Author's response to reviews

Title: immunological predictors of CD4+ T cell decline in antiretroviral treatment interruptions

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Author's response to reviews: see over
Madrid, Thursday, 13 December 2007

Dear Sir/Madam,

RE: Manuscript MS: 1534220967157131 (Seoane et al. Manuscript entitled: “Immunological predictors of CD4+ T cell decline in antiretroviral treatment interruptions”).

Thank you very much for your e-mail of November 13, 2007. Please find enclosed a point-by-point response to the Editor’s comments and the new revised version of our manuscript. Furthermore, English language usage has also been revised.

We hope that the current revised version of our manuscript is apt for a second review. Please feel free to contact us regarding any further questions about our work.

Thank you very much for your consideration. We look forward to hearing from you at your earliest convenience.

Yours sincerely,

Mª Ángeles Muñoz-Fernández, PhD
Reviewer 1: Daniel Skiest
Major Compulsory Revisions

A) Methods
1. Second paragraph-
   a) How often were clinical parameters e.g. T cells and HIV RNA et checked?

   We have included in M&M: “Patients were evaluated monthly during the first three months and every two months thereafter.”

   b) What were the specific criteria for reintroducing HAART? What clinical events are the authors referring to? AIDS defining events, CDC category B events. Did they include non AIDS related conditions e.g. renal, liver, cardiac events?

   We have changed a paragraph in M&M: “Criterion selected for reintroducing HAART, if needed, was the appearance of a clinical event or experienced declines in CD4+ cell count <350/µL.” By “Criterion selected for reintroducing HAART, if needed, were: (i) patient's choice, at any level of CD4+ count; (ii) appearance of symptoms that could be manifestations of HIV infection (CDC category B events and/or AIDS defining events); and (iii) a decreasing CD4+ count before falling below <350 cells/µL.”

B) Results
1. First paragraph- How did the authors define thrombocytopenia e.g. what platelet count?
   We have included in this paragraph of M&M “thrombocytopenia (<50,000 platelet/mm³)”

2. Second paragraph- fourth line- There appears to be a typographical error. What was the percent < 500/µL?
   We apologize for our mistake in the text. We have changed the paragraph: “A decrease in the CD4+ count was observed in all the patients, 23 (85%) patients experienced declines in CD4+ cell count <350 µL <500/µL, 19 (70%) in CD4+<400/µL, and 16 (59%) in CD4+<500/µL.” By “A decrease in the CD4+ count was observed in all the patients, 23 (85%) patients experienced declines in CD4+ cell count <350/µL, 19 (70%) in CD4+<400/µL, and 16 (59%) in CD4+<500/µL.”

3. Last sentence of second paragraph- How do the VL increases after TI compare to the pre-ART VLs?
   We wish to clarify Reviewer #1’s concerns regarding VL increases. We have added a new paragraph in M&M: “This was considered as the duration between the baseline timepoint (HAART interruption) and the appearance of a decrease in CD4+ (values of CD4+ <350/µL, CD4+ <400/µL, and CD4+<500/µL) and increase of VL (values of VL >10,000 copies/mL and VL >30,000 copies/mL).”

C) Discussion
1. First sentence- The authors statement that TI poses little if any risk of clinical complications is too broad based on their very limited study. Other studies e.g. SMART and others have shown that there may be complications with TI.
We have removed the first sentence of this paragraph: “This study confirms that discontinuation of antiretroviral therapy in selected patients has little, if any, risk of clinical complications.”

2. Second paragraph- Where is the data showing a positive association with starting HAART and subsequent HIV RNA load?

We apologize for our mistake in the text. We have added a new paragraph: “Interestingly, the patients with high HIV-RNA load when started HAART had high likelihood to achieve a rebound HIV-RNA load >30000 copies/mL (RR: 1.85 per log10; CI95%: 1.02; 3.35; p= 0.04) and to achieve a rebound of HIV-RNA load >100000 copies/mL (2.45; 1.12; 5.36; p= 0.02) after ART interruption”

3. First paragraph page 11- While LPR to PPD may have been predictive of outcome in this small number of patients it is premature to conclude that it is clinically useful for selection of patients to discontinue HAAART. The authors have not shown it to be predictive in a multivariate analysis, it is not clinically available and it is not practical. At most they can suggest further studies to look at this as an independent predictor. The authors should include a discussion of limitations of this study.

We agree with the reviewer about the small number of patients, but we think that LPR \textit{in vitro} is a good test to measure the quality of immune response, in the general population and HIV patients.

