Reviewer's report

**Title**: Immune correlates of CD4 decline in HIV-infected patients experiencing virologic failure before undergoing treatment interruption

**Version**: 4  **Date**: 17 December 2007

**Reviewer**: Adriana Perez

Reviewer's report:

This manuscript is a resubmission presenting the results of patients and frozen cell samples collected at the Immune Deficiency Treatment Center at McGill University Health Center in Montreal, Quebec, Canada.

Major Compulsory Revisions. General:

The authors are assuming that the readers are very well familiar with their terminology and notation when reporting the results of their study as well as familiar with the methods implemented by the authors and the population of study.

Abstract:

-What is IQR in this abstract? THIS WAS PARTIALLY CORRECTED. THE AUTHORS STILL ARE PRESENTING TWO VALUES FOR IQR WHICH IS INCORRECT. THE INTERQUARTILE RANGE IS ONLY ONE VALUE AND NOT TWO VALUES. AUTHORS ARE MISLEADING READERS WITH THE CURRENT TERMS USED. Authors indicate that they are reporting the 25% and 75% percentile, then they should say that. Authors should consult any statistical textbook to identify that the IQR is ONLY 1 number.

Authors indicate in the abstract that the objective of the study was to DETERMINE the PREDICTIVE VALUE of (a) pre-treatment interruptions proliferative capacity AND (b) cell surface markers for CD4 count change. This objective of the study in the abstract does not match what is presented in the entire manuscript. In statistical terms, a predictive value is a conditional probability and the results do not present such probability for any of the cases (a) and (b). Authors need to be extremely clear to readers on their research. Is the purpose of their research to determine that predictive value? The authors seem to use PREDICTIVE VALUE not with a statistical definition but probably as association or maybe as prediction. This is unclear with the terms used by the authors. Are the authors concluding that the use of pre-TI immune proliferative responses are helping to predict the CD4 counts decline during TI or/and the cell surface marker are helping to predict the CD4 counts decline during TI? The methods implemented by the authors do not allow them to measure PREDICTION in statistical terms. Again is unclear for this reviewer what was the objective of the research in this manuscript.
Methods:

Again, authors used statistical methods for the description and interpretation of their findings. Authors used statistical inference techniques to report their findings. The use of statistical methods in inferential process is based on the assumption that there is a sample selected from a population of interest. Statistical methods are also known for samples that were randomly selected from the population of interest with some known probability to proceed with the inferential process. Because the authors did not inform the readers if this is a sample or not, this reviewer is assuming that the patients were selected without a randomization procedure and furthermore this reviewer is assuming that they collected information in these patients for a particular period of time. Assuming that authors collected ALL patients in that particular time period then they will have is the full collection of patients and will consist of a CENSUS. These individuals will not constitute a SAMPLE but a CENSUS. Under these assumptions, authors should not use statistical inference methods for a sample of individuals in a CENSUS. It is unclear for this reviewer if these assumptions are correct or not, but it raised methodological concerns that until the authors do not explain to the readers is unclear what the authors collected. After re-read the answers on Response 12, if these are ALL the patients available then authors can not use test of hypothesis here. They have a census of their patients. They did not select a sample and therefore all the statistical analyses for census data should be used.

Authors still lack to document many methods components already requested previously by this reviewer:

- Authors still lack to describe the population of interest. What is the population of interest for these authors? All patients with HIV? All HIV patients with treatment interruptions in the city of Quebec? Why did the authors selected this particular hospital to conduct their research? Is this a referral hospital and the only one where this could be conducted? Is this a pilot study in this particular hospital without a particular population of interest? The authors do not inform the readers all these aspects and the current version of the manuscript does not reflect how the readers may get answers to these questions. Authors wrote that this a retrospective study. It is unclear from where these individuals were identified as retrospectively. Did the authors review charts, review blood collected? Of what study? What design did the authors used to retrospectively collect the data? Why was a retrospective study chosen and from where was that retrospective information collected? Which format, form, or questionnaire was used to collect systematical information on these patients besides their frozen cells samples? What was informed to the individuals that their samples will be used for? What about the lab results? What machine and kits did the authors used to run each one of the markers? Would not this make a difference? The authors mention the brand but they do not provide the specifics on how each one of them was used and protocols implemented. Did the authors have lab controls?

