load markers did not explain all of the risk increase and there were other factors – yet to be identified – that also played some role in increasing the risk of OIs or death.

Ref. [19] examines the statistics surrounding the elevated OIs and death in the DC group. They suggested that the overall hazard ratio was 2.6, which means that participants in the DC were more than twice as likely to experience OIs or death. Among patients with viral load levels of 400 copies/ml or less, the rate of OIs or death was 3.2 in the DC arm, compared with 0.8 in the VS group. However, among patients with HIV RNA levels higher than 400 copies/ml, there was no significant difference. For these reasons, the DC arm of the SMART study has been stopped.

One of the recommendations in [18] is that treatment interruptions must be avoided unless motivated by some significant need, such as serious antiretroviral toxicity. This reference also suggests that there must be a "missing link" that would explain the unexpectedly high risk of adverse outcomes in patients undergoing treatment interruption, some "impairment of immune function not reflected in peripheral blood CD4+T cell count".

Ref. [19] concluded that across a range of baseline demographic characteristics, the risk statistics for the DC arm were similar. The only baseline characteristic that had a different outcome was baseline viremia, i.e., the risks due to treatment interruption were most pronounced in patients who entered the study with a viral load below 400 copies/ml. According to [20], the incidence of both serious and non-serious events was greater in the DC arm than in the VS arm.

A substudy to examine quality of life among 1225 SMART participants [21] concluded that episodic use of therapy did not improve quality of life of the patients. Moreover, physical functioning, general health perception and energy scores were

Table 3 – Clinical trials on STIs.

CD4	VL	No.	Treatment	Results	Ref
>300	<50	10	7 days on/7 days off for up to 68 weeks	Viral control maintained side effects ↓	[32]
$\frac{CD4}{CD8} > 1$	<20	12	Off until VL >3000 copies ml^{-1} or max 30 days off	Viral control maintained side effects ↓	[33]
>500	<50	8	Off until VL >5000 copies ml^{-1}	↑ HIV-1-specific T-cell response	[35]
>200	<5000	68	8 weeks off	↑ CD4 T-cell count ↓ Viral load	[36]
>400	<400	8	30 days on/30 days off for 7 months	↑ HIV-1-specific T-cell response. No viral control	[37]
Varied	<500	14	Range 14–196 days off	Viral rebound to pre HAART levels	[38]
>150	>5000	10	28 days on/28 days off	No ↑ drug resistance	[39]
Varied	<50	11	Varied	Resistance ≠ interruptions	[40]
>350	<50	18	Off until VL >5000 copies ml-1 or CD4 <25% from baseline	Viral rebound within 2 – 3 weeks	[41]
>300	<50	133	2 weeks off/8 weeks on for 4 cycles	Viral load similar to pre-HAART levels	[42]
>300	<50	14	2 weeks off/8 weeks on for 4 cycles	Viral rebound within 8 days	[43]
>300	<50	52	4 weeks off/8 weeks on	↑ drug resistance	[44]
Varied	Varied	40	Median 214 days off	↑ AIDS events	[45]

Table 4 – Adverse outcomes from the SMART study [13].

	VS	DC
Time on treatment	93%	33%
Median of interruptions	–	3
Disease progression	47 (1.5%)	117 (3.7%)
Patients more likely to die	0.9%	1.7%
Serious progression of disease	0.1%	0.6%
Risk of serious complications	1.4%	2.1%
Person year of follow-up % patients	72.3% of 3701	28.8% of 3666
Risk of serious complications	1.4%	2.1%
HIV RNA level	400	400
Therapy during follow-up time	94%	33%
Fatal or non-fatal OIs	47	120
Median proximal CD4 count	540	343
Follow-up time with CD4 < 350	7%	32%
Overall median viral load (logs)	2.6	4.0
Types and severity of clinical events—[20]		
Clinical events occurred	20	70
Patients in OFF therapy %	30	57
Serious events with CD 4< 350	0	9
Non serious events with CD4 < 350	7	34
Serious events with CD4 = 350	4	6
Non serious events with CD4 = 350	11	26

worse among patients during treatment interruption in the DC group compared to the VS group.

