

An In-Silico Analysis of the SMART Study of HIV Infection ^{*}

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Abstract: A mathematical model is developed to further examine the dynamic interaction of uninfected T cells with Human Immunodeficiency Virus (HIV) mutations. We study how the dynamics are affected by immune response to infected cells under intermittent antiretroviral therapy. Our goal is to analyze the SMART (Strategies for Management of Anti-Retroviral Therapy) study outcomes and based on that try to identify possible causes of its failure. We mathematically describe the HIV infection and perform numerical simulation to approach the course of the disease. Preliminary results suggest that the scheduling of follow-up visits and working range of CD4⁺ T cells count used during the SMART study could explain the observed adverse outcomes in that trial.

Keywords: Modelling and simulation, Biomedical systems, Control theory, HIV dynamics.

1. INTRODUCTION

HIV infection takes place when the glycoprotein gp120 on surface of HIV fits the protein marker cluster designation 4 (CD4) on the surface of most immune cells. Cells with this marker are referred to as being CD4 plus (CD4⁺) or helper T cells, Annah (2006). HIV recognizes CD4⁺, attaches to it and to a chemokine co-receptor (CXCR4 or CCR5) to facilitate to enter into the host cell by fusion between the HIV membrane and the host immune cell. Since 1996 when the use of protease inhibitors (PI) were acknowledged as effective against HIV, new antiretroviral drugs have been developed as part of an ongoing effort to try to control or prevent the replication of HIV, Panel (2006). The combination of three or more different antiretroviral drugs, commonly referred to as Highly Active Antiretroviral Therapy (HAART) has proven to bring enormous benefits for most HIV positive patients.

HAART slows remarkably the replication of HIV in the blood and in many cases reduces the amount of active virus (viral load) to undetectable levels. The International AIDS (Acquired Immune Deficiency Syndrome) Society of USA recommends therapy for all symptomatic patients and for asymptomatic patients with CD4⁺ below 200 cells/mm³. Further, therapy should be considered and discussed with patients whose CD4⁺ are 200-350. Therapy should typically be deferred for asymptomatic patients with CD4⁺ > 350, Brian (1998). Although several studies to determine the appropriate moment to start therapy have been proposed, Jeffrey et al. (2003), 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 as discussed in next section.

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2. THE SMART STUDY

The risk of significant toxicity and side effects have been a motivation for researchers to investigate new strategies to deal with HIV infection. As one of the options to HAART, the so called Structured Treatment Interruptions (STI) was developed with view to relieve HAART medication-related toxicity, to make HIV more sensitive to drugs (since continuous therapy may cause drug resistance) and to re-stimulate the immune system by allowing a controlled rise in the viral load. In this context, SMART was proposed as a large international trial designed to determine which of two distinct HIV treatment strategies yields a better clinical outcome over the long term.

The SMART study was supposed to run for over 9 years, but it was prematurely stopped after 2 years due to a significantly greater risk of opportunistic infections (OIs) or deaths in those who were assigned to receive interrupted therapy. SMART trial set out to recruit 6000 patients, all with CD4⁺ count ≥ 350 cells/mm³ who were randomized in two groups: First, *continuous drug therapy* or *Viral Suppression* group (VS), aimed at suppression of the viral load to undetectable levels, irrespective of the CD4⁺. Second, *episodic therapy* or *Drug Conservation* group (DC), designed to stop HAART when patients' CD4⁺ reached 350, and resumed when counts fell back down to 250 or less. Before randomization, participants' antiretroviral therapy history and medical history were obtained. Follow-up visits were scheduled at 1 month and 2 months, every 2 months thereafter for the 1st year, and every 4 months in the second and subsequent years, SMARTgroup (2006). Other trials based on CD4⁺ cell count-guided therapy are: *i) Staccato study*, Ananworanich et al. (2003). *ii) Trivacan study*, Danel et al. (2006), *iii) BASTA trial*, Maggiolo et al. (2003) and *iv) Short-cycle*, Cardiello et al. (2005). These trials involved a number of patients considered too small to allow for the reliable assessment of effects of treatment interruption on clinical outcomes.

2.1 Clinical outcomes

The SMART study enrolled 5472 patients (2720 assigned to DC and 2752 to VS) from 318 research sites in 33 countries. 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 1 presents the relationship between adverse outcomes, proximal CD4⁺ and viral load levels, see Sadr and Neaton (2006). The primary finding revealed a 2.5 fold increased risk of disease progression or death in DC group as compared with the VS group.

