HIV reservoirs and immune surveillance evasion cause the failure of structured treatment interruptions: an *in silico* clinical trial

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Introduction

Continuous antiretroviral therapy is currently the most effective way to treat HIV infection. The increase in life expectancy of HIV positive individuals raised both costs and side effects of combination Anti-Retroviral Therapy (cART), stimulating research into Structured Treatment Interruptions (STI). STI aimed at discontinuing the therapy according to a schedule so as to minimize the side effects without losing substantial protection. In a large, randomized clinical trial [1,2] STI were associated with an increased risk of death and opportunistic diseases connected to treatment interruptions. Many clinical studies on different STI schedules resulted in generally neutral or negative outcomes [3-5], although the reasons are still not fully understood and often attributed to drug resistance.

Although clinical studies proved that there are increased risks associated to cART interruptions, patient-initiated unstructured treatment interruptions are quite common in the clinical practice [6]. A recent systematic review [7] of cohort studies and clinical trials indicates a proportion of unstructured treatment interruptions ranging from 5.8% to 83.1% with a median of 23.1%. The mean duration of cART interruptions ranges from 11.5 days to 18 months with a median of 150 days. The main reported reasons for treatment interruptions are laboratory toxicity and clinical side effects.

Both frequency and duration of unstructured treatment interruptions observed in clinical practice are a good reason to reconsider treatment schedules alternative to the continuous treatment in order to reduce the amount of drugs administered to the patients.

Our aim is to gain a better understanding of the reasons behind STI failure: are they inherent to the HIV dynamics or consequential of other mechanisms like the emergence of drug resistance? We resort to a well-established and validated agent-based model (ABM) of HIV infection [8-12] to investigate the reasons of STI failure. By excluding to model the resistance to the drugs we restrict the possible causes of STI failure to mechanisms such as virus reservoirs in macrophages and resting/memory $CD4^+$ T lymphocytes.

Methods

In the present work we investigate treatment interruptions with a set of *in silico* simulations: we test three STI used in clinical trials and compare their efficacy to that of the continuous (i.e., uninterrupted) treatment. We simulate the disease progression for a group of 250 *virtual* (i.e., *in silico*) HIV positive patients. For each group of *virtual* patients we compare the effects of different treatment strategies on the HIV infection over a therapeutic period of 48 weeks, three years after seroconversion. We finally evaluate the efficacy of each STI schedule by challenging the immune system of the virtual HIV patients with a simulated opportunistic bacterial infection at the end of the treatment period. The immune system reaction against those bacteria depended on its efficiency at injection time.

To measure the effectiveness of the different STI we monitor the survival curve in a population of 250 virtual HIV+ and compare them with the continuous treatment and the void treatment (i.e. untreated virtual patients). Subsequently we look into the infection dynamics associated to each STI and find that CD4+ cell counts and provirus levels correlate significantly to the number of deaths in the cohort of virtual patients. At last, using a simulated annealing algorithm, we search for an optimal STI schedule that performs better than the STI tested so far in clinical trials.

Results

We validate the results of our model by comparing the simulations for the 8 weeks on / 4 weeks off STI and for the continuous treatment with data from a clinical trial performed over a period of 48 weeks using similar treatment schedules [4]. In Figure (1A) we compare the simulations outcomes with the clinical trials. The simulation results for CD4+ and CD8+ cell counts are within the ranges observed in the clinical trial for both the continuous therapy and the STI.

Results of the STI strategies tested in clinical trials are shown in Figure (1B). As observed in the clinical trials, all the STI strategies are associated with an increased number of deaths. The ratio of deaths associated to the WeekOn/WeekOff strategy over that of continuous therapy is 1.95 at the end of the 30 days after opportunistic bacterial infection. The same ratio is 2.05 for the 8WeeksOn/4WeeksOff and 3.52 for the 4WeeksOn/4WeeksOff strategy.

The effects of the different STI on virologic and immunologic parameters are shown in Figure (1C). As expected, the parameters that correlate more with the survivability of the virtual patients are CD4+ cell count and provirus. The “4 Weeks On / 4 Weeks Off ” STI has the worst survival curve even though it has a CD4+ cell count comparable to that of the “Week On / Week Off ” STI. The “8 Weeks On / 4 Weeks Off ” STI has the highest CD4+ cells count but has a survival rate comparable to the “Week On / Week Off” STI. In both cases the provirus level is much higher in the former STI, leading us to point out the importance of HIV reservoirs as one of the main causes of STI treatment failures.

