Author’s response to reviews

Title: CKR-L3, a deletion version CCR6-isoform shows coreceptor-activity for limited human and simian immunodeficiency viruses

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Author’s response to reviews: see over
Replies to Editor's comment:

"The manuscript in the present form is not suitable for publication, and it needs of a deep rewrite, addressing the many compulsory revisions as requested by reviewers.

We think that modification of the manuscript in light of these comments will improve the paper. Please consider these comments carefully and submit a revised manuscript.

The revised manuscript should be accompanied by a letter indicating the manuscript number and stating your disposition of each of the reviewers' suggestions, item by item. Your rebuttal letter should duplicate the comment, followed by your response."

RE: MS 1548721024116274  
April 18, 2014

Dear Editor,

We do sincerely thank for sparing your valuable time, efforts and deep insight towards improving our manuscript. We also gratefully acknowledge the reviewers role for their concerted efforts, hard labor and essential suggestions and valuable recommendations that have assisted us in improving this manuscript. We remain deeply indebted to them as well.

In response to such perceptive and judicious comments from both the reviewers, we have tried to incorporate all the information wanted and suggestions rendered point-by-point in the attached rebuttal letter. We have tried our utmost to explain all the queries and addressed each of their comments and thus modified all the related sections of this manuscript accordingly. We sincerely hope our coherent feedback comply with both the referees’ remarks and queries.

Furthermore, we have managed a qualified native English professional (Dr. Damani Pigotte, Assistant Professor, School of Medicine, Johns Hopkins University, USA. dpiggot1@jhmi.edu) to go through the whole manuscript to review it very carefully where we then have rewritten as he suggested,
accordingly (please find in red color font). We hope, these revisions will provide greater clarity to the paper and increase its eligibility for publication. Please allow us to acknowledge Dr. Damani Pigotte in our revised manuscript for his intense contribution there.

To wind up, we took a privilege of sharing that an abstract from our research work has been accepted recently for the ensuing ISHEID 2014 (International Symposium HIV and Emerging Infectious Diseases) where I am going to present a poster on May 21-23, 2014 at Marseille, France (a part of abstracted data from this manuscript, MS 1548721024116274). Therefore, we have made an official declaration in our revised manuscript (please vide page 2 in lines 33-35). Please let us know if your journal has some conditions or confinement on disseminating data from this under-review manuscript.

Lastly, we do hereby affirm that the revised manuscript have been formatted according to your journal’s recommended style.

Sincerely Yours,
-Salequl Islam
Replies to Reviewer(s)' Comments
(Reference: MS 1548721024116274)

A. Reviewer 1: Alfredo Garzino-Demo

Major compulsory revisions:

1. There are two major issues with this manuscript. One is that there is no information on the growth characteristics of the different cell lines. Even though all cells were derived from the same initial cell line, NP2/CD4, it is possible that different clones or transfected cells may have different growth rates. Since RT is observed more than 10 days after infection, even a small difference in cell growth rate may reflect on viral growth. So, data on cell growth rates must be included to correctly interpret the data.

Authors’ Response:

We do thank reviewer-1 to bring this issue into discussion. To get congruent growth of the NP-2/CD4 and its derivatives cell lines, we made single cell cloning immediately after selecting the positive transfection. We preserved enough number of clones from each derivative and started our infection assay with equal aged (identical passaged number) and equal number of different cells. In each passage of infection, cells were trypsinized and counted. We always detected very similar concentration of derivative cells. Therefore, growth rate of the cells remained similar to each other. To avoid further discrepancy, we seeded equal number of cells in all of the subsequent passages of infection assay. Please vide modification of our manuscript incorporating detail procedure shown as highlighted on page 7-8, line 155-158.