Lundgren (2006) presented an analysis to determine why patients in the DC group, who spent very little time below 200 cells/mm³ presented very bad results. OIs and death occurred more often among patients with lower CD4⁺ and higher viral loads. It was found that combining both CD4⁺ T cell count and viral load was more predictive of the risk than either marker alone, demonstrating that the risk of OIs or death is reflected by both lower CD4⁺ and higher viral loads. However, it should be noted that CD4⁺ 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 when interrupting therapy.

El-Sadr (2006) examined the risk factors that might help explain the elevated OIs and death in the DC group. They showed 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.

2.2 Conclusions from SMART study group

Treatment interruption guided by the CD4⁺ count, significantly increased the risk of OIs or death, as compared with continuous therapy, largely as a consequence of lowering the CD4⁺ and increasing the viral load. Treatment interruptions should be avoided unless motivated by some significant need, such as serious antiretroviral toxicity. The conclusion from Lundgren (2006) is that there must be a “missing link” that would explain the unexpected high risk of adverse outcomes in patients undergoing treatment interruption, some “impairment of immune function not reflected in peripheral blood CD4⁺”. El-Sadr (2006) concluded that across a range of baseline demographic characteristics, the findings were similar. The only baseline characteristic that had a different outcome was baseline viremia, i.e., the risk of treatment interruption was most pronounced in patients who entered the study with a viral load below 400 copies/ml, which suggests that viremic patients, whether ON or OFF therapy, carry a similar risk of adverse events. According to them, the rates of adverse outcomes were higher in DC group across all analyzed subgroup, and none appeared to benefit from treatment interruption. However, some groups did experience particularly inferior outcomes. According to Cohn (2006) the incidence of both serious and non-serious events was greater in the DC arm than in the VS arm.

Table 1. Adverse outcomes - SMARTgroup (2006)

	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	72.3% of	28.8% of
% of patients	3701	3666
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 - Cohn (2006)		
Clinical events occurred	20	70
Patients in OFF therapy (%)	30	57
Serious events with CD4 ⁺ < 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

However, among patients with proximal CD4⁺ of 350, the proportion of serious events was similar in both groups. A substudy to examine quality of life among 1225 SMART participants is presented by Burman (2006) concluded that episodic use of therapy did not improve quality of life of the patients. In treatment interruption, however, physical functioning, general health perception and energy scores worsened among patients in the DC group compared to the VS group. The different studies on SMART trials suggest that the type of treatment provides little if any benefit and considerable risk of both minor and life-threatening adverse events.

Finally, the SMART study has provided an answer to its primary goal, demonstrating that CD4⁺-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 because of a safety risk in the DC group and because it appeared to be very unlikely that the superiority of the drug conservation treatment would be shown. All patients were advised to restart continuous treatment, SMARTgroup (2006).

3. MATHEMATICAL MODELING 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⁺ over the course of the study. Despite this explanatory verification, we seek a more detailed analysis by using mathematical modelling, that may lead to a clearer explanation. We use dynamic analysis from a control theoretic point of view, looking at the interaction of HIV infection and its treatment as a process continuously evolving over time. This is done by describing CD4⁺ and viral load and using well established ordinary differential equation models for the dynamics of HIV infection. We also include the infected cells produced when free virus infects target CD4⁺.

Also, many studies have proven that the immune system can provide selection pressure for or against viral diversity, Nowak and Charles (1996). Therefore, we include in the model a simplified description of the genetic mutation of HIV. Ferreira and Middleton (2008) studied the dynamic properties of viral mutations of a model with a single point mutation in wild type (HIV-1). In their analysis of all possible equilibria and local stability properties, they proved that generically, there exists a locally unstable fixed point corresponding to an uninfected state and a locally stable equilibrium corresponding to the infected steady state. Ferreira et al. (2009) made a generalization of their technique to systems of m viral variants. They concluded that despite viral diversity, there exists two different valid equilibria: *i*) Uninfected state (locally unstable equilibrium) and *ii*) Infected state (m possible equilibria), where only one (corresponding to the fittest strain) is locally stable.

