Author’s response to reviews

Title: PD-L1, FGFR1, PIK3CA, PTEN, and p16 expression in pulmonary emphysema and chronic obstructive pulmonary disease with resected lung squamous cell carcinoma

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Version: 1 Date: 01 Jan 2019

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Response to the review comments

Manuscript Number: PULM-D-18-00560

Journal name: BMC Pulmonary Medicine

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I appreciate reviewers for very positive and useful comments. On the basis of their comments, we have revised our manuscript.
BMC Pulmonary Medicine operates a policy of open peer review, which means that you will be able to see the names of the reviewers who provided the reports via the online peer review system. We encourage you to also view the reports there, via the action links on the left-hand side of the page, to see the names of the reviewers.

Reviewer reports:

Yanhong Liu (Reviewer 1): General comments

This study evaluated PD-L1, FGFR1, PIK3CA, PTEN and p16 expression in 59 SCC patients associated with emphysema and COPD. The authors concluded that PD-L1 expression level was inversely associated with severity of emphysema in SCC patients. Overall, this is a comprehensive and well designed and executed study. The manuscript is very well organized and presented.

Here are some minor comments the authors may consider to further improve the manuscript:

1. Patients' smoking pack-years and tumor stage information are important risk factors. This will need added to Table 1 and stratified expression analysis.

RESPONSE: As reviewer commented, patients' smoking pack-years and tumor stage information are important risk factors. Therefore, I added and stratified the smoking pack-year data, in addition to tumor stage in Table 1. We focused on the comparison with each expression level between emphysema level or COPD status in this paper. If we compared between pack year (for example, cut-off value are 20, 40, 60, 80) and each expression level (cut-off level are TC0, 1, 2, 3, IC0, 1, 2, 3), it would be another paper to show the relations and difference. We will plan to make another paper thanks to reviewer’s pointing out.

2. The discrepancy from this study (SCC and PD-L1) with previous studies (adenocarcinoma/NSCLS) warrants further discussion and research.

RESPONSE: As reviewer pointed out, I agreed with the need of further discussion. Therefore, I added and changed the sentence “Another is that our study focused on protein expression while other study focused on the gene mutation. These differences may…” in Discussion on page 11.

3. Tables 4 and 5 could combine to one (optional).

RESPONSE: As reviewer recommended, we combined Table 4 and 5.

Alyssa Gregory, PhD (Reviewer 2): Major:
1. Methods: Was antigen retrieval performed on the formalin-fixed tissues? If so describe this process in the methods section. Were any isotype or secondary-only control sections prepared to validate that the antibodies were specific?

RESPONSE: As reviewer recommended, I described some process in the methods section. I added the sentence “To improve the expression, the tissues were pre-treated with microwaves in ethylenediaminetetraacetic acid for PD-L1, or in retrieval solution (DAKO Target Retrieval Solution pH9, Glostrup, Denmark) for FGFR1, or in citrate buffer for PTEN and p16, before staining. Positive controls in IHC protocols were specimens from pulmonary SCC where the target molecules have been confirmed to be positive. To validate the specificity of the secondary antibody, negative reaction control sections obtained by omission of the primary antibody were used.” on Molecular and IHC analysis in Methods.

2. Figures: It would be helpful to add a representative figure showing the IHC staining for PD-L1. This will help the reader understand how the tumor cell and immune cell expression values were obtained.

RESPONSE: As reviewer recommended, I agree with adding the representative image of PD-L1 expression for tumor cell and immune cell expression. Therefore, we added the newly created Figure 1 that showed the representative image of PD-L1 expression for each staining level. Furthermore, we revised the former Figure 1 to Figure 2.

3. Results: Were immune cells scored for FGFR1, PIK3CA, PTEN, and p16 expression and just showed no expression, or were immune cells not analyzed for these markers? Explain why this portion of Table 3 is empty in the text.

RESPONSE: As you know it very well, a lot of studies that used the same antibody (SP-142) we used evaluated the tumor-infiltrating immune cell staining level and compared between tumor-infiltrating immune cell staining level and overall survival [27 Fehrenbacher L, et al. Lancet. 2016]. On the other hand, as far as I know, tumor-infiltrating immune cell staining level itself and relation between tumor-infiltrating immune cell staining level and overall survival are not evaluated for FGFR1, PIK3CA, PTEN, and p16. Therefore, we only evaluated tumor-infiltrating immune cell for PD-L1. That is why a portion of Table 3 is empty. So, I added the sentence “Immune cells were evaluated only for PD-L1, based on a previous study [27].” on Molecular and IHC analysis in Methods.

4. Statistics: I am assuming that Welch's t-test was used on Figure 1 and Chi-squared test were used on data tables, although this could be more clearly stated in the Methods or Figure legend to help the reader. Recommend statistics review particularly for Figure 1, to determine if this is the best test to use to measure Goddard score in relation to PD-L1 expression levels.

RESPONSE: I appreciate reviewer for pointing out the statistic problem. After discussing with our statistician who is one of the co-authors, we realized that it was equal variances between two
groups. So, we analyzed with t-test, not Welch's t-test for Figure 1 which is comparison between each classification of expression and Goddard score. The comparison was one variable between two groups. Therefore, we had to describe t-test. We analyzed with t-test originally, so the results is completely same. We revised and stated clearly to help the reader “P values <0.05 were regarded as statistically significant. The t-test was used to compare between each expression and Goddard score. Chi-squared test was used to compare between each expression and severity of emphysema, presence of COPD/ Gold staging. Groups…” on Data analysis in Methods.

Minor:

1. It would be more descriptive to say that PD-L1 expression "inversely" correlated with emphysema severity. (pg 10, line 54) (page 4, line 3)

   RESPONSE: As reviewer commented, I added the word “inversely” to each sentence in page 4 and 10.

2. Change "each expression" to "expression levels" or "expression category" (pg 3, line 45) (pg3, line 51)

   RESPONSE: As reviewer commented, I rephrased "each expression" to "expression levels" in page 3.

3. Change "...SCC are potential targets for therapy..." to "...potential targets for therapy for SSC include..." (page 5, line 13)

   RESPONSE: As reviewer pointed out, I changed “...SCC are potential targets for therapy...” to “potential targets for therapy against SCC including…” in page 5.

4. Change "COPD was based..." to "Diagnosis of COPD..." or "COPD score" (pg 7, line 16)

   RESPONSE: As reviewer commented I changed the sentence “COPD was based…” to “Diagnosis of COPD...”in page 5.