Moreover, the growth rates of all the derivative cells of NP-2/CD4 were validated since the system was introduced long before in our laboratory and subsequently, the method was used and described in many previous reports (Nedellec R et al., J Virol. 2009; Shimizu N et al., AIDS. 2009; Hoque SA, et al., Microbes Infect. 2012).
2. A second issue relates to the overall relevance of the data to pathogenesis. The authors show that only two isolates can use CKR-L3 (or CCR6- which is not new as the information was in their previous article); and RT data are shown only on one virus. However, the viruses can use also CCR5 (and CXCR4 in the case of the HIV-2 isolate), which are often coexpressed often with CCR6. Since the author show that CCR5 is a much more efficient receptor for the CRR-L3 isolates, one wonders whether the use of this additional receptor has really any impact on HIV infection/AIDS. Other coreceptor such as CCR3 had been shown to mediate HIV entry, and still overall the evidence for their role in pathogenesis is marginal. So the findings reported here on CKR-L3, while of some scientific interest, necessitate of some validation before broad conclusions on their impact. Accordingly, i recommend careful revision of the conclusions made by the authors.

Authors’ Response:
We sincerely appreciate the critical analysis and thoughtful insights. We fully agree with the reviewer on the shortcomings of CKR-L3 in HIV infection in vivo. Thus to comply with reviewer’s suggestion, we deleted the ambiguous part and revised our conclusion-section accordingly (please vide page 16-17, line 358-363).

3. English: Needs some language corrections before being published.

Authors’ Response:
Yes, we do agree that we may often have inadequacy in standard write up in English being non-native English spoken nation. However, we made our manuscript cross-checked by a native English professional and modified his correction as such (please vide modifications shown in red color font for your kind notice).
**B. Reviewer 2:** Svenja Steinfelder

**Major Compulsory Revisions**

1. Generally: the paper would greatly benefit, if a native speaker could be involved for an updated and corrected version.

   **Authors’ Response:**

   We also remain grateful to reviewer-2 for the thorough careful checking and the thoughtful suggestions. Again, agreeing that non-native English spoken nation may often have inadequacy in standard English write up, we made our manuscript cross-checked by a native English professional (Dr. Damani Pigotte, Assistant Professor, School of Medicine, Johns Hopkins University, USA. dpiggot1@jhmi.edu) and his modifications were incorporated in the final draft of this manuscript (please vide modifications shown in red color font for your kind notice).

2. Table 1: It is unclear how the authors quantified infected cells. Please provide a description how the frequency of infected cells was quantified. Accuracy would greatly improve if an automated method would be applied for quantification.

   **Authors’ Response:**

   Since it remains unclear to reviewer-2 on how we quantified infected cells, let me brief in this aspect below:

   During each cell passage, cell-concentration was measured and volume was adjusted to fix 5000 cells/well on the IFA slide. At low levels of viral infection, all antigen-positive cells was counted individually and calculated. At the later stage of infection cycle, both the antigen-positive and antigen-negative cells per microscopic fields were counted in triplicate and mean value was taken to calculate the percentage of cell infection. However, all these were carried out manually since due to lack of automated system. Nevertheless, part of our calculation for antigen-positive cells on the images of IFA was validated by ImageJ for windows, version 1.38 (NIH, USA).
3. Figure 2: Please provide a description how the frequency of infected cells was quantified.

Authors’ Response:
We sincerely hope that our above response to 2\textsuperscript{nd} query posed by the reviewer should be able to satisfy to answer regarding Figure 2.

4. Figure 4: Please provide data on how many experiments were performed with HIV-2MIR and SIVsmE660 passaging through the different co-receptor bearing cells with subsequent sequence analysis. In this form it appears it was done only one time.

Authors’ Response:
We ran two independent experiments to examine different coreceptor activities. We incorporated related clarifications in our modified manuscript (please vide: page 11, line 233-234).
However, for sequence analysis, provirus DNA was amplified from later infection assay.

5. Figure 2/3: Please perform statistics and provide information on tests used.

Authors’ Response:
We remain thankful to the reviewer and appreciate the critical appraisal. We have provided the concerned information on page 9, line 185-188; page 27, line 589-590; page 28, line 609-610, which we hope should satisfy to address this query.

Minor Essential Revisions

6. line 47 should read "CCR6" instead of "CCR5"

Authors’ Response:
Well, we checked it repeatedly. Since we found the statement being appropriate, we didn’t feel like to change that, as the reviewer suggested.

7. methods: please describe the quantification of RT activity, instead of referring to another article

Authors’ Response: Accepting the reviewer’s careful insight positively, we have modified our text accordingly by adding details of RT activity (page 8 in lines 164-173).
We do appreciate reviewer-2 concern, comment and suggestion in order to improve this manuscript, significantly.