Since SMART was designed to examine the effects of intermittent treatment, notably the impact of therapy on the immunologic system, we include the immune response to infection. Such consideration provides information to assess the role of Cytotoxic T lymphocytes (CTL) and how they are affected by viral diversity in the following model extended from Kwon (2007).

$$\begin{aligned}
\dot{T} &= s_T - d_T T - T \sum_{i=1}^m r_i \\
\dot{T}_1^* &= (1 - \mu) r_1 + \mu r_2 - d_{T^*} T_1^* - \delta T_1^* Z_1 \\
\dot{T}_i^* &= \mu r_{i-1} + (1 - 2\mu) r_i + \mu r_{i+1} - d_{T^*} T_i^* - \delta T_i^* Z_i, \quad i=2, \dots, m-1 \\
\dot{T}_m^* &= \mu r_{m-1} + (1 - \mu) r_m - d_{T^*} T_m^* - \delta T_m^* Z_m \\
\dot{V}_i &= p_i T_i^* - d_V V_i, \quad i = 1, \dots, m \\
\dot{Z}_i &= \kappa Z_i T_i^* - d_z Z_i, \quad i = 1, \dots, m
\end{aligned} \tag{1}$$

where $r \in \mathbb{R}^m = [r_1, r_2, \dots, r_m]^T$.

(1) describes the interaction between the replicating virus and host cells, as well as the immune system and the HIV mutations. T_1^*, \dots, T_m^* denote each cell infected with a different virus strain (or mutant), V_1, \dots, V_m that are produced from uninfected target cells T ($CD4^+$) at infection rates $r_i = T\beta_i V_i$, $i = 1, \dots, m$. Therefore, there are m viral variants in (1). Z_i denotes the magnitude of the specific CTL produced in response to presence of T_i^* which decay at rate $d_{T^*} T_i^*$. Clearance of infected cells is modelled by CTL at rate $\delta T_i^* Z_i$. The term $\kappa Z_i T_i^*$ represents the proliferation of CTL in response to antigen. CTL decay at rate $d_z Z_i$. Parameter μ denotes the probability of mutation to resistant mutant during reverse transcription (RT) of viral RNA into proviral DNA. T cells are produced by thymus gland at constant, s_T . Their interaction with free virus produce T_i^* at rate β_i , which in turn produce new virus particles at rate p_i . Parameters β_i and p_i are selected to satisfy $\beta_1 \geq \beta_2 \geq \dots \geq \beta_m$ and $p_1 \geq p_2 \geq \dots \geq p_m$ respectively. T , V_i and T_i^* die at rates d_T , d_{T^*} and d_V respectively. κ denotes the magnitude of Z_i against V_i . According to Nowak and Charles (1996), κ is defined as the growth rate of specific CTL after encountering T_i^* .

According to Wodarz (2001), helper-dependent response can be modeled in greater details by distinguishing between CTLp (precursors) and CTLe (effectors). This is because help seems to be required more for the expansion

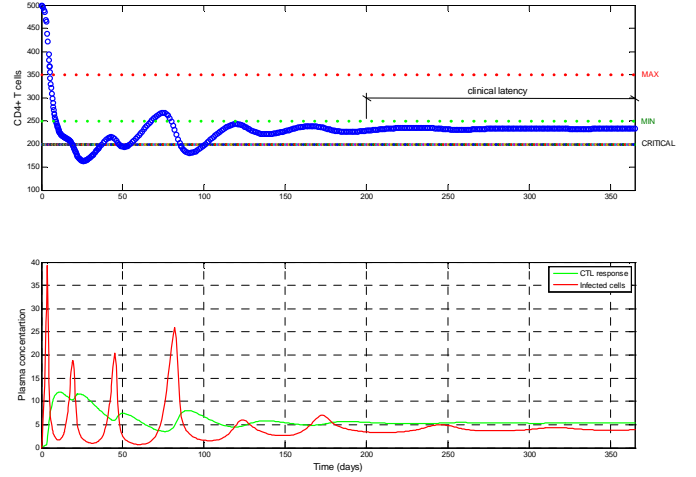


Fig. 1. Profile of CD4, CTL proliferation and infected cells

of CTLp population and not for the differentiation into effectors. Hence, if help becomes limiting, the rate of differentiation is greater than the rate of expansion, resulting in the extinction of the helper-dependent CTL response, see Doherty (1993). This does not apply to helper-independent response which can be captured by the single variable Z_i .

Annah et al. (2007) applied control theoretical concepts to study STI schedules with equal ON/OFF periods. They concluded that failure of most STI clinical trials could be attributed to failure to re-establish two conditions: *i*) whether or not the OFF therapy period is short enough and *ii*) whether or not ON therapy period is long enough to re-establish the conditions before the interruption. However, they do not consider explicitly the CTL and HIV mutation. Furthermore, their clinical trial was based on a much smaller cohort of 36 patients.

