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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Author's response to reviews: see over
July 24th, 2015

Doctor Dafne Solera,
Editor
BMC Cancer

Dear Doctor Solera,

This is in reply to your letter dated June 16, 2015 re our manuscript referenced “1289252299164815- P38 MAPK expression and activation predict failure of response to CHOP in patients with Diffuse Large B-Cell Lymphoma.” We wish to thank you and the reviewers for the excellent comments made to improve the manuscript as well as the Editor’s comment request for the revision. We have extensively edited the manuscript, found highlighted in yellow, and have also responded to the reviewer’s comments, point by point, and our responses also referred to the changes made in the revised manuscript. In addition, we have also added one supplementary table (Table 2) and one supplementary figure (Figure 1) to further clarify some of the reviewer’s comments. Clearly, in the revised manuscript we have also revised the reference list according to the revisions.

We trust that the revised manuscript is now suitable 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

Mario I. Vega, Ph.D
Associate Professor

Benjamin Bonavida, Ph.D
Professor at UCLA
P38 MAPK expression and activation predicts failure of response to CHOP in patients with Diffuse Large B-Cell Lymphoma.

Editors Comments:

We have extensively rewritten the rebuttal letter with specification on the changes made and the reference in the text, which are highlighted. We have also extensively revised the discussion section reflecting the changes made in the result section. The abstract has been rewritten with better clarification and reflecting the results. We have also revised the discussion regarding the issue of phosphorylated related and unphosphorylated fully related p38 MAPK.

Reviewers #1

The authors present a study describing the expression of p38 and phospho-p38 in DLBCL patient’s samples and their relationship with BCL2 and NF-κB expression and response to CHOP treatment. This study is well conducted, results are really convincing. The major result showing that phospho-p38 is a marker of CHOP resistance and that all the phospho-p38 positive tumors also express BCL2 is compelling. Nevertheless, manuscript would deserve more detailed commentaries about the setting up of the experiments and some better explanations on results obtained.

The setting up of the experiments was clarified in more detail in the Materials and Methods section on pages 8 through 10 and highlighted in the manuscript. We clarified the patients tissues clarified the disease stage and the assessment of the IPI scores.

The explanations of the result were modified as requested and found highlighted on pages 12 through 19 of the Results section. Briefly, we have characterized the patient population (Page 12 and supplementary Figure 1) and the performance status. We also clarified the responder(R) and non-responder (NR) subsets (Page 12 first paragraph). In addition, we clarified the four subgroups that were identified based on the expression p38 and p-p38 (Page 13 first paragraph). Likewise, we have also clarified the ROC analysis (Page 13 the end of the first paragraph).

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?

The relationship between p38 and p-p38 expression in tumor tissues and in response to CHOP therapy based on the IPI score were clarified in more detail and highlighted in pages 14 and 15.

The reviewers’ questions regarding the analysis of p-p38 in tumor patients after CHOP treatments. While these studies are of interest, however, the availability of tumor biopsies was not possible and also had ethical implications. In the response to whether CHOP or any other drug activate p38, a report by Germni A., et al. demonstrated that treatments of a colorectal cancer cells activate p38 and pharmacological inhibitors sensitized cells for chemotherapy. Our unpublished findings in B-NHL also demonstrated that treatment with
chemotherapy drugs did not show an increase of p38 activation after treatment and there no reports available about p38 signaling activation on lymphoma after chemotherapy (Germni A., et al Cancer Letter 2014)

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.

The analysis report showed the following:

- EFS 88% p38- and 83% p-p38
  34% p38+ and 33% p-p38
- OS 83% p38- and 74% p-p38
  47% p38 and 50% p-p38

These findings clearly demonstrated that there was a significant correlation between p38 and p-p38 in all of the patient’s tissues.

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?

We agree with the reviewer that the p38+/ABC and p-p38+/ABC are expressions of the same group of 22 patients the modified figure 4E represents the finding and clarify the findings. The figure legend was also corrected accordingly.

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

We and several other groups have used Santa Cruz antibodies for IHC (Lappas M. et al. Placenta 2014, Gonzalo S. et al Rev. Esp. Enferm. Dig. 2012, Vega M. et al Cancer Res. Abstract 2825, 2008 and Vega M. et al. Blood 108:2373, 2006) as they are all well standardized for IHC. In addition, we have used Cell Signaling Tech. and Abcam antibodies ( Cat. No. 197328, #4511, #9212) and obtained similar findings Result unpublished.

-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.

