Author's response to reviews

Title: Personalized medicine in psoriasis: developing a genomic classifier to predict response to Alefacept

Authors:

MAYTE Suárez-Fariñas (farinam@mail.rockefeller.edu)
KEJAL R SHAH (kshah@mail.rockefeller.edu)
ASIFA S HAIDER (haiderasifa@hotmail.com)
JAMES G KRUEGER (kruegej@mail.rockefeller.edu)
MICHELLE A LOWES (lowesm@mail.rockefeller.edu)

Version: 5 Date: 6 October 2009

Author's response to reviews: see over
October 2, 2009

Manuscript no: 1476 8037 0028 6155

**Personalized medicine in psoriasis: developing a genomic classifier to predict response to Alefacept**, by Mayte Suárez-Fariñas, Kejal R. Shah, Asifa S. Haider, James G. Krueger, and Michelle A. Lowes *

Dear Editors,

Thank you for your recent correspondence regarding our manuscript. We appreciate the time and comments of the reviewers and editors. Please find following our responses to the reviewer comments. Changes in the main manuscript are underlined as track changes, and a second “clean” copy of the manuscript is also submitted.

**REVIEWER COMMENTS:**

**Reviewer #1: W. Clark Lambert, M.D., Ph.D**

This is an excellent preliminary report. I have only one suggestion, as follows:
1. MS page 9, line 5: After "small", make a further statement emphasizing the limited value of your conclusions, due to your small data base.

   We have included an additional sentence reflecting this limitation.

**Level of interest:** An article of outstanding merit and interest in its field

**Quality of written English:** Acceptable

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

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

**Reviewer #2: Claes Enk**

This work attempts to define genomic classifier genes to predict responsiveness to alephacept by analysis of peripheral leucocytes in psoriasis patients. Alephacept, being highly effective but only in a minority of patients, is indeed an ideal candidate for such an analysis as described in the introduction.

**Major concerns**

1. The analysis is based on tissue samples collected in connection with previously published work. This work dealt with immune mechanisms of alephacept, and the division between responders and non-responders was based on histological response, i.e. histological
normalization of the individual psoriasis lesions. In clinical settings responders versus non-responders are usually defined by clinical scoring such as PASI. For genomic classifiers to have clinical impact, they should be able to predict which patients will respond to treatment and who will not. The authors appear to acknowledge the preliminary nature of the present work when they mention the need for “a prospective clinical trial”. I would like the authors to elaborate on this issue and suggest that they add "histological" to the title: “Personalized medicine in psoriasis: developing a genomic classifier to predict histological response to Alefacept”.

We recognize that PASI is the most commonly used clinical measure of response to any anti-psoriatic medication, and agree that: “For genomic classifiers to have clinical impact, they should be able to predict which patients will respond to treatment and who will not”. However, we specifically chose histological response to develop this genomic classifier as we wanted to have an objective, clear and reproducible end-point of treatment. In any future prospective clinical trial to test the ability of the classifier to predict response, we would envision that both histological response and PASI be tested. We have now included the PASI responses of the patients in Table 1, so that others may re-analyze the data using this outcome measure if they wish to do so.

We have elaborated on the preliminary nature of the present work. As suggested, we have added the word “histological” to the title.

2. Confirmative PCR was not performed due to lack of RNA. This is a serious flaw that limits the validity of the findings as pointed out by the authors themselves in the cover letter. Would it be possible perform PCR on pooled samples?

We agree, however, we simply do not have any remaining good quality RNA from this patient group to do any further analyses. We did actually approach the current producer of alefacept to see if they were interested in supporting a prospective clinical trial, but they were not in a position to do so.

In over 5 years of micro-array studies we actually find most of the Affy chip data to be quite reproducible. Where it is unreliable is in genes of low abundance, such as cytokines. We actually filtered out the low expression genes in our analysis. Also, in considering the use of this prediction classifier in clinical practice, it is likely that a micro-array platform would be chosen over RT-PCR, which also supports using this platform to develop the classifier.