We studied the relationship between HIV and the immune system during the natural course of infection, i.e., not taking into account any therapy. It was observed that HIV escapes from CTL response due to genetic mutation. 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 again the immune system reacts by eliminating this mutated virus. A new HIV variation arises and once again the immunologic system acts to clear it. These alterations occurring in the virus structure are characteristics during the acute HIV infection when the amount of HIV in the blood is very high. At this short stage of the disease the infected cells producing new viruses are almost completely destroyed either by the immune system (figure 1) or by natural death. Consequently, after the initial peak the viral load drops to very low levels causing a stabilization in $CD4^+$, leading the patient to clinical latency (figure 1), a phase of the disease in which the virus may remain dormant for several years and produce few or no new copies of HIV.

4. EXPLANATIONS FOR SMART FAILURE

In some cases patients present long-term nonprogressor status, i.e., HIV positive patients with relatively high $CD4^+$ and therefore high levels of CTL, Autran and

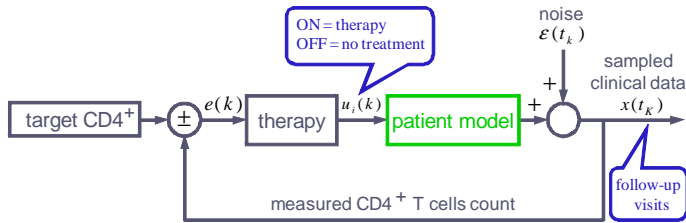


Fig. 2. Control system block diagram.

Garcelain (2000). However, because the immune system can not completely eradicate the virus in the long term, then most infected patients are expected to develop AIDS. A treatment strategy to reduce and prevent mutant virus particles and infected cells as well as to rise the uninfected $CD4^+$ T cells count is necessary. When the SMART study began, data indicated that the risk of AIDS was low among patients who had never received antiretroviral therapy and among those who had received it but who also had $CD4^+$ counts of more than 200 cells/mm³, Phair et al. (2002). Consequently, the SMART committee chose to use a $CD4^+$ T count threshold of 250 cells per cubic millimeter for initiation (or reinitiation) of antiretroviral therapy in the DC group, SMARTgroup (2006). The $CD4^+$ T cell count thresholds for stopping and starting antiretroviral therapy were chosen also on the basis of reported associations between $CD4^+$ counts and the risks of OIs and death, Bethesda (2006).

In control engineering terms, this corresponds to using a simplified “relay” switching scheme, also known as ON-OFF control and common in thermostatic systems. See Bennett (1993) for details. Such control systems have proven very effective in controlling low order (1 or two states) 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 the “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 should be faster than the system dynamics, which in the case of HIV, shows substantial dynamic behavior over intervals of days or weeks.

We model the input control, $u_i(k)$, corresponding to drug administrated by replacing β_i with $(1 - \eta u_{RT_i}) \beta_i$ and p_i with $(1 - \eta u_{PI_i}) p_i$, where u_{RT_i} denotes the effect of RT enzyme which acts to block new infections, u_{PI_i} denotes the effect of PI which causes the infected T cells to produce non-infectious virus and $\eta < 1$ is the maximum drug efficacy. Figure 2 shows diagrammatic representation of the relationship among the basic elements of the feedback control system. Each sampled data is compared with the working range defined for the $CD4^+$ block. $u_i(k) \in [0, 1]$ means that u_i is only allowed to assume OFF or ON, corresponding to stop or (re)initiate treatment respectively, depending on the difference, $e(k)$, and the scheduling defined for the follow-up visits. (1) is represented by patient model block and the noise corresponds to undesirable input signal (lack of accuracy in measurements, human error etc).

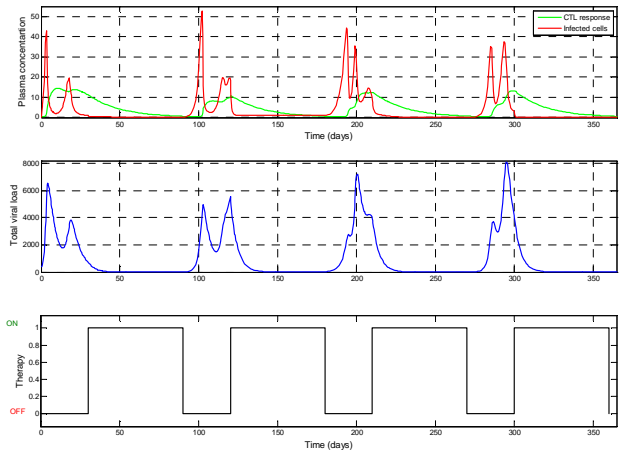


Fig. 3. Infected cells and viral load during OFF therapy.