The clinical outcome analysis by Kaplan Meir based on p38 and p-p38 were described in more detail and highlighted in Page 14 bottom and page 15 top. The detail descriptions were reflected on the impact of p38 and p-p38 and in both the EFS and OS.

The paragraph was clarified and as suggested it was split into two sections as recommended by the reviewer and are found highlighted in the bottom of page 14 and the top of page 15.

Authors should explain why they decide to perform BCL2 staining and to analyze the relationship of BCL2 and p38 staining. Rational is not clear.
We have briefly described the rationale for examining bcl 2 and B-NHL tumors in the text and is highlighted in the pages 15, 16 and 17. Bcl 2 s already know biomarker that has impact in the chemo-response in vivo and according to our previously reported in vitro results, p38 has impact in the Bcl2 expression (Vega, et al 2004).

**-Performance status (PS) should be better described.**

The scoring system for Performance Status evaluation was according to the Eastern Corporative Oncology Group (ECOG) (Oken MM, et al 1982).

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

A supplemental Figure 1 for NF-kB staining has been added and the text was modified accordingly (pages 9 and 12).

**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.

The data in the text were corrected and consistent with table 2 (page 13 line 14).

**Level of interest: An article of outstanding merit and interest in its field Quality of written English: Needs some language corrections before being published**

The manuscript has been edited for the English language and grammar.

**Reviewer #2:**

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

We have described in more detail additional information regarding the design of the study (page 8 line 9 and 11) and the finding with respect to other clinicopathological features were described in the results section (page 13 lane 5). We have increased the follow up to 5 years and the figures were modified accord and described in the results and discussion section (page 14 line 16, page 15, 16 lines 10, 15 and 16).

**Major Compulsory Revisions:**

- 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.
We agree with the reviewer’s comments, had the patients been in US or European hospitals; however the study was done with patients admitted in Mexican hospitals whereby the R-CHOP treatment is not the main regiment and the majority of the patients are just treated with just CHOP. We have made this statement in the introduction and discussion sections and whereby the rational of this study will emphasize. Pages 7, 20 and 23

-Usage of the references should be reviewed, in particular in the Background. The references have been reviewed and modified and are found highlighted in the text and in the reference list.

The References were reviewed and modified (page 5 and 6 Ref 4, 6, 7 and 10)

-The Method are not well-described. Page 10 includes results regarding response to treatment, whereas page 12 (Patient characteristics), describes methodological aspects.

The method section was revised and was described in more details (Pages 8 and 10). The methods and results section were corrected accordingly. The patients characteristics from the results section were moved to method section and the methodology the response (page 10 line 15) or no response to treatment was moved to the results section and the results regarding response to treatment were moved to methods (page 12 line 12).

-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?

The interpretation of the IHC results were clarified in more detail and highlighted in the methods section. The scoring system for the tissue staining was reported by the percent of positive cells with score 0-100 %. (Page 10 )

-How were the results of the multivariate analyses if IPI is included?

The results including the adjustment for IPI were included as a supplementary Table (Supp table 2) and described in the results and discussions section. (Page 18 line 16)

Minor Essential Revisions:  
1. Why are the authors using an IgG isotype control?

The IgG isotype control was used as specificity control in the IHC. The information was included at results section (page 12 line 22).

2. Figure 2D does not exist, as indicted in page 14.

The text was corrected for this typed error and the data are reported in Figure 2C. Page 14

3. Why do the authors recommend R-CHOP treatment if p38 is expressed?
We have extended the discussion section regarding our in vitro and in vivo findings to support the R-CHOP as treatment of choice. (Page 23)

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

The manuscript has been edited for the English language and grammar

Reviewers #3:
Major Revisions:

Figure 3 and 4 need number of each sample curve and p value indications. ROC curves are reported in the methods, but not in the text and results.

We have corrected Figures 3 and 4 and have added the number of each sample on the curve and the P-values in figure 3 (A-D) and figure 4 (A-F).

ROC curves are reported in the methods, but not in the text and results.

The ROC data were mentioned in the results section (page 13 line 19).

Minor revision

Please explain why p-p38 is the main prognostic factor even upon bcl-2 adjustment (Table 3 RR 2.93 versus 3.33).

These sentences were modified according to the results (page 18 line 22), we mentioned that p-p38 is one of prognostic factors in addition of Bcl-2 and after the analyses were adjusted for Bcl-2, p38 and p-p38 and remain as prognostic factor. Supplementary Table 2 was added and the discussion section modified accordingly.

In the conclusion the word "new biomarker" is too strong and not sufficiently demonstrated; I suggest substituting the word with the softer "additional marker".

The paragraph was modified according the recommendation. (Page 25 line 19)