3. Lacking confirmative PCR analyses, I suggest that a microarray analysis be carried out on an unrelated group of samples as a negative control to demonstrate the specificity of the genomic classifier for psoriasis

We have developed a genomic classifier to specifically predict response to alefacept in patients with psoriasis. To demonstrate both the sensitivity and specificity of this classifier we can only use psoriatic patients who received alefacept. An unrelated group of patients that do not have psoriasis or who do not receive alefacept is outside the population that this classifier targets. However, we have pursued this approach with other therapeutic agents for psoriasis unsuccessfully, which supports the specificity of the genomic classifier for alefacept in psoriasis. We consider alefacept particularly suitable to develop such a genomic classifier.

Minor concerns
4. Data on only 7 of the 10 non-responders are presented – what happened to the rest?
For the original clinical trial, there were 22 patients enrolled, 12 were histological responders, and 8 were non-responders. Two patients dropped out due to non-response, and so they were included in the non-responders based on intent-to-treat, although we do not have a complete set of biopsies on these two patients. We had good quality RNA and data for the discriminant analysis from 9/12 of the responders, and 7/8 of non-responders. Thus we had data from 16 patients, and this has been clarified in the manuscript.

5. The abstract could be more detailed and specific on the findings

The abstract has been improved by providing increased background and methods information, and presentation of more detailed and specific findings.

6. Page 7, line 9: At least in terms of expenses, treating a non-responder in vain appears more costly than abstaining from treating a potential responder

We agree that in terms of dollars, it would be more expensive to treat a non-responder in vain. However, the term “costly” was used in terms of patient treatment, rather than a dollar value. We meant to convey that it would be worse for a patient to predict that they would not benefit from alefacept, and not be offered treatment when they would indeed benefit, rather than to treat them without a response. This has been clarified.

Level of interest: An article whose findings are important to those with closely related research interests
Quality of written English: Acceptable
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’

Reviewer #3: Erna Snellman
Discretionary revisions:
Should the acknowledgement be completed?
Discussed further in section
Minor essential revisions:
None
Major compulsory revisions:
Methods should be given more carefully so that also a reader, who has not possibility to go to previous publications, can get an idea, how the patients were recruited, what was the initial aim of the study in 2003, the description of the methods used - at least the main points.
The limitations of the tiny cohort should be stressed further as well as the possibility of misinterpretations. The risk of multible publication bias of a tiny study should be considered.

Reviewers detailed further comments to the authors
1. Background information and the question posed are well defined and open the question well. The item is important and interesting.

2. Some major problems are found in description of methods.
a. The study is based on old material rooting form a study completed in 2003. Almost all data is already presented in pieces in at least three earlier published studies. These are referred and included in the list of references (n:o 5 Haider AS et al. 2005, n:o 14 Chamian F, Lowes MA et al. 2007 and n:o Chamian F, Lin SL et al 2007).
b. In the present manuscripts almost no data about the patients or their psoriasis is given. The referred earlier works either don’t tell all details. How the 22 patients were recruited in the study, from where they came, and what was the initial aim of the recruitment? Was it a pharmacy
industry paid study for the efficacy of Alefacept?

We have provided more detail on the following: patients' age, gender and ethnicity, methods of recruitment, inclusion and exclusion criteria, aim of the original study, examples of histological response and non-response, PASI scores for patients, and funding sources for the original study. We have compiled a new figure demonstrating histologic response and non-response (now Figure 1). We have also incorporated information summarizing the methods and results of the original mechanism of action study (refs no 5, 14 and 15).

3. As regards the soundness of the data the authors conclude already in 2005 in an other published paper that this is a preliminary study and based on a small cohort of only 22 patients, and that in future it is necessary to perform studies in a larger cohort to confirm the findings.
   a. I am a little worried that, despite of their previous conclusions, the same data is used again. Background description of the patients is negligible and using the same data many times may increase the risk of publication bias.
   b. The situation becomes even more uncertain as the classifier mis-identified two of the patients as responders although they were histological non-responders.
   c. The only new data in study seems to be the more detailed description and classification of the selected genes according to response and non-response. In addition the data on only 9 responders and 7 non-responders were available.

