Author's response to reviews

**Title:** Immune control of HIV-1 infection after therapy interruption: immediate versus deferred antiretroviral therapy

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**Author's response to reviews:** see over
Response to Reviewers

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First of all we wish to thank the reviewers for their comments. They have contributed substantially to improve the article.

Herein, each comment (in font italic) is followed by our answer (in blue) and the changes made to the manuscript (in red in the revised manuscript).
Reviewer 1

Question: 1) In general:
Paci et al have written an interesting paper describing the results of a computer model that predicts the optimal time of initiation of HAART. Although not stated clearly in the introduction, the study concerns the initiation of HAART during primary HIV infection. The structure of the abstract and manuscript lacks a section describing the goals and objectives for this study. The pages are numbered but the lines are not. A cohort of patients with primary HIV infection is used to provide data for the primary infection group. It’s unclear where the data comes from in the “late” HAART group, as well as the “cohort data” mentioned on page 4. The structure is actually background-results-discussion-conclusion-methods, lacking an objectives section indicating the purpose of this work. (minor)

Answer: The “late” HAART group in this article are actually data produced by simulations (the section is in fact named “in silico study”). Cohort data refer therefore only to early treated patients. The issue of the objective section is a minor: few sentences are included in the conclusions whereas a complete paragraph is not required by a BMC journal.

Question: 2) Page 2

Background: asymptomatic patients are mentioned. Authors must state clearly if they mean asymptomatic acute HIV infection or asymptomatic chronic infection. (minor)

Answer: Right. The word asymptomatic is misleading.

Action taken: We cut the sentence.

Question: 3) Page 3

Background: immediate therapy: can mean initiation of HAART after a maximum of 12 months after infection (the 6 months seroconversion interval + the maximum 6 month therapy initiation delay). Table 1 gives days before therapy, but it’s not clear what it means. (minor)

Answer: The column “days before therapy” indicates how many days elapsed from diagnosis (enrollment) of the patient and the initiation of HAART.

Action taken: We modified the column title to: elapsed time (days) and added a note in the figure caption to clarify the meaning.

Question: 4) Page 4

Authors describe how simulated data compares to “cohort data” after treatment interruption. Since the results from cohort data are so diverse, authors should provide references to the data they used. If they used only one small cohort, I wonder why they did not test the external validity and robustness of the model against other larger cohorts (published or presented by Desquilbet, Steingrover, Koegl). By selecting only one small cohort as the basis for the model, the choice of this cohort influences the model greatly. Same goes for the data of deferred treatment strategies described on page 5. (major)

Answer: The cohort data coming from the Spallanzani Hospital were used to compute the statistics shown in figure 3 and 4. For what concerns the simulator we did not use those data (strictly speaking) since we calibrated the model on the basis of known facts reported from literature. This has been clearly indicated in the additional material. An example is the viral set point, the two slopes of viral decay during HAART. Our cohort data basically are in line with those estimations. We did use the cohort data for setting therapy specific parameters like the duration, the initiation of therapy, the level of viremia and of CD4, etc. This is reported in section titled “In silico study ...”
Question: 5) Page 6
Life long treatment from primary infection is a feasible option in patients that need it. Such was not tested in this study and hence not proven impossible. Authors may wish to remove or modify this statement. (minor)

Answer: Ok, true, but we were just discussing the issue in view of a possible emergence of side effects. In any case we can not realistically simulate side effects.

Question: 6) Page 7
CTL responses are related to viremia, its not clear however who's in control (Jansen/Miedema). Authors model rebound of viremia to similar levels in the groups and assume different CTL responses without any validation of this outcome. Viral rebound dynamics are very different in patients undergoing STI after early or deferred HAART (Steingrover/Pogany et al, AIDS) and rather contradictory to the results from the model in this study. This makes one wonder about the robustness of the model. (major)

Answer: Viremia and CD8 are surely correlated (through a non-linear relation) to CD4 since these cells constitute both a reservoir for HIV and a stimulus for CD8 proliferation. Our tentative interpretation (which is not the main message of the article) of the results produced by the simulations (fig 5, end of section results) is that a higher level of CD8 in lately treated patients with respect to those who receive early treatments is a consequence of the fact that the immune system has spent considerable time to control viremia before HAART initiates and therefore there is a higher level of cytotoxicity: more CTLs (case of late therapy) \(\rightarrow\) less viremia, and the opposite, less CD8 (case of early therapy) \(\rightarrow\) higher viremia.

Moreover, we are currently looking at unpublished clinical data for patients treated with late HAART and we are observing that the predicted-by-simulation effect of CTLs is less than what we expected (\(\sim 10\%\)). The differences in viremia are statistically irrelevant \((P < 0.05)\) as those of CTLs. These results will be submitted for publication in due course. This indicates that it is difficult to draw final conclusions from these data/simulations.

The message of Steingrover et al. shown in fig 1(a) is that there is a different rebound in viremia with the early HAART controlling better the rebound (they did not show the levels of CTLs though). Therefore, as the reviewer points out, the message seems to be opposite of ours. However, on second thoughts, this is not true. Actually, there are two main differences between our cohort and the one used in Steingrover et al. These differences, listed below, could explain the different conclusions we reached.

The first one is that Steingrove’s cohort patients have been recruited with a negative western blot for anti-HIV antibodies hence before seroconversion. In contrast our cohort data includes patients either during the inflammatory response and also during seroconversion.