- Authors lack to specify the inclusion criteria for these patients. They just mention
that there was an inclusion criteria without explaining to the readers those details. The authors indicates that the Materials and Methods section indicates that were just frozen samples however, those frozen samples came from some specific population of study, not just because they are frozen. This is important because readers need to be able to compare the individuals from these authors to other populations from the readers. Among how many these patients were available? For example, are these 13 patients out of 100 who originally collected but did not provide you frozen cells? How many visits/cell samples do the authors have available that it is limited to only 13 individuals? Does the center only have information on 13 samples=13 individuals ever? What was the timeframe for collecting these patients? The authors informed the reviewers but not the readers. What are the biases associated with this?

-Authors lack to document the exclusion criteria used for this research. Authors should document changes in sample size due to using their exclusion criteria. For example, women were eligible to participate in the study following the inclusion criteria, however, did authors exclude pregnant women? Why authors do not care from which individuals are the sample coming from? The authors informed the reviewers but not the readers that the center has a database with the results collected at each clinic visit. So it seems that they have selecting somehow the patients fro their analysis besides to the fact that 13 of those samples were frozen. In fact the authors indicate to the reviewers but not again to the readers that SUPPÓSUDLY the authorâ##s patients are representative of an HIV clinic based population. Authors can not assume that, but they need to provide the information to the readers. The readers are the ones who need to evaluate if their patients characteristics are similar to the authors and not vice versa.

-It is still unclear, what was the purpose of dichotomizes the count of CD4+ T cell decline? Authors only indicate that they split the results by the median of the CD4 count loss which is different than the CD4+ T cell decline. This was done without explaining to the readers why this was important to the authors. Their explanation to the reviewer is still unclear and it seems as just a fishing expedition. Why would they expect differences by only the median? Authors lack to describe to the readers what was their definition of a â##lesser CD4 count lossâ##.

-Which subpopulations were studied? It is unclear in this current version of the manuscript. The authors still mentioned subpopulations in the statistical analysis section and they informed the reviewer that they did not have subpopulations. This is inconsistent with the current version of the manuscript. If the authors do not want to invest time reading their own manuscript and having it completely clear why do you expect the reviewers and readers to read this manuscript?. The statistical terms for subpopulations are usually describe in terms of socio-demographic characteristics. This reviewer seems to assume that the authors do not mean a subpopulation but they lost power describing their individuals by two groups, collapsing them by a particular median value. Authors lack to inform the readers what was the median value to be used for comparison purposes with potentially the readerâ##s population characteristics. Why was this
median value important to evaluate differences in their markers? This was not defined by the authors as their objective or what is the relation of this subpopulation analyses with their objective? The manuscript just indicates they did it without explaining what its purpose was.

Authors lack to inform the readers, what test statistic or approximation was used to compute statistical significance of the spearman correlation coefficient. They informed readers that Spearman correlation coefficients were assessed. However, in the results section besides reporting the spearman correlation coefficient they report a p value associated to a hypothesis. There was not hypothesis expressed in the Statistical analysis section, it is unclear if the p-value is appropriate and valid and the authors do not report what is the test statistic used associated with the p-value of the Spearman correlation coefficient. What test did they do? Ho: Pho=0?? Any other value? If so, what test statistic was computed / approximated to the distribution of the spearman correlation coefficient?

Authors lack to inform in the methods section the purpose of their graphs. If the authors do not value their graphs to provide the detail of them why should the readers? What was the purpose of the individual contour plots? It is not sufficient to indicate that these graphs are just a standard way to show results. Authors need to inform readers, not just because they do not have a purpose. Why was not described in the methods of this manuscript? In fact the authors did not even mention in the methods that the outcome of the paper is to present figures with results from two patients.

- Which statistical method was used for testing trends as described in the results section? Authors: The same statistical methods. Trends were reported for observations of interest that did not achieve a p-value of 0.05. Unclear this answer in statistical terms. The Spearman correlation coefficient does not measure a trend. The answer by the authors indicates that they did not measured trends statistically. Therefore, what are they referring as a trend? Are they testing for a quadratic, a cubic trend? U shape trend? What trend?

