Author's response to reviews

Title: Effects of highly active antiretroviral therapy with Nelfinavir in vertically HIV-1 infected children: 3 years of follow-up

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Author's response to reviews: see over
Dear Sir/Madam,

RE: Manuscript MS: 1677251495997863 (Resino et al. Manuscript entitled: EFFECTS OF HIGHLY ACTIVE ANTIRETROVIRAL THERAPY WITH NELFINAVIR IN VERTICALLY HIV-1 INFECTED CHILDREN: 3 YEARS OF FOLLOW-UP).

Thank you very much for your e-mail of May 23, 2006. 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,

Salvador Resino, PhD
Reviewer 1:

Major Compulsory Revisions:

1. Introduction:
   1. We rather prefer to omit the word *selective*, so that the new sentence would be:
      Nelfinavir (NFV) is an HIV-1 Protease Inhibitor (PI)
   2. We develop every acronym in the paper at least once. We keep the acronyms ART and HAART to refer to the antiretroviral treatment because we assume that the former includes not only drugs but also other considerations like adherence or resistance and the latter only indicates antiretroviral agents.
   3. We add: The challenge to give it weigh twice daily …instead of the previous schedule of three times per day… to emphasize the fact that taking the same dose less times per day contributes to reinforce adherence. Nevertheless, when our study began the patients had a three times per day schedules so we clarify that in the Methods.
   4. We change the way we express our objectives: Instead of saying: However further analysis, like our study, to evaluate the use of this drug in pediatric age, are needed. We say: However further analysis, like our study to assess the association between baseline characteristics and virological or therapeutical failures are needed.

2. Methods:
   1. (25-35 mg/Kg, three times per day): We specify that our children received three doses per day because when the study began all our patients follow this regimen and as we suggested in the Introduction we thought that it would have been better to follow a BID prescription to achieve a higher adherence.
   2. We do not set the criteria to prescribe NFV in the hospital. Not that, we include all infants under NFV regimens in those centers. In order to clarify the way we recruit patients we add the next sentence: All vertically HIV infected children in Madrid are enrolled in the same cohort, which has 276 patients. We selected those patients who started HAART with NFV, 72 out of 276. Nevertheless, from these 72 infants, 42 had previous treatment and 14 were naïve. We selected only the 42 experienced children in this study.
   3. We add: The children were monitored under a standardized form at least every 3 months with physical examinations, and serial measurements of CD4+ T-cells and VL. All the hospitals included in our study follow the same guidelines for
ART and we monitor infants every three months with approximately the same approach.

4. We joint the definition of therapeutic and virologic failure in the methods because in the results when we talk about therapeutic failures, we include virologic failures. As a result the sentence would say: Therapeutical failure was defined as any of the following, instead of saying: Virological or therapeutical failure was defined as any of the following.

5. We announce clinical events in the methods: During the follow-up we recorded all the clinical events and side effects related to the use of NFV.

6. We include some p-value in the result section.

7. We perform a logistic regression analysis for end points with previous fixed times (12 weeks, 24 weeks). When we do not consider previous fixed times we use Cox regression. We have already included HR values.

8. We specify that we perform a multivariate analyses with the following sentence: We performed a multivariate logistic regression analysis to determinate the odds ratio (OR) of baseline characteristics (%CD4+, VL, and age at baseline, previous ART protocol, and new drugs in HAART regimen) on virological or therapeutical failure. Moreover, treatment failure was analyzed by Kaplan-Meier method and multivariate Cox regression analyses to assess the hazard ratio (HR) values of baseline characteristics.

3. Results:

1. We change the sentence: The HAART protocol used was 2 NRTI plus NFV and 28/42 of children had new NRTI in first line of HAART, into: The HAART protocol used was 2 NRTI plus NFV and 28/42 of children changed NRTI in first line of HAART, because all of them have taken previous NRTI regimens.

2. We specify the number of children who did not met inclusion criteria and the reasons for exclusion previously in the Methods.

3. We incorporated the median of follow-up: During the study period (median: 41 (min: 6; max: 71.3) months).

4. We explain in the Methods why we use a logistic regression.

5. Considering the few values of HR or OR that our study shows, we rather think it would be better not to make a Table.

6. We divided Figure 1 into Figure 1 and 2. We think that the number of figures that we made it is enough to show the evolution of our cohort clearly.
7. The description of measure of adherence was briefly done in Methods by the following sentence: Adherence was measured by each clinician by pill count methods and interviews with parents or tutors.

4. Discussion: We include some latest reference and we also improve our English style.

Reviewer 2:

**Major Compulsory Revisions**

1. **Discussion:**

We have included the following sentence to explain how age could have influenced rates of virologic failure: “Furthermore, our study recruit a high number of children older than 3 years, 74%, who have been taken ART for long periods of time and whose rates of virologic failure were higher that those reported in infant under 3 years, (77% vs. 56%)”

2. **Discussion:**

We have changed in the Discussion: “The combination of lopinavir (LPV) and RTV, has been widely used and it has demonstrated a high activity.” By “The combination of lopinavir (LPV) and RTV, has been widely used on pediatric patients and it has demonstrated a great activity, even higher as those reported with NFV [11]. Nevertheless, LPV/RTV was not approved for administration to pediatric patients when our study was started.”

3. We have checked grammar/spelling.

4. **Results (Clinical events):**

- We have changed “Overall, the adherence was high and HAART with NFV was well tolerated.” by “Overall, the adherence was >90% among all the infants included in our study during the follow-up. Besides, HAART with NFV was well tolerated.”

- In Table 1 we added 100% because all our patients have an overall adherence higher than 90% during the follow-up.

5. **Discussion:**

We have included the next paragraph in order to explain how other treatments could have influenced the virologic response.

“Moreover, at baseline only 1/3 of children included 2 new NRTI in HAART line and 1/3 did not take any new NRTI. High virologic failure at the end of our study is probably due to previous resistance mutations to NRTI. Besides, new NNRTI or PI were added as second HAART line and some patients changed NRTI during the follow-up. However, the global tendency of VL was not improved which could be explained by the appearance of new resistance mutations to NRTI or cross resistance to PI. It is probable that if a resistance assay would have been used to guide changes of ART, virological response would have been higher.”

6. Figures have been clarified.
Reviewer 3:

Major Compulsory Revisions

1. We add the following sentence to discuss how previous treatments could have influence our results: Only baseline VL and previous ART protocol [Combined therapy (CT) or Mono-therapy (MT)] were important factors to predict VL control during follow-up. The HR of baseline VL to achieve uVL was 0.33 (CI95%: 0.16; 0.68; p =0.003) per \( \log_{10} \) VL at baseline and HR of previous ART protocol (CT vs. MT) to achieve uVL was 0.18 (CI95%: 0.05; 0.69; p =0.013). HIV-infected children with VL >50,000 copies/mL at baseline had a negative likelihood of 3.34 (CI95%: 1.15; 9.67; p =0.014) to achieve uVL.

2. We mention briefly how we measure adherence in the Methods by adding: Adherence was measured by each clinician by pill count methods and interviews with parents or tutors.

3. In the light of the moment when patients stopped HAART with NFV (mean time 27.4±1.7 months), we think that our result are basically due to the use of NFV. However, we add in the Discussion the influence that the introduction of LPV/rit could have had in our patients during the follow-up.

“The combination of lopinavir (LPV) and RTV, has been widely used on pediatric patients and it has demonstrated a great activity, even higher as those reported with NFV [11]. Nevertheless, LPV/RTV was not approved for administration to pediatric patients when our study was started.”

4. We have already done an intention to treat analysis but as we found a very low virological response rate we thought it would be better to do an on-treatment analysis, where we obtained a moderate response. We think that taking into account our results an additional intention to treat analyses would give scarce new information.

5. We improve our Discussion section with the next paragraphs:

-The combination of lopinavir (LPV) and RTV, has been widely used on paediatric patients and it has demonstrated a great activity, even higher as those reported with NFV [11]. Nevertheless, LPV/RTV was not approved for administration to paediatric patients when our study was started.

-Furthermore, our study recruit a high number of children older than 3 years, 74%, who have been taken ART for long periods of time and whose rates of virologic failure were higher that those reported in infant under 3 years, (77% vs. 56%).

-Moreover, at baseline only 1/3 of children included 2 new NRTI in HAART line and 1/3 did not take any new NRTI. High virologic failure at the end of our study is probably due to previous resistance mutations to NRTI. Besides, new NNRTI or PI were added as
second HAART line and some patients changed NRTI during the follow-up. However, the global tendency of VL was not improved which could be explained by the appearance of new resistance mutations to NRTI or cross resistance to PI. It is probable that if a resistance assay would have been used to guide changes of ART, virological response would have been higher.

6. We omit the reference to the PACTG 377 study.

**Minor Essential Revisions:**
1. We improve our English style.
2. We check that every abbreviation in the manuscript was previously defined.
3. We correct in the first page the number of Figures.
4. We change the median value by mean in the table so as values are the same as those reported in the figure.
5. We simplified the Figure legends.
6. The percentage of children calculated by Kaplan-Meier are different to percentage calculated in a normal quotient.

**Discretionary Revisions:**
1. We add the following sentence to introduce our study in the Background: However further analysis, like our study, to assess the association between baseline characteristics and virological or therapeutical failures are needed.