

Risks and benefits of structured antiretroviral drug therapy interruptions in HIV-1 infection

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Background: Structured interruptions of antiretroviral therapy of HIV-1 infected individuals are currently being tested in clinical trials to study the effect interruptions have on the immune responses and control of virus replication.

Objective: To investigate the potential risks and benefits of interrupted therapy using standard population dynamical models of HIV replication kinetics.

Methods: Standard population dynamical models were used to study the effect of structured therapy interruptions on the immune effector cells, the latent cell compartment and the emergence of drug resistance.

Conclusions: The models suggest that structured therapy interruption only leads to transient or sustained virus control if the immune effector cells increase during therapy. This increase must more than counterbalance the increase in susceptible target cells induced by therapy. The risk of inducing drug resistance by therapy interruptions or the risk of repopulating the pool of latent cells during drug-free periods may be small if the virus population remains at levels considerably below baseline. However, if the virus load increases during drug-free periods to levels similar to or higher than baseline before therapy, both these risks increase dramatically.

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Introduction

Despite significant recent advances in the development of potent antiretroviral inhibitors, treatment of HIV infection still faces many obstacles. Although combination therapy leads to a sustained reduction of virus load in many patients, eradication of the virus from these patients may not be achievable with current antiretroviral drug regimens because the virus can remain latent in cell populations with very slow turnover [1,2]. As a consequence, patients have to remain, for an indefinite period, on a treatment regimen consisting of highly

potent, costly medications that may be associated with adverse side-effects. Because of the high costs, combination therapy is only available to a small minority of the HIV-1-infected individuals worldwide. Moreover, because of the low tolerability of these drugs and the general absence of overt HIV symptoms, patients, who receive long-term combination therapy often become less adherent to the regimens, which in turn may result in the development of multidrug-resistant virus.

In view of these concerns, there is clearly a need to consider new, imaginative treatment strategies using

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the currently available drugs. In particular, given the evidence in support of CD8 T lymphocyte (CTL) responses in controlling virus load [3–6], treatment strategies that aim to boost the patient's cellular immune responses during therapy deserve closer investigation. One such strategy, which has recently received considerable attention [7], is structured therapy interruption (STI), here defined as a precise schedule according to which patients are put on and off therapy over a defined period of time. The idea behind STI is to boost the patients' HIV-1-specific immune responses with autologous virus in a process similar to vaccination. The boosted immune response may subsequently help to increase the rate of clearance of the virus during drug treatment or may help to maintain the virus load at a low level once patients stop drug therapy altogether.

Encouraging support for the concept of STI comes from isolated case reports. There have been several reports of viral suppression after intermittent compliance and subsequent drug therapy [8,9]. One of these reports showed that cellular immune responses were associated with control of viremia to < 500 copies/ml for up to 2 years [9]. Furthermore, it has recently been reported that in some patients with chronic HIV infection, interruption of antiretroviral drug therapy can effectively boost cellular immune responses [9–11]. These observations have raised the question of whether STI can boost the cellular immune responses against HIV to levels that potentially may reset the original virological set point to a lower level.

However, as well as the potential benefits of STI, there could also be serious drawbacks, such as an increased risk of drug resistance and repopulation of the reservoir of latent infected cells. Currently, several clinical trials are being designed to evaluate STI. We believe that at the current state of affairs it is useful to assess the potential risks and benefits of STI in the framework of simple population dynamical models of HIV replication. These models may help to interpret forthcoming clinical data.

Results

Dynamics of the virus

The dynamical behavior of HIV infection is determined by a complicated web of non-linear interactions between several populations including those of free virus, infected cells, susceptible target cells, and cells involved in the immune response. The non-linear interactions make it difficult to predict how these populations react to therapeutic interventions such as starting or stopping therapy. To illustrate this, consider the population dynamics of a patient put on antiretro-

viral therapy (Fig. 1). Prior to treatment, the populations of infected cells and the immune responses are in a steady state [12–14]. When therapy is started the population of virus-infected cells decreases rapidly. Let us assume that concomitantly the population of immune effector cells (both 'armed' and 'memory' CTL) declines during treatment, but at a considerably slower rate such that the ratio of immune effector cells to infected cells increases during treatment. Is there a point at which effector cells outnumber the infected cells such that the effector cells could control the infected cell load when therapy is stopped? To address such questions it is useful to build a mathematical model for the dynamics of the interacting populations. Such a model provides a useful tool to study biological assumptions and their logical consequences and may help to interpret clinical data.