In figure 3 the immune system reacts to increasing of infected cells that springs back as consequence of intermittent rebound of HIV during treatment interruption. This simulation is intended to highlight that even in patients who have no easily detectable HIV in their blood, HIV does rapidly rebound to high levels if the drugs are discontinued. These observations are in agreement with clinical findings presented by Kalams et al. (1999) who studied the interaction between the host cellular immune response and HIV-1 infection before and after the start of HAART. Their results suggest that CTL numbers decline rapidly when viral load is reduced by drug therapy. According to Chun et al. (1997), there exists an HIV-1 latent reservoir in infected patients despite prolonged treatment with HAART. Another possibility is that HIV may stay hidden in $CD4^+$ T cells more susceptible to infection before initiation of therapy. Palmer et al. (2008) suggest that low-level persistent viremia appears to arise from at least two cell compartments, one in which viral production decays over time and a second in which viral production remains stable for at least 7 years. In figure 3 the treatment is able to keep viral load at very low levels throughout ON therapy. However, as drugs do not completely clear HIV, then during OFF therapy more copies of HIV are produced leading to a higher viral load.

One of the problems of this intermittent treatment is reflected in the profile of $CD4^+$ with monthly follow-up visits for the 1st year of treatment and different initial conditions, see figure 4. Notice that although the period under therapy is able to re-establish healthy cells ($CD4^+ > 350$), because of the long time till the next follow-up visits (every 30 days) there is an important decay of healthy cells before the therapy is reinitiated. Furthermore, even after re-commencement of treatment, the $CD4^+$ count declines further before recovering to a safe number. Therefore, the T cell count temporarily drops well below the critical level (200 cells/mm³). This suggests a much worst result if we consider the follow-up visits scheduled every 4 months as proposed for the 2nd and subsequent years of the trial.

Numerical simulations were performed with the following parameter values from Jeffrey and Xia (2002), Nowak and Charles (1996): $s_T = 10\text{mm}^{-3}\text{day}^{-1}$, $d_T = 0.02\text{day}^{-1}$,

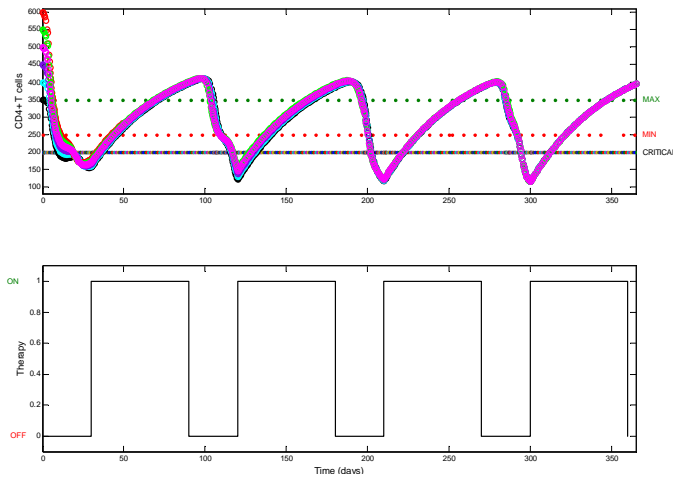


Fig. 4. Profile of CD4⁺ with monthly follow-up visits.

$$\beta_1 = 2.4e^{-6} \geq \beta_2 \geq \dots \geq \beta_m, p_1 = 100 \geq p_2 \geq \dots \geq p_m, \\ d_{T^*} = 0.24\text{day}^{-1}, d_V = 0.24\text{day}^{-1}, \kappa = 0.05, d_z = 0.05, \\ \delta = 1, \mu = 0.0001.$$