A second difference is that Steingrover et al. patients were treated with i) an intensive HAART, consisting of two or three nucleoside analogue reverse transcriptase inhibitors (NRTI), ii) a non-nucleoside reverse transcriptase inhibitors (NNRTI) and iii) a protease inhibitor (PI), whereas our patients were treated with just two NRTI and one PI.

These two features might be the cause of a lower level of viremia at the end of HAART accounting for a slower viral rebound. The opposite holds in our case. In conclusion, there are three different options for the beginning of the therapy: 1) during the hyper-acute phase (before seroconversion during the inflammatory response); 2) during the acute phase (i.e. within the first six months from primary infection); 3) during the chronic phase, once the viremia has reached the set point. In our study we focused on the differences between 2) and 3) and found none, whereas Steingrover et al.
studied the 1) and 3) alternatives and did found differences. We conclude that the results are not in contraposition, and suggest a simulation study to compare phase 1) and 2) instead.

**Action taken:** First of all we added the reference to the paper of Steingrover et al. AIDS 2008. Then, we have been more specific in the article in the definition of acute phase (section Results). Moreover, we added a little paragraph in the Discussion section (references included) to highlight these considerations. Finally, a new paragraph has been added to the Conclusions as well.

**Question:** 7) Page 8
Authors conclude that early therapy and subsequent STI leaves the viral rebound undisturbed. No argument is made why the results from the model is so different from multiple studies (Steingrover CROI 2007, Koegl CROI 2007, Steingrover et al AIDS, Steingrover CROI 2008). Also, authors conclude that the study should impact the current treatment strategies currently under study in randomized trials like Primo-SHM and Spartac. The undertaking of these trials shows how the need for robust data is felt in the field. Nonetheless, the authors imply that primary HIV infection needs not to be treated based on the results from the model. (major)

**Answer:** Once again, we highlight that our study focuses on a wider phase of the primary infection (acute-phase) with respect to the study reported by Steingrover et al (that focuses on the hyper-acute phase only). What is interesting is that our new analysis of (real world) late HAART rebound seems to confirm our present result although, as stated above, weakens the explanation we gave about CTLs control.

**Action taken:** We feel that a thoughtful discussion should be given together with the presentation of the whole picture, which is what we are going to do in a forthcoming manuscript. Nevertheless we added a small paragraph in the Discussion section and the reference to the paper of Steingrover et al. AIDS 2008..

**Question:** 8) Page 9
Predictive of the viral rebound is HIV-1 DNA intra-cellular concentrations (Yerly et al). Also predictive of the HIV-1 RNA concentration in plasma are genetic factors of the host. Neither is incorporated in the model. Author need to discuss why this results in an acceptable model. External validation needs to show that such a model is indeed capable of producing robust predictions. (major)

**Answer:** The computer model considers PI and TI treatments (their implementation is described in the method section). In the model, the transcriptase inhibitor leads to cells carrying a viral genome which does not replicate (hence a surrogate of the HIV-1 RNA) whereas the protease inhibitor leads to viral proteins that we call “inactive virus”. This form of inactive virus which is produced in infected cells accounts (among other things) for the HIV-1 DNA observed in real life in patients during HAART. Therefore they are incorporated in the model although we do not use HIV-1 DNA as an indicator of viral rebound (our viral rebound is given only by HIV-1 RNA like in a number of previous studies and, most importantly, because the clinical data available only included HIV-1 RNA counts). This critic gives a good suggestion that we might consider in the future, subject to the availability of proviral HIV-1 DNA clinical data.

**Action taken:** We have detailed the description of the computer model to account for the differences of infective (i.e., HIV-1 RNA) and un-infective (i.e., HIV- DNA) viral genome

**Question:** 9) Concluding remarks
This paper is an interesting effort to model complex data. The model is internally validated based
on a small cohort of patients with primary HIV infection undergoing early therapy. The outcomes involve both patients that undergo early and deferred therapy. The outcomes are contradictory to existing study data that is published and presented. An external validation of the model and its results is needed before any conclusions can be drawn regarding the treatment of primary HIV infection. The authors may consider other predictors of the dynamics of viral rebound to be added to the model to produce improved results. The authors may want to think and explain about the purpose of the model and its results.

**Answer:** The reviewer raised useful questions that helped us in understanding the range of our conclusions and to better shape future studies. In particular we are currently analyzing a small cohort of lately treated patients to see if the predictions of the simulator are confirmed. A discussion about using other predictors is also underway and might be considered in future works. We feel that the present revised version of the paper is a consistent piece of work that already deserves publication. We also wish to point out that the range of our conclusions should to be taken as suggestions that do need further investigations and clinical validation. There is no claim of absolute truth.
Reviewer 2

Question: This is an interesting study applying mathematical modeling to address issues in respect of optimal HIV treatment approaches. Appropriately for this journal the language used is plain English making the approach used easily understandable to non-mathematical audience such as HIV clinicians. It is a nice demonstration of how modeling is increasingly been used to answer clinical questions that otherwise are difficult to address, with the proviso that the predictions of the model still need to be tested in real life to see that they hold up.

Answer: We fully agree that the predictions of the model need to be tested. As a matter of fact, we are, currently analyzing a data set (source Spallanzani Hospital in Rome) of 20 subjects receiving treatment during the chronic stage of the HIV-1 infection. The detailed results will be published in due course. What we can tell, at this time, is that our predictions seem to hold: the viremia in 83% of the subjects returns to its pre-treatment set-point within one year off therapy. This is in good agreement with the simulation predictions that give 80% of the early rebounders. In summary everything seems to confirm that the differences between the two therapeutic regimes fade within one year from discontinuation.