We begin with a standard model of the rate of change of the population density of infected cells, I , as a function of the densities of susceptible target cells, T , and immune effector cells, E , given by

$$dI/dt = (bT - d_I - pE)I \quad (1)$$

The model is discussed in detail in the Appendix. Briefly, b represents the infectivity of the virus, d_I is the death rate of infected cells owing to viral cytopathicity and p gives the killing rate of infected cells by immune effector cells.

This equation represents the simplest form that describes the dynamics of infected cells as a function of the population densities of susceptible target cells and immune effector cells. It is similar to the equation used

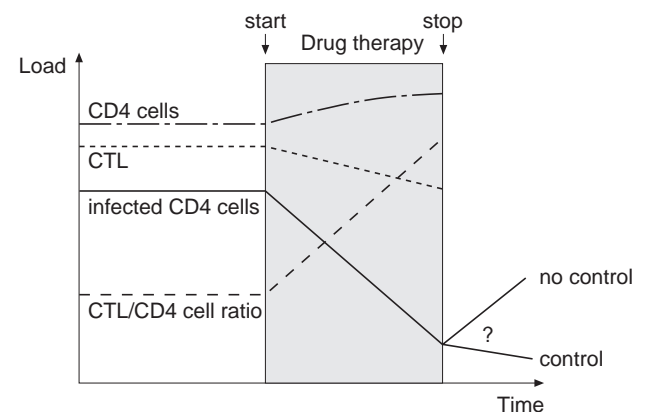


Fig. 1. The structured therapy interruption paradigm: if a patient's virus load declines much faster during highly active antiretroviral therapy than the decline in HIV-specific cytotoxic T lymphocyte (CTL) frequency, will there be a point at which the CTL outnumber the infected cells to an extent that the CTL can suppress the virus load when therapy is stopped?

in the models for the quantification of HIV dynamics *in vivo* [13–15] but is expanded by the term pEI , which reflects the contribution of the immune responses to the overall death of infected cells [16–19].

Dynamics during and after treatment

The left-hand side of equation (1) is the rate of change of I at time t . Thus the infected cell population grows whenever $dI/dt > 0$ and declines whenever $dI/dt < 0$. If $dI/dt = 0$, i.e. if the virus population is in steady state, then the steady-state densities of susceptible cells, T_{ss} , and immune effector cells, E_{ss} , must fulfill $bT_{ss} - d_1 - pE_{ss} = 0$. Since both reverse transcriptase inhibitors and protease inhibitors reduce the rate at which susceptible cells get infected, the viral infectivity, b , is reduced in the presence of drugs to some level $b_d < b$. Assuming that at the start of treatment the infected cell population is at steady state, the population of infected cells declines during treatment, because $b_d T_{ss} - d_1 - pE_{ss} < 0$. Both, common sense and experimental evidence suggests that the number of susceptible target cells increases concomitantly [20–22], because viral infectivity is reduced and the population of infected cells is declining. Therefore, we expect that at the end of a period of treatment the population of susceptible cells has increased to some level $T_e > T_{ss}$. At the same time, the number of effector cells will have changed from the steady-state level E_{ss} to a level E_e , which may be higher or lower than E_{ss} .

Above we have asked the question whether in the scenario shown in Fig. 1 the ratio of immune effector cells to infected cells may reach a threshold during treatment where the effector cells are able to control the viral replication when therapy is stopped. Equation (1) provides an answer to this question. Stopping therapy is equivalent to increasing the viral infectivity from its reduced level, b_d , back to b . Clearly, if during treatment both the number of susceptible target cells has increased ($T_e > T_{ss}$) and the number of effector cells has decreased ($E_e < E_{ss}$) then the conditions are favorable for the growth of virus when therapy is stopped, because $bT_e - d_1 - pE_e > 0$. Stated another way, the virus population will only continue to decline after therapy if the number of effector cells has increased during treatment to a new level given by $E_e > E_{ss} + b/p(T_e - T_{ss})$. This increase has to more than counterbalance the increase of susceptible cells during treatment.

Equation (1) implies that unless the effector cells increase during treatment to a level higher than the pretreatment equilibrium, there will never be a point after which the immune response can suppress virus growth in absence of therapy, whatever the ratio of effector cells to infected cells. Why is this so? One might expect that a ratio of 1000 effector cells to 100

infected cells is more favorable to the virus than a ratio of 500 to 10?