Results:

- Same as before regarding the numbers besides IQR. Authors need to be clear and precise on their use of statistics and notation

- The authors still did not provide clarification in this aspect: What are the responses evaluated in the figures presented in this paper? They are described differently than in the text or they are different variables presented? For example, figure 1A notation shows CD4. However in the text they have the following options: CD4 counts, CD4 count nadir, CD4+, CD4 + T cell, CD4 proliferative, etc. What of all of these options are the authors referring in figures and tables, the notation are not consistent across all tables and text. This makes the manuscript confusing and raised concerns to this reviewer. Please notice the inconsistency between the title of the table, the text and the information within the figures. This reviewer expects that ONCE notation is given for each term from
beginning to end in the manuscript, across tables, figures and text.

-What are the values reported in each contour plot for figures 1, A? What does the acronym CFSE means? Authors need to explain their paper to the readers. They answer this question to the reviewer but not to the readers. They have to keep in mind that the readers are who need all of these clarifications made by the authors!. What are the units of the CD4 and CD8 reported in those figures? Are those counts only? Are those CD4+ etc? Please standardize the notation. The answers to the reviewers are informing the reviewers that they are CD4+ cells however they do not appear clear in their figures and text consistently. Authors used a different notation in their figure legends. For example they described CFSElo cells, etc. Please notice that the authors answered the reviewer that the figure represents %CFSElo. What is the real value and correct notation across the entire manuscript?

-Authors indicated that #there was no missing data. If samples were not available for time points that met the inclusion criteria, then the time points were not included in the study. However, due to the limited amount of cells available for each time point tested, not all immune parameters could be tested for every time point.## This raised too many flags to these reviewers. Which time points are the authors referring to? It seems buy this statement that they selected patients using a criteria beyond having the frozen samples and therefore not longer a CENSUS. This is too confusing . They did not used any sample size calculation so if it is a census is still unclear because methods are inconsistent. If the authors have a census then the statistical test are incorrect.

- Figure 1 B still shows a line. What is that? The authors mentioned that they will remove showing linearity but this is not presented in this version. Again, the authors are assuming that Spearman correlation coefficient measures linearity and this is incorrect.

-Authors should inform the readers about the limitation of this research not only the reviewers.

-What is the race/ ethnicity of this individuals or nationality or nativity? -What are the socio-demographic characteristics of these patients? Authors: This not relevant for our study and does not influence the conclusions we draw. Please inform the readers. They are the ones who will decide if the demographic characteristics of these 13 patients are relevant to them or not to use your findings.

-What is the lower and higher than the median from which one? Page 7, 2 paragraph.

-There is new information not descried in the methods about an anti-CD3. Infom the readers in the methods section.

-Page 8, 1st paragraph. Authors are confused with the way statistics works. The authors wrote â## When the percentage of CFSE lo T-cells (notice different notation) was plotted versusâ¥ we FOUND a STATISTICALLY â#î,. Authors
need to understand that statistically significance is not FOUND using a plot. They should have used a test statistic and a hypothesis. They do not inform the readers this correctly, indicated their abuse of statistical terminology and test. PLOTS do not measures statistical significance.

-A p-value of 0.03 in Table 1 in text was not found on table. The second association is not presented in table 1 CD8+. Why do the authors need to report their results in Figure 1B and table 1 as well as in the text. Too many repetitions.

-Previous comments made for figure 1 are applicable to figure 2, authors lack to inform the readers what is their purpose. It is not sufficient just to do it because other authors do it, without explaining the readers the Authorâs purpose.

-Page 8, 2 paragraph, last sentence. Authors are incorrect in stating that CD8+ T cells did not correlate with CD4+ Assuming that the authors are using the p-value for testing an association, just because they did not reject the null hypothesis it does not ALWAYS mean that there is not association. Statistically, it could mean that there is not ENOUGH sample size to detect it or that yes, it is not there. It is unclear with the information presented in this manuscript because it does not show if this is a sample or a census of patients. Authors need to rephrase this that they could not detect a correlation.

Discussion:
-Authors lack to inform the readers of the limitations not only the reviewers. Please do not include that the sample size is a limitation in the conclusion.
- The epidemiological biases will vary depending if these patients are a sample or a census and authors need to address those as potential limitations in this section.

Tables and figure: please use the same acronyms and names across all the figures and text and table in one ONLY notation.

What next?: Reject because scientifically unsound

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Not suitable for publication unless extensively edited

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:
I declare that I have no competing interests