All the text modifications are yellow highlighted

Specific Comments to Authors:

Manuscript No.: 68607 Title: Effects of viremia and CD4 recovery on the gut "microbiome-immunity" axis in naïve HIV-1 patients undergoing ART therapy. In this study, the author has studied "Effects of viremia and CD4 recovery on the gut "microbiome-immunity" axis in naïve HIV-1 patients undergoing ART therapy." A lot of studies have already been carried out on a similar topic, and comprehensive data is available in the literature. The present manuscript investigated the fecal microbial composition, serum, and fecal microbial metabolites and serum cytokines' profile of naïve patients before starting ART and after reaching virological suppression after 24 weeks of ART therapy. It was found that the English language used in the manuscript needs major improvements as there are some punctuation and grammatical mistakes present throughout the manuscript. The figures required the proper explanation and caption. Moreover, research models are not discussed in an understandable manner, the introduction section is poor, which reflects that the author needs a more comprehensive way of thinking. It is obvious that the quality of the manuscript does not fulfil the standards of the journal, therefore should be reconsidered after major revision.

Specific comments:

1. The Abstract needs to be revised, especially the results section.

As rightly suggested, we revised the abstract focussing on the result and conclusion sections

2. Please add more strong keywords.

In agreement with the suggestion, we inserted more strong keywords

3. Page 2: "The microbiota-immune axis springs up from a mutual interplay between human microbiota and the immune system." Please revise the sentence; this is just like the author uses an online spinner tool to remove the plagiarism.

Thank you for the suggestion, we have revised the sentence in "The mutual interaction between the human microbiota and the immune system defines the so called the "microbiome-immune axis"

4. Page 3: "Converging data from many cross-sectional studies suggest that the gut microbiota (GM) changes over the progression of HIV infection [11], [12], [13], [14], [15], [16]." This is not the right way to insert so many references in one place, please omit unnecessary references.

In accordance with the reviewer, following also the revision point N. 9, we massively reshaped the introduction section, adding more literature data, illustrating the state of the art with the main results of the cited studies, revising also the language.

5. Page 3: "It is known that increased .relative abundance of pro-inflammatory bacteria has been associated with increased plasma viremia." Can you please add the name of that bacteria?

Reshaping the introduction, we have erased this sentence.

6. Page 3: "It is known that increased relative abundance of pro-inflammatory bacteria…." Please emerge this paragraph to the previous paragraph.

Following the right reviewer's advice, we merged the two paragraphs

7. Page 3: "Its metabolites are also involved in regulating vital host activities." Please add few activities to support this statement.

In agreement with the reviewer, we added some supporting activities as energy metabolism, cell-to-cell communication, and host immunity

8. Page 3: "Serum and fecal microbial metabolites and serum cytokine profile of naïve patients before starting ART…" Please make sure it is naïve or native?

Yes we confirm that "naïve" is the right word

9. Page 3, The whole introduction section is general. Authors are advised to revise the introduction section carefully and add more data to support the problem statement. It is advised to add literature in the introduction section to create a research gap.

Thank you for this critical suggestion; we have reshaped the introduction following the reviewer indications

10. Page 5: Total genomic DNA was extracted from frozen (-80°C) stool samples, collected at different timepoints (weeks 0, 24)…" Why was the sample not taken after every week of dose administration?

Thank you for the adequate question. We think that the sampling after every week of dose administration is very relevant but this is a preliminary study and we decided that 0 and 24 weeks could be the most representative times to get significant results. The purpose of this pilot is to obtain preliminary data for a future study, extending the sampling times and number of patients.

11. Page 5: "Statistical analyses on ASVs representing the bacterial community were performed in R (R Core Team, 2014) with the help of the packages phyloseq 1.26.1…." All information should be moved to at the end under the statistical analysis section.

Thank you for the suggestion, the paragraph regarding "Statistical analyses on ASVs…" has been moved on "statistical analysis section"

12. Page 6: "Each sample was thawed, weighted (between 0.5-1.0 g) and added to sodium bicarbonate 10mM…." There is always a space between a unit and a value (10 mM).

As rightly suggested, we inserted a space between unit and value

13. Page 7: "Free fatty acids determination by GC-MS analysis: This heading contains repeated information, please merged with the previous heading or omit.

Thank you for this advice, we merged the two paragraphs. As the reviewer noted, we analysed fatty acids from two different biological samples (serum and faeces) and we need to specify the two different extraction protocols, however, we used the same detection methodology that is GC-MS.

14. Page 7: "A value under the Low Limit of Quantification (LLOQ) was considered as 0 pg/ml." Somewhere the author use 'ml' and somewhere 'mL', please follow the same trend throughout the manuscript.

 The reviewer is right, and we are sorry for being imprecise. We corrected with "mL" though the manuscript

15. Page 9: ". In particular, the genera Ruminococcus 2 and Succinivibrio were …" Please make sure that genera Ruminococcus 2 is correct.

Yes, it is correct, the reviewer can check it also here http://dx.doi.org/10.1136/bmjdrc-2019-000717

16. Page 9: "The slight changes to the composition of the microbiome we observed (only at order and genus level) led us to ask whether the GM metabolic activity might also be changed...." Please revise the sentence.

Many thanks for this suggestion we corrected the sentence.

17. Page 9: "Did not reveal any significant change after 24 weeks of therapy for each patient (data not shown)." There is no need to mention 'data not shown', it decreases the worth of study.

We erased the sentence, following the reviewer suggestion.

18. Page 10: "As known, gut microbial dysbiosis is linked to aberrant immune responses …" Can you please add more data to support this statement in relation to your results?

As requested, we inserted more supporting explanation adding a couple of references.

19. Page 10: "We documented significant changes in isobutyric (p=0,01), isovaleric (p=0,04) and 2-methylbutyric (p=0,04) acids …" Please use full stop (.) between values (p=0.01) instead comma (,).

We corrected the comma with the full stop

20. Page 11: "In order to shed the light …" Please revise these words.

We revised with "clarify"

21. Page 12: "Some authors have implied that ART may enhance dysbiosis, which is consistent with the high frequency of gastrointestinal side effects with this treatment {Lozupone, 2014 #3582}, [17], [42]." Reference is missing, please correct it.

Thank you for the indication, we inserted the correct reference

22. Page 14: There is no need to separately add a conclusion section in this type of study. It should be merged to the discussion section at the end and should be a single paragraph with key observations and strong future recommendations.

As suggested by the reviewer, we merged the two paragraphs.

23. Authors are advised to proofread the whole manuscript to overcome grammatical mistakes.

We have finely revised all the manuscript (please see the grammatical correction in green)

24. Authors are advised to add a list of abbreviations.

We inserted a list of abbreviation as suggested

25. The figures and table need appropriate captions.

On page 23, the reviewer can find figure legends

26. The headings and subheadings need to be revised especially in results section.

Thank you, we corrected headings and subheadings