According to Equation (1), the rate of killing of infected cells by immune effector cells is pE per infected cell. Therefore, a density of 1000 effector cells (per unit volume) leads to twice the rate of killing per infected cell than a density of 500 effector cells. This is because the term pIE assumes random interactions between effector cells and infected cells. Klenerman *et al.* reported increasing rates of target cell killing for increasing effector to target ratios [23]. However, since different effector cell concentrations were added to a fixed number of target cells, Klenerman *et al.* actually demonstrated a dependence of the killing rate on the effector cell concentration and not on the effector to target ratio. In fact, they conclude that their data support the concept that the killing rate is proportional to the effector cell concentration.

Two questions emerge at this point. Would a similar conclusion be reached if the main effect of the effector cell population is prevention of infection rather than killing of infected cells? Under what conditions do we expect to see an increase in effector cells during treatment?

To address the first question we note that an inhibitory effect of effector cells on infection can be modeled by replacing the term bTI by $bTI/(1 + KE)$ in Equation (1), where K is a constant. In the absence of effector cells ($E = 0$), the two terms are identical. As the effector cell population increases, the infectivity effectively decreases. In analogy to the argument above, we see that an increase in T and a decrease in E results in an increased rate of infection of susceptible cells and thus in a positive growth rate of the virus when therapy is stopped. Hence, in this case also the virus load will only continue to decline after therapy interruption provided the effector cell population increased during treatment.

To answer the second question is more difficult since the feedback between infected cells and immune effector cells is complex. On the one hand effector cells contribute to the death of infected cells, on the other hand effector cells require stimulation by the infected cells for proliferation. Most models of HIV dynamics that explicitly incorporate an effector cell population are of the form

$$dE/dt = KI - d_E E \quad (2)$$

where d_E is the death rate of effector cells and K is a function describing the stimulation of the proliferation of effector cells. In these models, the function K may either be a constant, k , or depend itself on the effector cells (for example $K = kE$ or $kE/(a + E)$, where a and

k are constants [16–19]). Although these models make different assumptions about the proliferation of immune effector cells, in all of them the proliferation of effector cells depends on the density of infected cells. Therefore, during treatment, as the infected cell population declines over several orders of magnitude, the stimulation of proliferation of the effector cells declines as well (see Fig. 2). Therefore all these models predict a decline of the effector cell population during treatment and, as a consequence, a positive growth rate of the virus at the end of therapy. Under what circumstances, then, do we expect to observe an increase in the effector cell population during treatment?

The population of effector cells will increase during treatment if either the ‘production’ of effector cells increases or the ‘death’ of effector cells decreases. For both possibilities there is some biological support. The production of effector cells could increase, for example, because of the increase of CD4 T helper cells or because of peripheral redistribution from lymph nodes. Wodarz *et al.* proposed a model in which the production rate of memory effector cells increases during treatment because of increasing CD4 cell help [24]. However, this requires that CD4 cell help increases faster than antigenic stimulation declines. Whether this is likely to occur *in vivo* is debatable, since the viral load usually decreases rapidly over several orders of magnitude during treatment. Another possibility is that the virus has an inhibitory effect on the effector cells. For example, it was demonstrated that the death rate of effector cells increases with increasing virus load in SIV

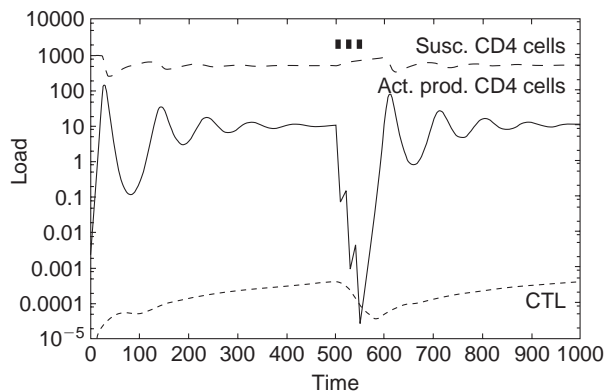


Fig. 2. The simulation of model 1 (see the Appendix) describing HIV dynamics during structured therapy interruption. Although this leads to an increase in the ratio of cytotoxic T lymphocyte (CTL) to infected cells, the virus load always grows in drug-free periods because the population of susceptible target cells (Susc. CD4 cells) increases and the population of effector cells decreases in presence of drug, thus making the condition favorable for virus growth in absence of drug. Population densities and time are given in arbitrary units; the bars show periods of drug treatment. Susc. CD4 cells, susceptible target cells; Act. prod. CD4 cells, cells actively producing virus.

[25]. A decrease in the load of infected cells may, therefore, decrease the inhibitory effect on the effector cell population. Any of these factors or a combination of them may result in a positive growth rate of the effector cell population during treatment. However, in order to result in an increase of the effector cell population during treatment, these factors must more than make up for the reduced stimulation of proliferation of effector cells that is associated with the decline of infected cells during treatment.