Finally, the SMART study has provided an answer to its primary goal, demonstrating that CD4+T guided treatment interruptions were inferior to continuous treatment within the study. Therefore, on January 10, 2006, the board recommended stopping enrollment in the SMART trial and all patients were advised to restart continuous treatment [13].

4.3. *Simulation of SMART*

A preliminary analysis of the SMART's results have suggested that the increased progression risk in patients undergoing treatment interruption may be explained in part by their lower CD4+T cell count over the course of the study. Here, we seek for a more detailed analysis, by using mathematical modelling, that may lead to a clearer explanation.

The mathematical description (1) is first used to study the relationship between the immune response and genetic mutations that may arise as HIV tries to escape the CTL response. To do so, the system (1) is simulated during the natural course of infection, i.e., not taking into account any therapy as shown in Fig. 3.

The CD4+T and CD8 population in uninfected humans have a typical range of equilibrium values, from 500 to 1500 cells/mm^3 for CD4 and from 300 to 1000 cells/mm^3 for CD8. The ratio of CD4/CD8 should stay between 1.2 and 2.2. However infected patients with HIV the ratio CD4/CD8 is inverted. As the immune system responds to HIV-1 (non-mutated virus) a first mutant arises and becomes dominant. This mutant is then recognized by CTL and the immune system reacts to eliminate this mutant viral strain. A new HIV variation arises and once again the immune system acts to clear it. These alterations occurring in the virus structure are characteristics during the acute stage of HIV infection when the amount of HIV in the blood is very high. At this early stage of the disease, infected CD4+T cells producing new viruses are almost completely destroyed either by the immune system

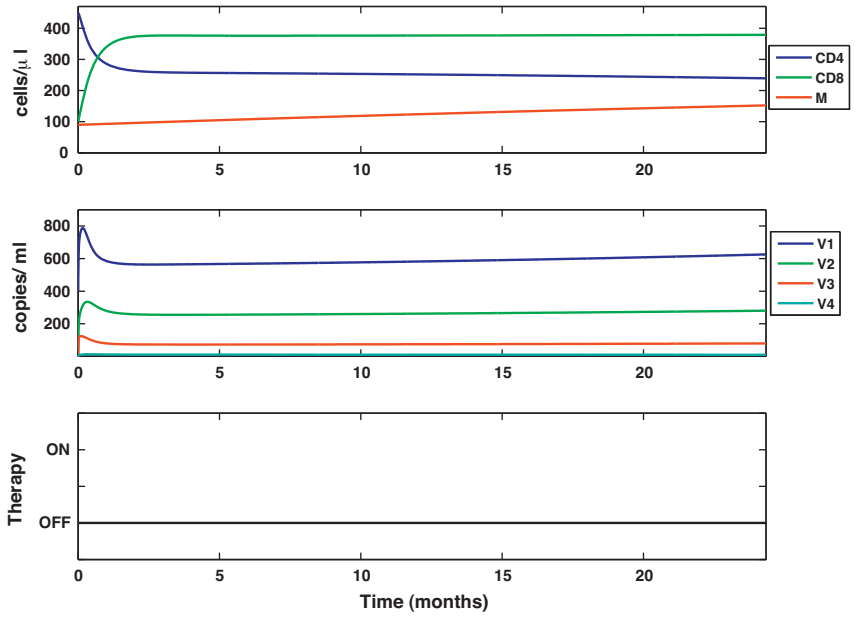

Fig. 3 – Simulation results for untreated HIV infection. These plots show the HIV behavior for patients without treatment. They illustrate the low levels of CD4+T cells, high viral concentrations slow drift upward in viral load and slow drift downward in CD4+T cell count, as observed in clinical practice.

or by natural death, see Fig. 3. Consequently, after the initial peak the viral load drops to low levels causing a stabilization in CD4+T cell count, in practice this may correspond to clinical latency or the 'asymptomatic phase', a phase of the disease in which the side effects and symptoms are slight. However infected macrophages are not affected by the immune response, and therefore they may produce virus in the last stages of infection.

