Author’s response to reviews

Title: A viral genome wide association study and genotypic resistance testing in patients failing first line antiretroviral therapy in the first large countrywide Ethiopian HIV cohort

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Dear Editor,

A viral genome wide association study and genotypic resistance testing in patients failing first line antiretroviral therapy in the first large countrywide Ethiopian HIV cohort (INFD-D-18-01147R1)

Thank you. Now, we have point-by-point replied to the comments forwarded by Reviewer 3 and made some essential revisions as suggested by the third Reviewer. We hope that the current revised version of the manuscript is suitable for publication in BMC Infectious Diseases.

Response to comments by Reviewer 3:

Reviewer 3: I did not review the first submission, thus, I take the liberty to add some suggestions/comments that might further improve the revised manuscript.
Response
We thank the Reviewer for the positive comment and suggestions/comments provided that we believe have improved our manuscript.

Comment 1. In agreement with reviewer 2, I would delete "cost-efficient/-effective" in the abstract (last sentence) and discussion (line 260) describing the NFLG assay. Cost efficiency was not determined in this study.

Response
We authors acknowledge for the valid comments and as per the Reviewer suggestion we have now deleted the phrase “cost-effective” both in the abstract and discussion parts of the revised version of the manuscript (abstract section, last sentence; discussion section page 12, line 263).

Comment 2. I might have missed it. If not, please add the years of enrollment of the study participants. Is this a historical group of patients starting ART in 2005 or rather a recent group of patients?

Response
Our cohort is a prospective real-life cohort, not a historical one. We published articles from the same cohort where we have briefly described the cohort and in “Materials and Methods” section of this manuscript we have provided our previous articles as a reference so that readers would have a chance for more complete information about the cohort including the time of sample collection. As per the Reviewer’s recommendation we have now specified the period when the sample collection was done in the revised version of the manuscript as follows (Abstract, line 4 – 5 and Materials and methods section, page 1, line 23, respectively):
• “virologic treatment failure patients who started first line ART during 2009 – 2011”
• “during 2009 – 2011”

Comment 3. In table 2 (as already done in table 3) it would be very beneficial to always show all figures, i.e., xx/yy (zz%), since the denominators vary a lot. Actually, it would be also helpful to add always the denominator in the text.

Response
We agree that the figures should be consistent throughout the tables and text. Accordingly, we have used the denominators for Table 2 and in the text as well when it is relevant to specify the denominator.

Comment 4. The 16 randomly selected patients (line 58) were chosen from the group of patients with VL >1000 at month 6, weren't they?

Response
As the Reviewer pointed out, the 16 patients were randomly selected from those who had virologic treatment failure outcome at month 6 (with VL > 1000 copies/ml). We have reflected this fact in the revised version of the manuscript by including the following (page 3, line 59):
• “among virologic failure patients with VL >1 000 copies/ml at month 6”

Comment 5. In line 94 I would repeat "using the 150 copies/ml and 1000 copies/ml, ". Since two categories of virological failure have been defined, it is also helpful to repeat the information of the categories in lines 112 and 197.
Response
As the Reviewer recommended we have stated the two viral load cut-off values by including the following (Result section on page 4, line 95):
• “using the 150 copies/ml and 1,000 copies/ml cut-off values”
In addition, we have included the following phrases (Result section on page 5, line 114; and page 10, line 199–200, respectively):
• “with VL > 1,000 copies/ml”
• “of virologic failure patient samples with VL > 1,000 copies/ml”

Comment 6. Figure 1 would gain value by adding distances.
Response
We acknowledge the Reviewer for the valuable comment and as per the recommendation we have now added distance for the Figure 1 and added a description in the figure legend as follows (page 20–21, line 503–509):

Figure 1. Maximum likelihood phylogenetic analysis of the baseline and month 6 NFLG sequences showing proper matching. A Neighbor-Joining tree was generated in MEGA with the Kimura 2-parameter method and full-length sequences of all successfully assembled samples. All final branches display a full bootstrap support of 100% confirming proper sample matching without cross-contamination and therefore all samples could be used for longitudinal analysis. The scale bar corresponds to 0.01 change per nucleotide.

Comment 7. Comparing the data displayed in figure 2 with the data given in supplementary table S1, some discrepancies appear. For instance, the M184V mutation was detected at month 6 in patient ET02 based on GRT, however, the virus was susceptible for 3TC/FTC at month 6 based on NFLG, i.e., the M184V mutation was not detected. A comparison of sequence results derived by GRT and NFLG should be included.
Response
The GRT and NFLG tests are two separate analyses. The ET02 ID for the GRT and the ET02 ID for the NFLG analyses refer to two different samples from two different patients. As the Reviewer pointed out we have also understand the potential confusion that could be created by this discrepancy if one considers the overlapping IDs. Hence, we have now modified the ID in the Supplementary Table S1 in order to avoid the potential confusion as follows:
• * For example ET02P instead of ET02 (the letter P in the ET02P is to indicate for the PBSS or population based assay).

Comment 8. It would simplify comparisons by reordering supplementary table S1 by ID (as done in figures 2 and 3).
Response
As the Reviewer recommended we have reordered the Supplementary Table S1 by the ID.

Comment 9. Please add the ID of the missing patient in line 512.
Response
As the Reviewer indicated it is also important to add the ID for the missing patient in Figure 3.
Therefore, as per the recommendation we have added the following point in the legend of Figure 3 (page 22, line 516 – 517).

- “(due to amplification failure for ET16 month 6 sample)”