The conclusion drawn from Equation (1) is that the virus population grows when therapy is stopped unless the effector cell population has increased during treatment. Note, however, that at least theoretically there is also the possibility that virus growth could be controlled in the absence of an increase in the effector cell population, provided the effectivity of killing of infected cells increased during STI. Mathematically, this would require an increase of the parameter p during STI. Note, however, that an increase in the value of p implies that the effectivity of killing of infected cells has to increase per effector cell.

Transient or sustained control

Even if the immune response increased during treatment such that the virus growth rate is negative when therapy is stopped, the question remains whether this control of virus replication is transient or sustained. A sustained control implies that therapy shifts the virus load from its pretreatment equilibrium to a new, lower equilibrium level, which is sustained even in the absence of therapy. Mathematically, this means that in addition to the pretreatment levels of susceptible cells, T_{ss} , and effector cells, E_{ss} , there is a second set of values, T_c and E_c , for which Equation (1) equals zero.

Although many models of the combined dynamics of susceptible, infected cells and effector cells do not show control of virus growth after therapy, it is possible to construct a reasonable model that results in sustained control after STI. Figure 3 shows the dynamical behavior of such a model in which only STI of a certain duration and spacing of treatment intervals shifts the dynamics in favor of sustained immune control. In this model, the death rate of effector cells depends directly on the load of infected cells. Thus a decrease of the infected cell load also results in a decrease of the death rate of effector cells. Interestingly, if treatment is left uninterrupted over a long period of time the patient may revert back into the ‘virgin’ state from which the virus can grow back to its high pretreatment value (Fig. 3). At the heart of the model underlying Fig. 3 is the fact that this dynamical system allows for two stable steady states; transitions between these steady states can be achieved by STI. The model and its assumptions are explained in the Appendix (model 2).

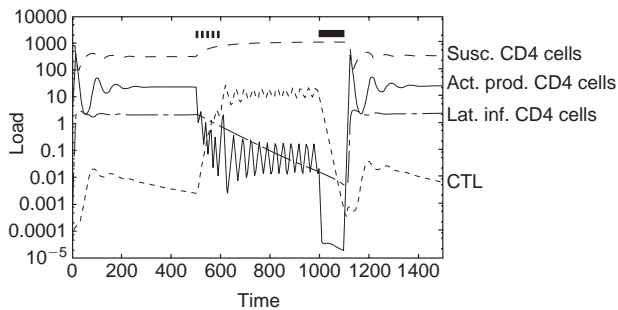


Fig. 3. The simulation of a dynamic model of HIV replication in which structured therapy interruption (STI) can shift the balance in favor of sustained suppression of virus load (model 2). Details of the model are given in the Appendix. The model is an extension of model 1 and incorporates a latent infected cell population. Furthermore the model includes a term reflecting virus-related impairment of cytotoxic T lymphocyte (CTL) function. The simulation shows that for this model STI may lead to an increase in the CTL population that leads to sustained suppression of the virus load even when treatment is stopped. Interestingly, too long a phase of continuous treatment may revert the patient to a state of high virus load and low CTL frequency. This happens because virus load is suppressed to levels that are too low to sustain a high enough level of stimulation of CTL proliferation. Note also, that the latent cell population continues to decline during STI even in periods when no treatment is given. Population densities and time are given in arbitrary units; the bars show periods of drug treatment. Susc. CD4 cells, susceptible target cells; Act. prod. CD4 cells, cells actively producing virus; Lat. inf. CD4 cells, the latent infected cell population.

As stated above, one can easily construct a model (based on justifiable biological assumptions) that exhibits the required behavior for sustained control after therapy. There is, however, a large body of equally reasonable models that do not result in control of virus growth. Which of these models best reflects the biological reality is debatable. However, provided Equation (1) gives a satisfactory description of the dynamics of infected cells, then any form of control of the virus growth after therapy requires that the effector cell population increases during treatment. Because this argument is only based on Equation (1) and the fact that the susceptible cells increase during treatment, this result is highly robust with regard to the assumptions of the detailed population dynamical interaction between the virus and the immune responses.

Consequently, for the evaluation of the clinical data from STI trials it is crucial to obtain accurate quantitative measurements of the effector cell populations (both armed and memory effector cells) before, during, and after therapy, using assays such as the limiting dilution and/or chromium release assays [12,26] the elispot assay [27], and the MHC-tetramer assay [28]. These data

must be interpreted with great care in the light of the above discussion.

