Reviewer's report

Title: IP-10 response to RD1 antigens can be a useful biomarker for monitoring tuberculosis therapy

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Reviewer: Payam Nahid

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The authors report interesting findings related to differences between QFT-IT and home-brew in vitro assays using multiepitopic RD1 peptides selected by computational analysis in monitoring change in IFN-gamma and IP-10 in active TB patients on treatment. The authors report a significant quantitative decrease in the level of IP-10 in response to the RD1 selected peptides between the baseline and end of TB treatment, including a significant decrease in the proportion positive. Overall, this exploratory study is hypothesis generating and provides some of the groundwork needed for investigating the role of IP-10 monitoring in treatment of active tuberculosis.

Major Compulsory Revisions:

1) Given the small sample size, and the lack of data/analysis on clinical and microbiologic outcome measures, please consider highlighting that this study is intended to be exploratory and hypothesis generating. This should be done in the introduction and discussion, and in the abstract if possible.

2) Whereas the authors state as their conclusions in the abstract and discussion: "In this study, we demonstrated (for the first time to our knowledge) that IP-10 can be a useful marker for monitoring therapy efficacy in response to RD1 antigens in patients with active TB", this conclusion as stated is not supported by the design of the study as presented. What has been reported is that IP-10 secreted response to selected RD1 peptides decreases during treatment. This conclusion is distinct from stating that this biomarker is useful for monitoring treatment efficacy given that no clinical, microbiologic, failure/relapse treatment efficacy data is provided/analyzed. Would the authors kindly rephrase their primary conclusion re: changes in IP10 during treatment?

Minor Essential Revisions

1) Can the terms "the TB Antigen of the Quantiferon TB Gold In Tube" and "RD1 selected peptides" be improved for clarity, in the abstract and throughout the manuscript? The peptide antigens in the QFT TB G IT are more than one, and the repeatedly stated "the TB Antigen of the Quantiferon TB Gold In Tube" suggests only one of the antigens in the commercial kit was assessed.

2) Overall, the abstract could be improved for clarity - for example, please clarify what the "dichotomous outcome" is in the results section of the abstract - "When the data were analyzed for the dichotomous outcome,...". The reader may
anticipate comparisons by clinical outcomes, i.e., responded well to treatment versus did not.

3) Page 4, end of first introduction paragraph: "The performance of these assays is extensively reviewed [5-7] indicating that they are more sensitive than (or at least as sensitive as) the tuberculin skin test (TST) in detecting latent TB infection (LTBI) and active TB cases." Based on my understanding of the literature, I'd be more conservative about this statement and rephrase as "they are at least as sensitive as (some studies say more sensitive) the TST".

Note - "In studies that compare the sensitivity of QFT-GIT to that of TST in patients with culture-confirmed active tuberculosis, pooled QFT-GIT sensitivity was 83% and pooled TST sensitivity was 89%" (see and consider citing Mazurek MMWR 2010).

4) Methods, page 6, study subjects - Kindly clarify the reasoning behind the study design as it relates to the exclusions. Was the cohort originally designed for a parent diagnostic study? For example, as the authors note, prior studies of IP10 in HIV infected showed high sensitivity as a diagnostic. Then, why exclude HIV-infected patients in terms of looking at changes in IP-10 or IFN-gamma secretion on treatment? Similarly, why exclude patients who had undergone TST or silicosis patients? How were these characteristics relevant to evaluation of a biomarker of treatment response?

5) Page 6 methods - Kindly define/spell out the definition of "standard category 1 regimen" for the benefit of readers without expertise in TB treatment.

6) Page 7, first paragraph - Currently, it is stated that "plasma were collected after centrifugation and stored at 4°C until assayed" I believe the manufacturer of QFTIT recommends no more than a maximum of 8 weeks at 4C. Kindly report the maximum lapse in time from refrigeration of plasma to running the ELISAs.

7) Page 8 measurement of IP-10 - Kindly clarify if the IP-10 ELISA assay used for this study was indeed made by BD, as stated in this section. Referenced papers by the authors list R&D Systems Inc, MN, USA as the IP-10 assay manufacturer. This is important to clarify because the ROC used for this study where developed from data using R&D kits.

8) Page 8 - Assuming that unstimulated background secretion was always substracted out, please consider deleting the words "In general" from "In general, the data from stimulated whole blood reported in the text and figures are reported after the subtraction of the relative unstimulated control,..."

9) Did the authors intend to write p<0.05 for significance in place of < or + to 0.05? Later on, on page 11 (Results Section), the authors state "The proportion of positive responders between baseline and the end of therapy (20/27, 74.1%) was almost statistically significant (p=0.050)" which creates some confusion in regard to the p value cut-off used.

10) Figures 1 and 2 - The inclusion of months 2 through 5 on the x axis in the
figures gives the impression that there is a linear change across time, which in fact may not be the case (some pts may have had IP10 go up, then down, some down then up etc). Would recommend using only T0 and T6 for the figures.

11) In line with my earlier comment in the major compulsory revisions section, Table 1 also needs to be reconsidered, unless the conclusions of the paper are adjusted. Table 1 currently shows the difference between populations in whom the assay was performed versus not performed - it would be more informative for it to report the demographic, clinical, radiographic and microbiologic characteristics of those patients in whom IP10 and IFN gamma significantly changed and those that it didn't, or more preferably, those in whom sputum smear or culture conversion occurred early versus late again as it relates to evaluating IP10 as a potential marker of treatment efficacy.

Discretionary Revisions:

1) Page 4 - Kindly consider changing the words "surrogate marker" to "biomarker of treatment response" in the introduction.

2) Page 6, bottom - Kindly confirm that no intermediate sputum sample were collected, for example at the end of the intensive phase, as recommended by WHO Treatment Guidelines. If collected, would the data on smear or culture status at the intermediate time points be interesting to consider in terms of concordance with IP-10 changes?

3) Page 7 re: concentration of RD1 peptides selected by computational analysis - kindly consider commenting on any assessments of purity for peptides (if done) and comment on any titration experiments performed prior to the selection of the final concentrations used in the assay.

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

Quality of written English: Needs some language corrections before being published

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests.