Author's response to reviews

Title: HIV-1 Drug Resistance-associated Mutations among HIV-1 infected Drug-naive Antenatal Clinic Attendees in Rural Kenya

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Response to Reviewers

Title of Manuscript:

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Reviewer 1:

- Abstract: Please correct the word “Standford” in “Stanford”.
  Answer: The word has been corrected as recommended.

- Background, page 1, please correct the following words: “There is need” (in “There is a need”; “in need ART” (in “need of ART”); “is hence fewer data” (in “is hence less data”).
  Answer: This has been corrected accordingly.

- Results: Please correct the sentence “A major protease inhibitor-associated mutation was found at position M46L...”. Indeed M46L is not a position but is the mutation at amino acid position 46.
  Answer: This has been corrected and now reads “A major protease inhibitor-associated mutation was found in one strain. This occurred at amino acid position 46 in the HIV protease gene (i.e. M46L)”.

- Discussion: Please correct the verb: “there appears” in “there appear”.
  The verb has been corrected accordingly.

Reviewer 2

Page 2 – Conclusion, 1st paragraph: “The prevalence of drug resistance among
drug-naïve pregnant women in rural Kenya in 2006 was below the World Health Organization (WHO) threshold.” This is incorrect, as this does follow the WHO participant mandatory eligibility criteria (Bennett et al. Antiretroviral Therapy 13 Supplement 2: 25-36).

Answer: Thank you very much for pointing out this. Indeed it is true we did not follow the WHO threshold eligibility criteria. The section has now been revised according to observations and reads “The prevalence of drug resistance among drug-naïve pregnant women in rural north rift Kenya in 2006 was 3.2%.”

Material and Methods section:
Page 4 - It will be helpful to the reader if the authors provide the epidemiologic characteristics of studied population (see reference Bennett 2008, age group < 25 years of age are more likely to be recently infected).

Answer: The study participants have been previously described. We have provided this information (as a reference).

Page 4, Specimens: The use of single-dose nevirapine for PMTCT in the country is not mentioned in the introduction, did the ART history (pre-exposure to ART for primigravidae or multigravidae and etc…) screen for this? Either way, it should be noted. If screened for and individuals included in the sample received sdNVP, it should be included in Table 1 as this would certainly affect the prevalence of primary NNRTI resistance mutations.

Answer: Using a structured questionnaire, information on prior exposure to ART was obtained from participants. Those with ART experience were noted and excluded from the final analysis. This information has been provided in “Methods” and in “Results” sections.

Page 5, Drug resistance analysis:

Answer: In this analysis, we did not use the CPR. This might have been a limitation. Further our participants did not meet the WHO threshold on population characteristics. As such this analysis was based on basic information obtained from availed samples.

Reviewer 3

Background
The latest data on ART coverage are confused. NASCOP should be spelled

Answer: This has been addressed and corrected

Results
Some questions on the numbers:

Answer: This section has been corrected and all numbers have been correctly accounted for according to the reviewers’ advice.
In the second paragraph there is probably a typing mistake in the number of recombinants: it is written 29, but they are 26, as correctly written in the abstract and in the table.

Answer: Thank you for pointing this out. We have now corrected the number accordingly.

Discussion
A key point is missing in the discussion: the data were gathered in the years 2005-2006, when ART coverage was around 20% in Kenya. Now ART coverage is estimated to be around 72% (maybe more), so that the scenario could have completely changed. This point should be clearly stated in the discussion and stressed as a limitation of the study; it could be also seen as a strong point in favour of fostering research on resistance surveillance in order to have results on naïve population rapidly available.

Answer: Thank you very much for stressing this out. We have now included a statement stating limitation of the study due to changing times and rapid upscale of ART in Kenya.

Reviewer 4

Background: Paragraph 3; line 6 and 7 “In Kenya, several studies have been done to document drug resistance among drug-naïve HIV-1 infected individuals [6, 7, 8]”, reference 8 does not address ARV resistance among treatment naïve study participants.

Answer: Thank you for noting this. We have now revised and included a relevant statement on transmitted and acquired drug resistance.

Methodology: Methods are well described, however lack information on how the selection of study participants was conducted i.e. inclusion and exclusion criteria. Furthermore, how was the blood sample transported, processed and stored before analysis? The authors must add this information.

Answer: This has now been addressed and a reference stating previous description of study participants included.

Results: On the data given it is indicated that 298 samples were available for analysis and it is not indicated on what happened to 11 blood samples collected to make the total number of 309 enrolled participants. Authors must add information to indicate why 11 samples were missing for analysis. The total number given for different of identified subtypes is 191 instead 188. The distribution must be corrected and be the same as those given in the abstract.

Answer: The numbers have been corrected to reflect the findings of the study.

We have revised the entire section on “Results”. We tried constructing a phylogenetic tree comprising all sequences. However this was very crowded to be included in the draft. Given that we used REGA to analyze for subtypes we
have not included the phylogenetic tree since this was thought to be a repetition of what we found using REGA.

Discussion: Paragraph 2: Limitations of the study could be best when put at the end of the discussion. Furthermore, line 7 reading “However, no recombination analyses were done”, the authors must remove this sentence as the recombinants were given in the results generated from the REGA software.

Answer: This has been addressed and corrected according to the reviewer’s observations.

Reference 15 gives only the Ethiopian prevalence of mutations and does not represent Tanzanian frequencies; hence the authors must give the reference for Tanzanian prevalence for justifying the statement.

Answer: We have now included a reference on the Tanzanian prevalence at the time of this analysis according to reviewer’s advice.

The authors must include in the discussion the implications/significances of the detected mutations associated with NRTIs, NNRTIs and PIs resistance.

Answer: This has now been included in the conclusion.