In the majority of subjects started on antiretroviral drug therapy, there is a decrease in the frequency of HIV-specific immune cells and their activation status, as measured by MHC class I tetramers, limiting dilution, or intracellular cytokine staining; the reduction in frequency is directly correlated with initiation of antiretroviral drug therapy and the suppression of viral replication [9,29–31]. HIV-specific T cell clones have been shown to persist at the reduced postviral suppression frequencies for long periods after antiretroviral drug therapy is started, possibly representing long-lived memory cells [32]. In patients who interrupt regular drug dosing, there have been documented increases in HIV-specific cellular immune response frequency, as measured by limiting dilution assays, T lymphoproliferative responses, and cytokine secretion in response to HIV antigen [9–11]. As of yet, there have been no documented cases of spontaneous increase of HIV-specific immune effectors after suppression of viral replication, consistent with the immunological principle that T cells proliferate in response to specific antigen. The implication of these observations is that it will require specific antigenic challenge to boost the cellular immune responses.

Repopulation of the latent reservoir

Apart from the potential benefits of STI, there is concern whether the pool of latently infected cells may be rapidly refilled during drug-free periods. Since the latently infected cell population is only decreasing with a very slow half-life, the fear is that even short therapy interruptions may set back the clock of therapy by many months.

We see two reasons why the refilling of the latent virus pool may be less of a concern. First, from a pragmatic point of view given the very slow half-life of the latent virus population, it is uncertain whether eradication of the virus may be achievable with standard drug therapy. Therefore, the question is whether an increase or decrease of the small and slowly replicating pool of latently infected cells actually contributes significantly to pathogenesis. We emphasize that we raise this point with regard to the contribution of the viral load in the latent cell compartment to disease progression and not with regard to what the latent cell pool may contribute to the evolution of resistance.

Second, it is conceivable that the latently infected cell population does not increase during drug-free periods if the interruption of treatment occurs early after the initiation of STI. This is best illustrated by the simulation shown in Fig. 3. After the initial viremia following infection, the virus load settles down at an equilibrium level. Once STI is started, the load of cells actively

producing virus declines rapidly during phases of treatment and increases again when treatment is interrupted. Interestingly, however, the latently infected cell population continues to decline even in phases in which the cell population actively producing virus increases. Only when the load of cells actively producing virus is high and the load of latently infected cells is low, does the latent cell pool begins to increase again. Therefore, early after the start of STI, the latent cell pool may decline even during phases of therapy interruption. A detailed mathematical reasoning for this observation is given in the Appendix.

Drug resistance

There is concern that STI may increase the risk of induction of drug resistance. It is obvious that for an idealized drug regimen, which can completely abrogate new infection of cells during treatment, continuous treatment would minimize the risk of development of resistance, since any ongoing infection of cells during treatment increases the risk of producing a drug-resistant mutant [33–37]. But the more relevant question is by how much STI would increase the risk of resistance relative to continuous treatment. So, for example, is STI with ten therapy interruptions a hundred times or just twice as likely to generate resistance?

To address the resistance problem, we distinguish between two alternative cases in which a patient's virus population at the start of therapy does or does not harbor resistant virus. In the former, we are concerned with how the growth of the resistant virus population is affected by STI. In the latter, we are concerned with how the likelihood that a resistant mutant is produced during therapy is affected by STI.

It is reasonable to assume that in absence of drugs a resistance mutation confers a fitness cost in comparison with the sensitive wild type, since otherwise the resistant mutant would have been selected prior to the first use of the drugs. In the presence of drugs, the fitness of sensitive virus is reduced and, if it is reduced below that of resistant virus, the resistant virus will grow in relation to wild type. The rate of increase in frequency is inversely proportional to the fitness difference between sensitive and resistant virus [15,38]. Unless the resistance mutations involve a high cost or the drug regimen is only weakly effective, the fitness difference between sensitive and resistant virus will typically be larger in presence than in absence of drugs. Therefore, the resistant virus will typically increase faster during periods of drug treatment than decline during drug-free periods. Consequently, an STI regimen with long phases of treatment and short phases of interruption will delay but not prevent the rise of resistance.

