Author’s response to reviews

Title: P38 MAPK expression and activation predicts failure of response to CHOP in patients with Diffuse Large B-Cell Lymphoma.

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Doctor Dafne Solera
Editor
BMC Cancer

Dear Doctor Solera,

This is in reply to your letter dated June 27, 2014 re our manuscript referenced “2564892461063217- P38 MAPK expression and activation predict failure of response to CHOP in patients with Diffuse Large B-Cell Lymphoma.” We greatly appreciate the reviewers’ comments that we have found very informative and answers to which strengthen our new findings. We have revised the manuscript and answered all of the comments that you will find attached below. We are submitting a revised manuscript as well a copy of the manuscript which highlights the revisions and additions.

We hope that you will find the revised manuscript satisfactory for publication in BMC.

This manuscript has not been previously considered for publication elsewhere and all of the authors have agreed for its re-submission in BMC Cancer.

Thank you for your consideration, we remain

Sincerely yours
P38 MAPK expression and activation predicts failure of response to CHOP in patients with Diffuse Large B-Cell Lymphoma.

Answers to Review #1

The reviewer requested that we add details about the setting-up of the experiments and better explanations of our results. We have added more details about setting-up and experimental designed in the introduction section and extended our results discussion.

Minor Essential Revisions

- IHC staining of p38 and phospho-p38 has been performed on clinical samples from untreated patients. Have you done the same experiments in on clinical samples after CHOP treatment? Or, at least, do you know if CHOP treatment (or one of the four molecules) activates p38?

R= It’s not possible to determine the p-p38 or p38 expression on clinical samples post-treatment, because biopsy post-treatments are not indicated and have ethical implications. Recently, it has been shown that p38 signaling is activated in cisplatin-treated colorectal cancer cells, and the pharmacological p38 blockade sensitizes chemoresistant tumor cells (Germni A., et al Cancer Letter 2014). However, we have analyzed the expression of p38 and p-p38 on lymphoma cell lines treated with chemotherapeutic drugs and did not show an increase of p38 activation after treatment and there are no reports available about p38 signaling activation in lymphoma after chemotherapy.

- Authors never comments or describe if all the phospho-p38 positive samples are also positive for p38. Even if the answer seems obvious, it is important, as a control, to confirm that the samples displaying p38 phosphorylation are also positive for p38.

Moreover, in Figure 4E, p-p38+/ABC and p38+/ABC display different pattern. As there is 22 patients p-p38+/ABC and 22 patients p38+/ABC (table 2), to my opinion, these patients should be the same one. Could you explain this discrepancy?

R= We are agree with the reviewer and the samples for p-p38+/ABC and p38+/ABC are the same; we have corrected the graph in the figure

- Why using Santa Cruz antibodies to assess p38 and p-p38 staining by IHC. CST antibodies seem to be more widely used. Did you compare the antibodies from
the 2 companies?

R= We and several other groups have used Santa Cruz antibodies for IHC, as they all are well standardized for IHC. In addition, we have used Cell Signaling tech. antibodies with similar results.

-page 14: paragraph “Clinical outcomes according to the expression of p38 and p-p38 MAPK” is a little messy and should be better explained. Authors should divide the text in two paragraphs, one describing p38 and the other describing p-p38.

R= The paragraph was modified and it was split into two section as recommended by the reviewer.

-Authors should explain why they decide to perform BCL2 staining and to analyze the relationship of BCL2 and p38 staining. Rational is not clear.

R= We have examined Bcl-2 due to its clinical implication and in addition, we have discussed the correlation between p38 and Bc12 and according to our previously reported in vitro results.

-Performance status (PS) should be better described.

R= We have described in detail the description of PS

-IHC staining of NFkB should be shown, at least in “supplementary data”

R= A supplemental figure for NF-kB staining has been added

Discretionary Revisions

-page13 lane 10: “In the GC subtype, only 2/58 (3%) were p-p38+” should be “only 2/24 (8%)”, if the table 2 is exact.

R= The data in the text were corrected.

Answers to Review #2

The study has a number of weaknesses including retrospective design, lack of information regarding other clinicopathological findings such as clinical impact of IPI, immunophenotype profile and bcl-2, and very short follow-up of the patients.

R= We have described in more detail additional information regarding the design of the study and the finding with respect to other clinic pathological features and we have increased the follow up to 5 years.

Major Compulsory Revisions

1. The results are based on a series of patients treated with CHOP, and the paper would be much stronger if the series include patients treated with current schemes of treatment, that include rituximab.

R= The study was done with patients admitted in Mexicans hospitals whereby R-CHOP treatment is not the principal regimen. The majority of the patients are treated with just CHOP. Rituximab is only included in private hospitals and we have explained this in the discussion and emphasized the importance of our
study.

2. Usage of the references should be reviewed, in particular in the Background. R= The references were reviewed and modified.

3. The Methods are not well-described. Page 10 includes results regarding response to treatment, whereas page 12 (Patient characteristics), describes methodological aspects. R= The methods and results section were corrected accordingly.

4. In page 9, the description of the interpretation of the immunohistochemical results for p38 and p-p38 should be clarified. How many cases were scored based on the intensity and how many based on the density values of the staining? Did the scoring system use the density values? R= The section was clarified and the information was included.

5. How were the results of the multivariate analyses if IPI is included? R= The results included the adjustment for IPI and was included as a supplementary Table.

Minor Essential Revisions

1. Why are the authors using an IgG isotype control? R= The data were explained

2. Figure 2D does not exist, as indicted in page 14. R= The text was corrected

3. Why do the authors recommend R-CHOP treatment if p38 is expressed? R= We have extended the discussion section regarding our in vitro and in vivo findings to support the R-CHOP as treatment of choice

Answers to Reviewer #3

Major revision

figure 3 and 4 need number of each sample curve and p value indications. R= The data were included

ROC curves are reported in the methods, but not in the text and results. R= The ROC data were mentioned in the text of the results section

minor revision

please explain why p-p38 the is the main prognostic factor even upon bcl-2 adjustment (Table 3 RR 2.93 versus 3.33). R= These sentences were modified according to the results

In the conclusion the word "new biomarker" is too strong and not sufficiently
demonstrated, I suggest to substitute the word with the softer "additional marker". 
R= The paragraph was modified