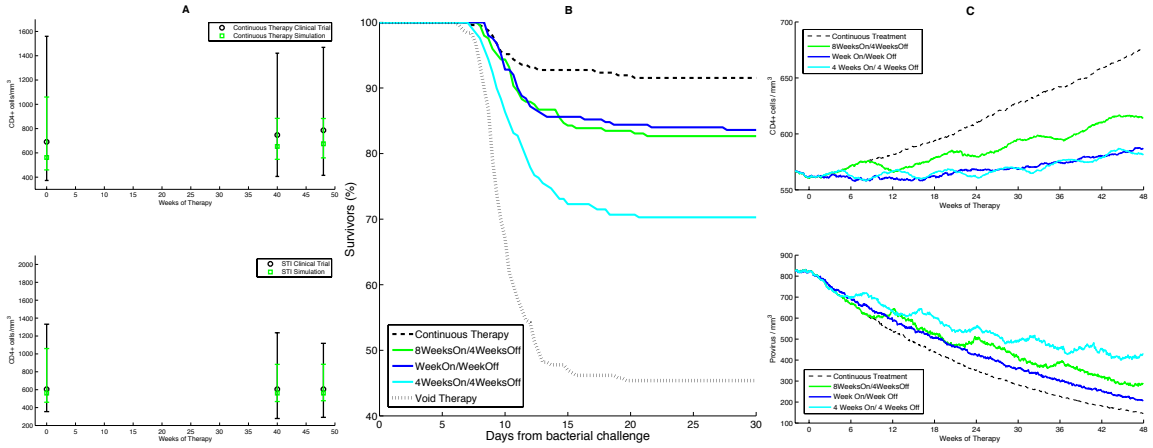

Figure 1. (A) Validation: In this plot we compare the median simulated CD4+ cell counts (shown in black) with the experimental values observed in a clinical trial (shown in green) for both continuous therapy (upper panel) and the 8 Weeks On/ 4 Weeks Off STI (lower panel). The bars indicate the ranges of maximum and minimum cells counts observed in the virtual (green) and real (black) patients. (B) STI Survival curves: percentage of survivors over time in a population of 250 HIV+ virtual patients. 30 days after bacterial challenge, survival rates are the following: Week On/Week Off STI 83.60%, 8Weeks On/4 Weeks Off 82.66% and 4 Weeks On/4 Weeks Off 70.28%. Survival rates for continuous therapy and void therapy are 91.53% and 45.38% respectively. (C) Simulated CD4+ cells count (upper panel) and provirus levels (lower panel) during the 48 weeks treatment period for the different STI and the continuous therapy.

Discussion

The three STI strategies tested in clinical trials have lower performances compared to continuous treatment, since the ratio of death associated to a STI strategy over that of continuous treatment is between 1.95 and 3.52. Since in the model we don't allow the HIV virus to develop resistance to cART drugs, the failure of STI seems to be inherent in the HIV infection dynamics, rather than caused by the emergence of drug resistant strains. We conclude that specific mechanisms of the HIV infection like HIV reservoirs both in macrophages and latently infected resting/memory CD4+ T lymphocytes and immune evasion, are sufficient to cause a failure of the STI treatments tested so far in clinical trials regardless of drug resistance.

The ethical problems associated to further studies of STI in light of the previous failures make the use of modeling techniques appealing. The possibility of simulating STI *in silico* to predict the success or failure of a given STI schedule is a powerful tool that can support the clinical studies without having impact on human beings. For the HIV infection the lack of fully predictive animal models ("mice lie and monkey exaggerate") [12] makes it difficult to validate accurately the prediction of computational models. It is very difficult to address the ethical implications of testing computational predictions on humans, given the potential loss of human lives that could be caused by a wrong prediction. Yet clinical trials are still needed and any tool that could be used to assist those trials should be considered. Actually, it is this the lack of predictive animal models for HIV that makes computational models one of the best tool at disposal of clinicians.

The use of *in silico* simulations allows searching for an optimal STI using a simulated annealing algorithm. We found indeed an optimal STI schedule that performs better than the STI tested so far in clinical trials, which will be published elsewhere. Our modeling approach for HIV is valuable since it is practically and ethically impossible to test many different STI on humans.

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