Based on theoretical considerations, it has been argued

that (at least in chronically infected patients) the likelihood that resistant mutants are present at the start of therapy is typically considerably larger than the likelihood that a mutation to resistance occurs after continuous antiviral treatment is started [34,35]. To determine by how much STI increases the risk of generating resistance, we need to estimate how many cells get newly infected during treatment. In our mathematical model, this number is given by the integral of bTI from the start of therapy to the endpoint of the period of observation. In Fig. 4, we show how the ratio of the number of cells infected during therapy to the number of infected cells present at the start of therapy increases with time after therapy. As long as the virus load during treatment is small in comparison with baseline before treatment, this ratio is considerably smaller than 1. This implies that the likelihood that a resistant mutant is present at the start of therapy is larger than the likelihood that this mutant is first produced during therapy. Only when the virus load increases to levels similar to baseline, does the ratio exceed 1. Therefore, as long as the virus load is low during STI the most likely origin of resistance is the selective outgrowth of a resistant mutant that is already

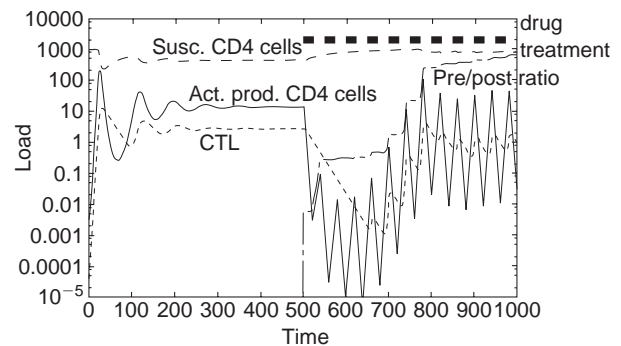


Fig. 4. The simulation of model 3 (see appendix). The curve denoted pre/post ratio shows the ratio of the number of cells that became infected after the start of therapy and the number of cells that were present at the start of therapy. As long as this ratio is much smaller than 1, the likelihood that resistant mutants were present prior to therapy exceeds the likelihood that a resistant mutant appears for the first time during therapy. In other words, resistant mutants are more likely to pre-exist in the viral population prior to treatment than to be generated for the first time during treatment. This simulation shows that, provided the virus load is held at a low level during structured therapy interruption, the likelihood of generating resistance remains small. The rise of the virus load after the transient decline is not caused by resistance but is the return of the virus load to a new equilibrium. Population densities and time are given in arbitrary units; the bars show periods of drug treatment. Susc. CD4 cells, susceptible target cells; Act. prod. CD4 cells, cells actively producing virus.

present at the start of therapy. Only if during STI the virus load grows to levels similar to those at baseline before therapy, will therapy interruption significantly increase the risk of treatment failure owing to resistance.

Discussion

Despite the initial optimism, it has become widely accepted that eradication of HIV from infected patients is not achievable with conventional combination therapy within a period of several years. However, the clear association between the viral load and disease progression [39] implies that patients should remain on therapy as long as possible. Because of the high cost of therapy and the low tolerability of the drugs, indefinite continuation of therapy poses severe problems both for the individual patient and the general public health system. Therefore, new approaches to therapy are urgently needed. The reasoning behind STI is to boost the patient's immune response with autologous virus during short phases of therapy interruptions. Support for an approach based on STI comes both from the similarity of this process to vaccination with live virus and from isolated case reports that have demonstrated the potential of therapy interruptions to boost the immune response. Therefore, STI deserves closer investigation. Clinical trials are currently being designed or are already underway. In this paper we presented a population dynamical analysis of STI with particular regard to viral dynamics of the immune response and the latent cell population and the risk of induction of drug resistance associated with STI.

We have argued, based on standard kinetic models of HIV population dynamics, that STI may lead to sustained control of virus load at low levels only if the immune effector cells increase during STI to a level higher than baseline at the start of therapy. This increase must more than compensate the increase in susceptible target cells that is induced by therapy. A decrease in the effector cell population is unlikely to lead to control of viral growth even if the ratio of effector cells to infected cells increases during treatment. This conclusion is very robust with regard to the detailed mechanism of interaction between the populations of effector cells and infected target cells. More specifically, the conclusion rests only on the assumptions for the dynamics of infected cells (as described by Equation (1)) and is independent of the dynamics of effector and susceptible target cells.

There is concern that short interruptions of therapy may rapidly refill the reservoir of latent infected cells and thereby reset the clock of treatment by many months. Here, we argued that the latent cell population is likely to be refilled only if the load of latent cells has decreased

by a larger factor than the free virus. For example, if a patient's virus load during STI is kept at all times by at least a factor ten below the baseline of the virus load before treatment, then the decline of the latent infected cell population will continue until itself has decreased by a factor ten or more. Hence, STI may not lead to a repopulation of the reservoir of latently infected cells, particularly in patients who have only been on continuous combination therapy for a short time. Note, however, that this does not imply that the dynamics of the repopulation of the latently infected cell pool are generally slow. On the contrary, the dynamics of repopulation can be very fast if the virus load is high (as can be seen by the increase of latently infected cells in Fig. 3 after the second phase of therapy).