4.4. *Simulations and possible explanations for adverse STI outcomes in the SMART study*

The DC arm of the SMART study can be understood in control engineering terms as a relay switching scheme, also known as ON-OFF control and common in thermostatic systems [22]. Such simple control techniques have proven very effective in controlling low order systems with simple dynamics, provided rapid measurements (ideally continuous) are used. In the case of the SMART study, there are two major potential shortcomings of this control (i.e., STI regime) design.

Firstly, the dynamics of HIV infection are quite high order, exhibiting non-linear and complex damped oscillatory responses. Such systems are unsuitable for simple relay control systems. Secondly, the measurement regime is, for good clinical reasons, not very frequent. However, the sampling rate for relay control should be faster than the system dynamics, which in the case of HIV, shows substantial dynamic behavior over intervals of days or weeks. In view of both of these limitations, poor regulation of healthy CD4+T cell counts can be expected, and therefore the immune system (CTL) response to the virus is also suppressed.

Fig. 4 shows numerical simulation results for the DC arm of SMART. Note that during periods when treatment is applied, the viral load drops to less than 400 copies. These observations are consistent with clinical findings presented in [23]. Their results suggest that CTL numbers decline rapidly when viral load is reduced by drug therapy. In Fig. 4, HAART is able to decrease the viral load levels throughout ON therapy. However, as the viral load is not completely cleared, during OFF therapy, HIV reproduces rapidly leading to a rebound in the viral load.

One of the problems of this intermittent treatment is reflected in the profile of CD4+T with monthly follow-up visits for the first year of treatment. Notice that during therapy the CD4+T cell count recovers to healthy levels (greater than 350 counts/μl). However, due to the long time till the next follow-up visits (every 30 days or more), there may be an important decay of healthy CD4+T cells before HAART is re-initiated. Furthermore, for a short time after re-commencement of treatment, the healthy CD4+T cell count declines further before recovering to a safe value. Hence, the CD4+T cell count could temporarily drop below the critical level (200 cells/μl). This reasoning also suggests a worse result if follow-up visits were to be scheduled every 4 months as proposed for the second and subsequent years of the trial.

As reported in some clinical studies, the interruption of therapy may allow re-emergence of drug-sensitive wild-type HIV [25]. This is illustrated in Fig. 4, which shows that drug-sensitive virus may reemerge in patients' blood following cessation of therapy.

Another potential problem with STI is that intermittent employment of HAART may also lead the appearance of resistant mutant strains that may cause many serious health risks. This is particularly dangerous in HIV-1 infected patients, whose CD4+T cell count is low, because the immune system may fail to respond to the resistant mutant. Fig. 4 illustrates that if macrophages are infected in early stages, then these long-term reservoirs would replicate HIV in later years of the infection. This numerical simulation also cautions against the