5. CONCLUSIONS FROM THEORETICAL STUDIES

Dynamics from (1) in figure 3 qualitatively shows that during ON therapy the CTL response declines as the viral load drops to very low levels, in agreement with Wodarz (2001) who verified that at the initial stage of therapy, the degree of immune impairment is reduced while levels of virus load are still sufficiently high to induce an initial expansion of the helper-dependent CTL response. However, viral load subsequently drops to very low levels, insufficient to maintain the CTL response. This could be due to scheduled follow-up visits that contributes notably to decline CD4⁺ to below 250 and consequently may expose patients to OIs. By making a relation between these conclusions and the results from clinical data of Lundgren (2006) in which the authors wondered why patients under SMART did so poorly with CD4⁺ < 200 cells/mm³ for a short time, the following issues are open for further investigations:

i) Recall from table 1 that patients spent 32% of their follow-up time with CD4⁺ < 350 cells/mm³ and that in figure 4, patients are prone to serious events because of exposition to critical levels of CD4⁺. It would be therefore of interest to consider STI strategies with briefer OFF therapy intervals. This could be achieved either by more frequent diagnostic visits or by replacing “OFF” therapy by a short term (eg., 1 week) interruption followed by resumption of therapy.

ii) SMART study recommended that treatment interruptions should be avoided unless motivated by some significant need (serious antiretroviral toxicity). Further, if an interruption occurs, patients should be closely monitored and therapy preferentially restarted at higher CD4⁺ (about 350) than those used in the SMART. This clearly highlights that, effectively, the follow-up time originally scheduled made the patients more likely to experience OIs due to low CD4⁺. In addition, the treatment design may have given better clinical outcomes had it been aimed at keeping the minimum predicted CD4⁺ count above 250.

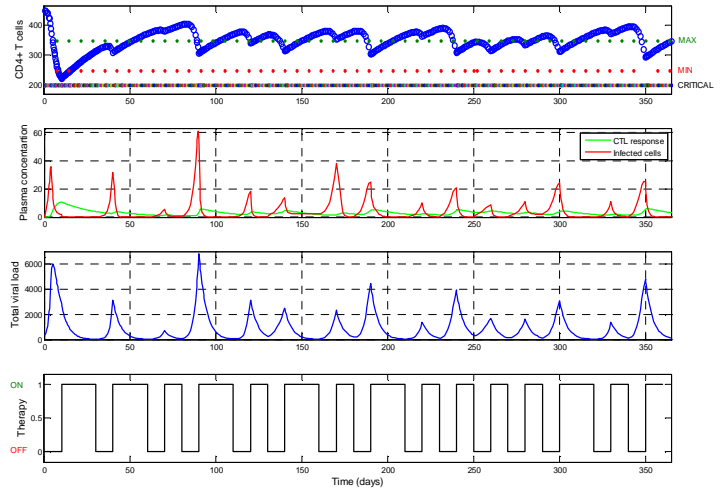


Fig. 5. Guided CD4⁺ therapy combined with viral load.

This of course requires simple formulae of software capable of short term extrapolation of sampled CD4⁺ levels.

iii) Sadr and Neaton (2006) suggest that the decision to (re)commence and stop therapy based on a combination of both CD4⁺ T cell count and viral load is more predictive of the risk than either marker alone, demonstrating that the risk of OIs or death is reflected by both lower CD4⁺ and higher viral loads.

Figure 5 combines an every 10 days of follow-up evaluation assuming both guided viral load (60-400 copies/ml) and guided CD4⁺ (250-350 cells/mm³) therapy. Notice that CD4⁺ T cell count does not drop to the critical level (200 cells/mm³) during the 1st year. This alternative scheme provides better immunologic control to prevent OIs while at the same time limit exposure to HAART with its attendant side effects. Our conjecture is based on the fact that the onset of AIDS occurs, on average, about 5 to 10 years after infection. This is supported by Richard (2008) who observed that the beginning of AIDS has is correlated with the diminution of the number of CD4⁺ but the major loss of T cells occurs late in HIV infection. Therefore, from a hypothetical point of view, CD4⁺ marker used in SMART study was not the most appropriate predictor of serious OIs.

The mathematical description developed for the disease progression has emphasized the important role played by immune response during the first stages of the disease (primary infection and asymptomatic phase). Our results suggest that the long follow-up visits (monthly) does not allow to preserve a satisfactory immune response in patients during withdrawal of therapy.

We are currently working on a more realistic approximation to describe the shift from clinical latency to symptomatic infection and subsequent progression from HIV to AIDS. The new approach will be also used as platform for the development of control strategies based on the body’s own defences to drive patient’s state to LTNP.

(Chapter head:)*

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