Finally, numerical simulation of STI (Fig. 4) shows that the number of cells infected at the start of therapy may be considerably lower than the number of cells that become newly infected during STI, provided that the virus load stays considerably below baseline during STI. This implies that the likelihood that a resistant mutant is present in the virus population before therapy exceeds the likelihood that a resistant mutant is first produced during STI. Consequently, the main factor contributing to the emergence of resistance may be the virus population at the start of therapy and a therapy regimen with few interruptions may not increase the risk of emergence of resistance significantly in comparison with continuous therapy. However, it must be noted that this supposes that all drugs can be phased out and started on a similar time scale. If the virus population is at some times controlled only by a single drug then in these periods the virus may obtain resistance mutations to each drug successively and eventually become multidrug resistant.

In summary, some of the major ethical concerns with clinical trials of STI, namely the repopulation of the latently infected cell pool and the risk of promoting drug resistance, may turn out not to be the major obstacles for controlled therapy interruptions. While this is encouraging, it certainly needs to be tested in carefully designed clinical trials. Furthermore, population dynamical models show that under specific conditions STI may lead to a sustained control of viral load at low levels. However, control of virus replication by the immune system requires that the effector cell population is stably increased to levels beyond baseline at the start of therapy.

Appendix

Dynamics of infected cells

The rate of production of infected cells, I , is given by the product of the population densities of free virus, V ,

and susceptible target cells, T , times a proportionality constant, i , describing the infectivity of free virus. Since the turnover of free virus is fast compared with that of infected cells [40–42], it is reasonable to assume that the free virus is at all times to a first approximation proportional to the infected cells, i.e. $V = kI$. Therefore, the rate of production of infected cells is given by bTI , where $b = ki$. Infected cells may die either as a result of viral cytopathicity or through immune response-mediated cytotoxicity. The rate of loss of infected cells owing to viral cytopathicity is given by d_1I , where d_1 is the death rate per infected cell. The rate of loss of infected cells owing to immune response-mediated cytotoxicity is given by pEI and depends on the population density of effector cells, E , and a proportionality constant, p , describing the killing rate by the immune effector cells. Altogether we obtain Equation (1) for the dynamics of the infected cell population

$$dI/dt = (bT - d_1 - pE)I \quad (1)$$

where dI/dt is a mathematical notation for the rate of change in the variable I at time t (loosely interpreted as a change in I of magnitude dI in a short time interval dt).

Equation (1) makes a number of assumptions that are worth pointing out. First, the population denoted by I , here loosely termed infected cells, is more precisely defined as the population of virus-producing cells and, therefore, does not include the population of cells infected with defective virus. The population of infected cells, I , can be further divided into cell types, such as CD4 cells or macrophages, or into types of virus producer cell, such as active, latent, or chronic. For the general discussion that follows below, however, these different types of virus-producing cell can be grouped together into one population. Second, the term pEI reflects the immune-mediated cytotoxic effect on infected cells, performed mainly by ‘armed’ CTL. However, this is not the only immune-mediated mechanism of interference with viral replication. Other mechanisms include the prevention of infection (for example through the release of suppressive factors or antibodies). Such mechanisms are not contained in the above equation, but their effects on STI are considered in the main text.

Model 1

The simulation shown in Fig. 2 is based on the following model: $dx/dt = s - d_T T - bTI$; $dI/dt = bTI - d_1I - pEI$; $dE/dt = cI/(I + K)E - d_E E$. Here the variables T , I , and E denote the population densities of susceptible CD4 cells, active virus-producing CD4 cells, and CTL, respectively. The parameters are s for the rate of immigration of susceptible cells, b for the infection rate, p for the killing rate, c for the

stimulation of CTL proliferation, and d_T , d_1 , and d_E for the death rates of the corresponding cell populations. The parameter K describes a saturation of immune stimulation at high virus loads. The parameters used in Fig. 2 are $s = 10$, $d_T = 0.01$, $b = 0.001$, $d_1 = 0.3$, $p = 0.05$, $c = 0.05$, $K = 0.1$, $d_E = 0.1$. At periods when drug is given the parameter b is set to 0.