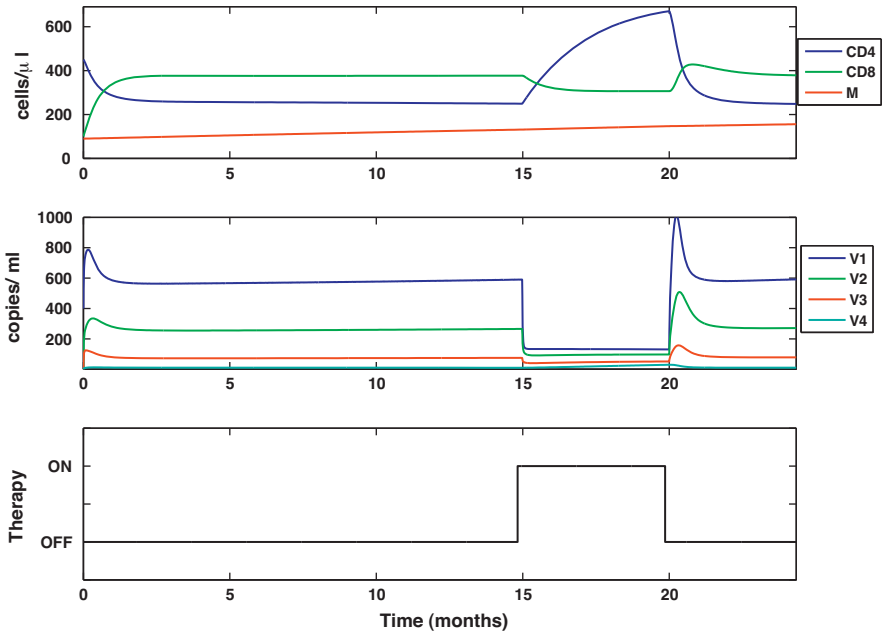

Fig. 4 – Numerical results for the drug conservation therapy arm of SMART. CD4+T cell dynamics exhibit the poor performance of this strategy, this is because therapy is just provided for a short period, which promotes viral proliferation and cell infection.

use of STI because of the risk of the re-emergence of resistant strains after HAART interruption.

Numerical results in Fig. 5 show that VS therapy greatly reduced the viral load and the CD4+T cells are maintained at over 250 cell/μl. Moreover, these results present the importance of continual therapy in suppressing macrophage infection. Macrophages may have an important role in the late stages of HIV infection.

There is also clinical evidence to support the view that STI may increase the risks associated with resistant viral strains. [26] suggested that mutations pre-existent in proviral DNA before HAART interruption, may emerge and become resistant to drugs, particularly to reverse transcriptase inhibitors. A similar conclusion is presented in [27], based on a randomized trial of 46 patients aimed at identifying and characterizing the emergence and persistence of drug resistant mutants during STI. The results of [27] show that STIs may select for drug resistant mutations, depending on regimen, virological and host factors.

5. The Staccato study

We now consider the Staccato trial [14,28]. The Staccato trial was a randomized study that recruited approximately 500 patients with healthy CD4+T cell counts greater than 350 cells/μl and viral loads <50 copies/ml. These patients were then randomly allocated to either continuous therapy (CT, 146 patients); treatment interruption (TI, 284 patients) or, 112 patients who were treated during 8 weeks in a week on, week off manner (WOWO). The one week interval was chosen on the basis that previous studies of treatment interruption have shown that viral load does not rise above 500 copies until at least a week after discontinuing treatment.

5.1. *Findings and conclusions on WOWO*

In patients whose HAART had been successful, the healthy CD4+T cell count had increased to above 350 cells/μl, with an HIV RNA viral load less than 50 copies/ml. When such patients are treated continuously with established HAART, future viral load failure (defined as two viral load measurements above 500 copies/ml) is rare, occurring in less than 5% of patients per year [29]. In the CT arm there were only two failures, while in the main WOWO arm, 53% of those 112 patients who completed the study experienced virologic failure in a short period of follow-up (eight weeks or four cycles of interruption). Such failures were verified also in simulations, see Fig. 6. Notice that since the entry criteria includes HIV RNA viral loads <50 copies/ml, then patients are not treated in the first week. However, because the viral dynamics are fast, when therapy starts (first cycle of week ON) the viral load is already higher than 500 copies/ml, causing the patient to experience virologic failure. Despite the progressive decrease verified in the next 3 cycles of interruption, drug resistant mutation strains may appear and cause other virologic failures.

When projected over the planned trial duration of 108 weeks, these high number of failures together with other findings [30], raised doubts about the feasibility of the WOWO approach to treatment interruption. For this reason, the WOWO arm of the Staccato trial was prematurely terminated.

6. A possible alternate STI regime

If, despite the earlier cautions based on both computer simulation studies and clinical evidence, there are situations where clinical trials of STIs are to be pursued, then careful attention