We have changed the paragraph: “Our results indicate that, not only the quantification of CD4+ (quantitative immune response) for the purpose of selecting the best candidate for treatment discontinuation, not only the quantification of CD4+ (quantitative immune response) is important, but also more the LPR response (quality of the immune response)”. By “LPR to PPD appears to be a useful marker to measure the quality of immune response, for selection of patients to discontinue HAART but further study is required to confirm this data.”

We have added at the end of discussion a new paragraph: “The main limitation in our study is the small number of patients. Thus, while LPR to PPD may have been predictive of outcome in this small number of patients it is premature to conclude that it is clinically useful for selection of patients to discontinue HAAART. We have not shown it to be predictive in a multivariate analysis. It is, in fact, quite possible that those individuals with lower CD4+ nadirs are also those with poor CD4+ T cell function and these assays simply identifying the same individuals. Further studies to look at this as an independent predictor should be carried out with a bigger number of patients to be able to carry out. In spite of this, we consider that ours data are very interesting and never previously described in non-structured treatment interruption.”

It is, in fact, quite possible that those individuals with lower CD4+ nadirs are also those with poor CD4+ T cell function and these assays simply identifying the same individuals.

\textbf{Minor Essential Revisions}
1. There are problems with the use of English and grammatical errors.

Language has been reviewed by experts.
**Reviewer 2: Jonathan Angel**

**Major Compulsory Revisions**

A) Major Concern:

The one major concern that I have with this manuscript is with the interpretation of the data. The authors state that CD4 positive T cell function is an “independent” predictor of CD4 T cell decline. In fact, this has not been demonstrated. It is, in fact, quite possible (if not likely) that those individuals with lower CD4 nadirs are also those with poor CD4 T cell function and these assays simply identifying the same individuals. Related to this, the authors state that they did not do a multivariate analysis because the numbers were small and therefore a statistical analysis was not done to suggest that there was independence.

We have changed a paragraph in discussion: “Not surprisingly, we have found that other immunological factors are *independent* predictors of the CD4\(^+\) decrease after treatment interruption.” By “Not surprisingly, we have found that other immunological factors are predictors of the CD4\(^+\) decrease after treatment interruption.”

We have added at the end of discussion a new paragraph: “The main limitation in our study is the small number of patients. Thus, while LPR to PPD may have been predictive of outcome in this small number of patients it is premature to conclude that it is clinically useful for selection of patients to discontinue HAART. We have not shown it to be predictive in a multivariate analysis. It is, in fact, quite possible that those individuals with lower CD4+ nadirs are also those with poor CD4+ T cell function and these assays simply identifying the same individuals. Further studies to look at this as an independent predictor should be carried out with a bigger number of patients to be able to carry out. In spite of this, we consider that ours data are very interesting and never previously described in non-structured treatment interruption.”

B) Additional Concerns:

1) It is unclear how patients were identified (were they consecutive patients that attended a clinic?), if there were subjects that were offered enrollment but declined participation or why the investigators chose an “n” of 27. All of these issues regarding the study design and conduct should be included.

We have changed the paragraph: “ART interruptions were decided by a joint decision between the physician and the patient, written informed consent was obtained from all patients, and the Ethics Committee of the participating Hospitals approved the study”. By “ART interruptions were decided by a joint decision between the physician and the patient, During two weeks, offered to be included in this study to all the HIV patient of different Hospital. Responded affirmatively 27 patients and written informed consent were obtained from all patients, and the Ethics Committee of the participating Hospitals approved the study”.

2) It is stated that three of the 27 subjects re-initiated antiretroviral therapy (1 for acute retroviral syndrome and 2 for thrombocytopenia) but it is not stated how CD4 counts from these individuals were handled in the data analysis. This needs to be explicitly stated.
We have changed the paragraph: “One patient developed an acute retroviral syndrome and two patients presented thrombocytopenia, for which they had to restart HAART.” By “During follow up, one patient developed an acute retroviral syndrome and two patients presented thrombocytopenia (<50,000 platelet /mm$^3$) for which they had to restart HAART (with CD4+ >350 cell/μL).”

3) In the Conclusion it is stated “However, the results are in agreement with previous findings on the safety of treatment interruptions…”. The number of individuals studied here is actually small to suggest safety but more importantly, over 10% of subjects in this study experienced clinical events that prompted re-initiation of therapy. At this rate, I am not so sure that this study confirms the safety of this approach.

We have changed this sentence in Conclusion: “However, the results are in agreement with previous findings on the safety of treatment interruptions in similar clinical studies, and suggest that ART interruptions may be useful in a specific subset of patients with a minimal CD4$^+$ nadir and a good LPR response”. By “However, the results suggest that ART interruptions may be useful in a specific subset of patients with a minimal CD4$^+$ nadir and a good LPR response”.

**Minor Essential Revisions**

There are problems with the use of English and grammatical errors.

Language has been reviewed by experts.