Model 2

The simulation in Fig. 3 is based on a dynamic model of HIV replication in which STI can shift the balance in favor of sustained suppression of virus load. The model is defined by $dT/dt = s - d_T T - bTI$; $b dI/dt = bTI - d_1I - pEI - q_1I + q_a I$; $dI_1/dt = q_1I - a_1I_1 - q_a I_1$; $dE/dt = cEI/(I + K) - d_E E - d'_E EI/(K' + I)$. This model is an extension of model 1. Here, the additional variable I_1 denotes the population density of latently infected CD4 cells. The additional parameters are: q_a and q_1 for the rates at which actively producing cells turn latent and turn quiescent, respectively; d_1 for the death rates of latently infected cells; and d_E for virus-related impairment of CTL function (for example by reduced helper T cell function). The term $d'_E EI/(K' + I)$ describes a saturation of immune impairment at high virus load. This model has two steady states corresponding to controlled and uncontrolled viral replication. Figure 3 shows that for this model STI may lead to an increase in the CTL population, which itself leads to sustained suppression of the virus load even when treatment is stopped. If treatment continues for too long a time, the dynamics can revert back to uncontrolled viral replication. The parameters used in Fig. 3 are: $s = 10$, $d_T = 0.01$, $b = 0.001$, $d_1 = 0.3$, $p = 0.05$, $q_1 = 0.001$, $q_a = 0.001$, $a_1 = 0.01$, $c = 0.3$, $K = 0.1$, $d_E = 0.1$, $d'_E = 0.25$, $K' = 5$.

Model 3

The model underlying Fig. 4 is given by $dx/dt = s - d_T T - bTI$; $dI/dt = bTI - d_1I - pEI$; $dE/dt = cI - d_E E$. Note that the stimulation of the growth of the effector cell population is directly proportional to the infected cell population and is simpler than in model 1. The ratio of the total number of cells infected during therapy to the total number of infected cells at the start of therapy is given by

$$1/I_0 \int_{t=0}^{t=T} bTI dt$$

where $t = 0$ is the start of therapy, $t = T$ is the endpoint of the period of observation and I_0 is the number of infected cells at the start of therapy. As long as this ratio is much smaller than 1, the likelihood that resistant mutants were present prior to therapy exceeds the likelihood that a resistant mutant appears for the first time during therapy. In other words, resistant mutants are more likely to pre-exist in the viral popu-

lation prior to treatment than to be first generated during treatment. Figure 4 shows that, provided the virus load is held at a low level during STI, the likelihood of generating resistance during STI remains small. In the simulation, STI was started at day 500 with a drug phase of 20 days followed by a drug-free phase of 20 days. The parameters used in the simulation are: $s = 10$, $d_T = 0.01$, $b = 0.001$, $d_I = 0.3$, $p = 0.05$, $q_1 = 0.001$, $q_a = 0.001$, $a_1 = 0.01$, $c = 0.3$, and $d_E = 0.1$.

Decline of the latent cell pool during interrupted therapy

The pool of latently infected cells may continue to decrease during therapy interruptions despite a concomitant increase of the actively producing cells for the following reason. The total production, P , of latently infected cells is proportional to the virus load, I . Therefore, $P = \alpha I$. The total clearance, C , of latently infected cells is proportional to the death rate, a_1 , of latently infected cells, I_1 . Therefore, $C = a_1 I_1$. At the steady state prior to treatment, production of latently infected cells equals its clearance, $\alpha I = a_1 I_1$. During treatment, the active infected cell population, I , declines rapidly, and therefore the total production of latent cells, P , decreases rapidly as well. However, since the latent infected cells decline only slowly, the total clearance of latent infected cells, C , decreases slowly. Assuming that STI leads to a tenfold reduction of virus load compared with baseline before therapy, the total production of latent cells decreases tenfold, while the total clearance only declines slowly. Hence, the latent infected cell pool will continue to decline until it has fallen by a factor of ten in comparison with its baseline value; at this point, clearance of latent infected cells is balanced by production. Consequently, particularly in patients who have only been on combination therapy for a short time, early therapy interruption may not involve a high risk of repopulating the reservoir of latent cells.

Model robustness and choice of parameters

Generally, good estimates of the parameters used in the models are available only for the turnover kinetics of virus-producing cells, latently infected cells, susceptible cells, and CTL [1,4,5,9,15,25,29,40,43]. Reliable estimates for other parameters, in particular those that describe the non-linear interactions between two populations, such as the virus infectivity, b , or the immune killing rate, p , have not yet been obtained. Therefore, simulations in this paper serve to illustrate qualitative behavior. However, we have checked carefully that the qualitative results of the paper are robust with regard to parameter choice.

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