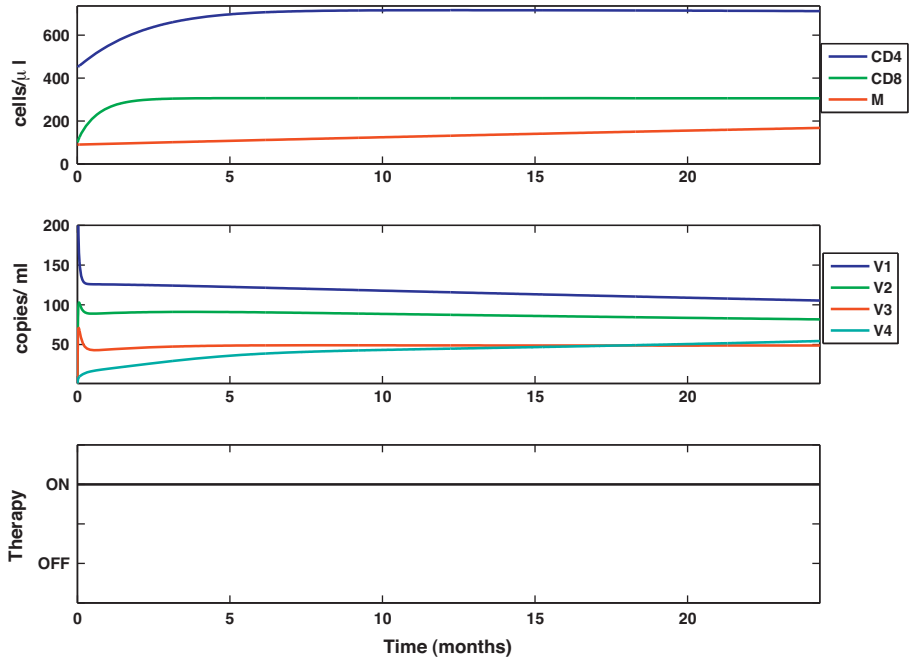

Fig. 5 – Numerical simulations of viral suppression therapy arm of SMART. HAART is always supplied which results in high CD4+T cells levels and viral load is always suppressed, this means we may reduce the appearance of new strains.

to the treatment regime is warranted. [17] suggests that the decisions to (re)commence and to stop therapy should be based on a combination of both healthy CD4+T cell count and viral load. This is qualitatively supported by the earlier computer simulation studies, that the combination is more predictive of the risk of OIs or death than either marker alone. In addition, as noted earlier, from the perspective of regulating healthy CD4+T cell count, more frequent sampling of this count and the viral load is desirable.

Based on these considerations, we propose an alternate regimen for decisions of when to re-initiate therapy, and when to withdraw therapy as follows:

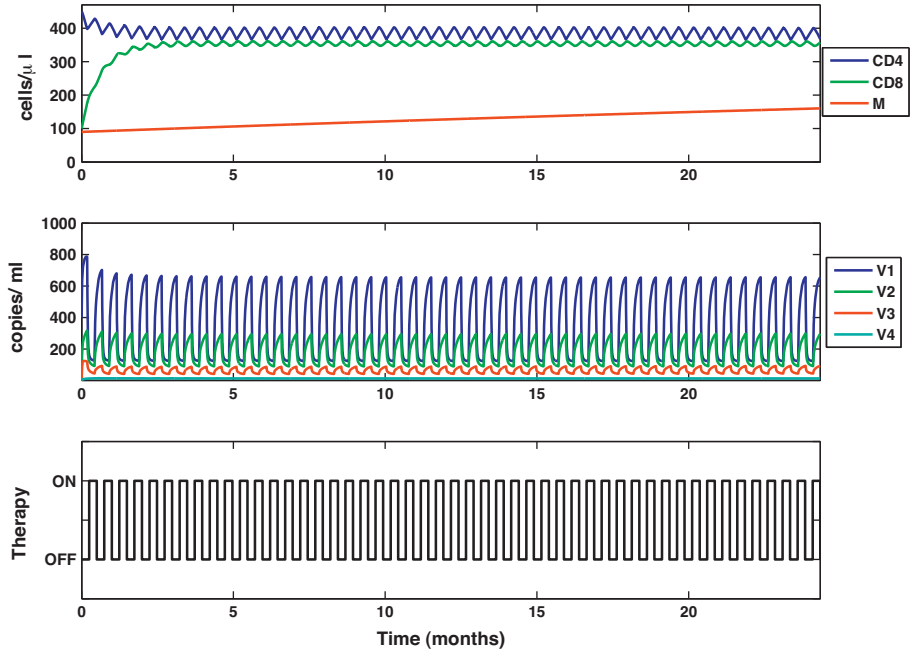

Fig. 6 – Simulations results for the WOWO of the staccato study expose the bad behavior of interruption treatments. Viral load shows high levels of oscillation.