In this present manuscript, we have analyzed the data using different statistical methods. This is not simply a more detailed description of the genes that are different between responders and non-responders as in our 2007 paper by Haider et al. Rather than establish what were the differences between responders and non-responders, we asked the data a different question: was there a set of genes that could predict response. We have clarified this in the manuscript. The background description of the patients has now been provided. We have clarified that this data has been used before. The sample size has been explained above and clarified in the text.

4. The over-all picture of the manuscript is that it refers too much on previous studies of the authors assuming that the readers have all these studies available. Without reading the other manuscripts it is almost impossible to say if any standards are followed. Accordingly, the reader cannot be sure of the quality of the study.

   The methods and results of the original mechanism of action study (refs no 5, 14 and 15) have been incorporated into the present manuscript.

5. The discussion is reasonable and interesting. The results show that it is possible to try to predict a response e.g. to Alefacept in advance. Prediction of the response could be valuable in choosing a medication for patient and in avoiding unnecessary cost and health risks.

6. The uncertainties due to tiny groups of patients and mis-classifications should be pointed out more clearly. In addition, the description of the selection and demographic of patients as well as methods in general is too minimal and relies too much on reading of other previous papers.

   The uncertainties due to tiny groups of patients and mis-classifications have been pointed out more clearly. The methods and results of the original mechanism of action study (refs no 5, 14 and 15) have been incorporated into the present manuscript.

7. No one of the writers announces in the author contributions to have participated in the care of
the patients in 2003, or in the blood or biopsy samplings. Should they be included in the author list or mentioned at least in the acknowledgements? Did the patients know this study including their files continue this long and that new researchers are involved?

JGK was the principal investigator and conducted the clinical trial in 2002-2003, as was described in the authors contributions. MAL and JGK have been involved in all the publications about this clinical trial from our group. ASH was the first author of the first genomic analysis paper, and is also a co-author here. Thus the new authors are MSF and KRS. MSF is the biostatistician and she conducted the re-analysis, and KRS participated in data interpretation and writing of the manuscript.

In 2002 our consent forms did not offer an option to specify how long patients wanted to participate in studies. However, we do not feel that re-analyzing and mining of existing coded data would infringe on the consent given at the time, and is actually worthwhile use of extremely precious clinical data.

8. The title seems adequate and it gives an idea of the content. However, the abstract doesn't convince.

As mentioned above, the abstract has been expanded.

9. Writing is OK.

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

**Quality of written English**: Acceptable

**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

**Reviewer #4: Francesca Mascia**

The authors propose a genomic classifier to predict the response of psoriatic patients to Alefacept before starting the treatment. The list of genes identified with a new analysis performed on previously published data will allow physicians to classify patients as responders to the drug just by looking at their blood RNA profile of a subset of genes.

I have a few minor essential revisions to suggest:

In the background paragraph (page 3) the authors misspelled the company that owns Alefacept, (Astellas Pharma instead of Astellis) and the whole sentence that describe the drug efficacy needs to be rewritten.

The company name has been corrected. The sentence describing drug efficacy has been re-written.

Page 3, after reference 5, I suggest the authors omit the word `somewhat` and be more specific.

Somewhat has been deleted. More details of the results of reference 5 are now given in the results and discussion.

It is common practice to verify the major findings of microarray data by Real Time PCR. The authors state that they do not have sufficient material from their trial to verify these data. This additional material would add strength to the manuscript. I suggest they try to obtain new samples (or old ones from previous trials) in order to further test their genomic classifier. If this request is not fulfilled this does not preclude the authors right to publish in BMC Dermatology

Please see response to Reviewer #2, points 2 and 3.

Page 6, given the audience of this journal and the relatively new application of the centroid
method, I would add more explanations about the treatment of the data and the generation of Figure 2. The journal does not assign space limits to the text so any detail that can help the comprehension for a broader audience is welcome.

We have provided more detail about the centroid method, and the generation of Figure 2 (now Figure 3).

Level of interest: An article of importance in its field
Quality of written English: Needs some language corrections before being published
Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.
Declaration of competing interests: I declare that I have no competing interests

Thank you for the opportunity to resubmit our manuscript.

Sincerely,

Michelle A Lowes, MD, PhD
Laboratory for Investigative Dermatology
Rockefeller University