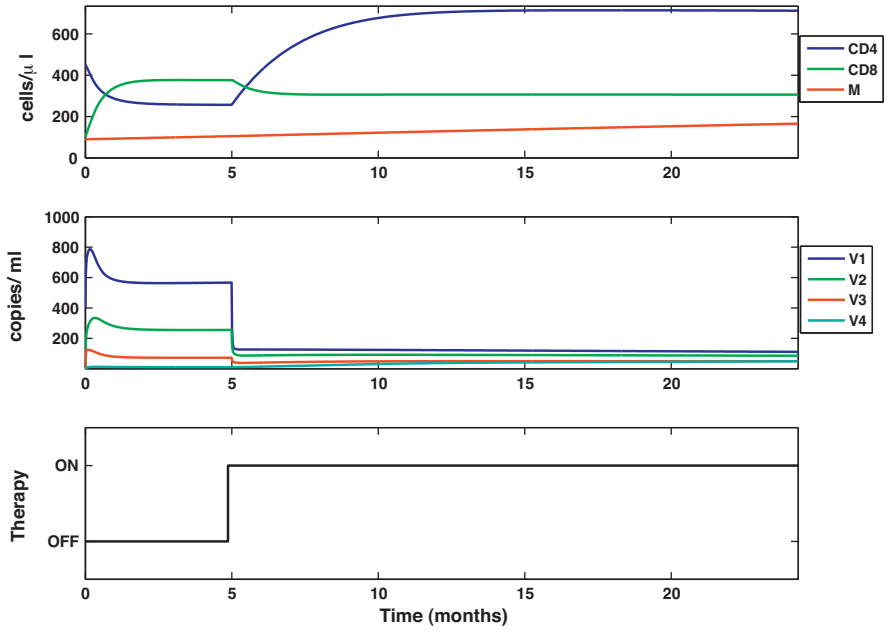

Fig. 7 – Numerical results of an alternate therapy with clinical decisions based on healthy CD4+T cell count combined with viral load present better dynamics than other STIs, however these results support the idea that treatment interruptions do not provide any improvement to tackle HIV.

1. Samples of viral load and healthy CD4+T cell count should be taken approximately 3 times per month.
2. HAART should be re-initiated whenever either: healthy CD4+T cell count drops below 250 cells/μl; or, viral load exceeds 400 copies/ml.
3. HAART can be withdrawn whenever both: healthy CD4+T cell count drops exceeds 350 cells/μl; and, viral load is almost undetectable (that is, less than 60 copies/ml).

Fig. 7 illustrates computer simulation of this alternative regimen. Notice that CD4+T cell count does not drop to the critical level (200 cells/μl). This alternative scheme seems to provide better immunologic control and thereby limit OIs while at the same time reducing the total exposure to HAART. Even though the exposure to HAART is reduced by this alternate STI regime, simulation results support that STI may lead to the appearance of resistant strains.

7. Conclusions

In this paper, we have briefly reviewed a number of clinical trials of STI approaches to treating chronic HIV infection. Two of the larger trials were the SMART study and the Staccato trial. Computer simulation studies of these two approaches to STI suggest that there may be several significant shortcomings in the approaches used to control HIV infection. Firstly, HIV infection shows rapid dynamic effects within a period of several days. These dynamics mean that to adequately regulate CD4+T cell counts, it may be necessary to employ a combination of more frequent measurements, perhaps as often as three times per month, together with decisions based on both CD4+T cell count and viral load measurements. Secondly, even with more frequent clinical decisions, viral mutation and the emergence of drug resistance appears to be significantly accelerated by STI. Furthermore, STIs may increase the infection of long-term reservoirs which will be important in late stages of HIV infection. These numerical results and supporting clinical evidences, cast doubt on the viability of STI.

Conflicts of interest

None declared.

Acknowledgements

The authors thank the support of Science Foundation Ireland. Grant number: 07/RPR/I177 and 07/PI/I1838.

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