Author’s response to reviews

Title: Heterogeneity of PD-L1 Expression in Primary Tumors and Paired Lymph Node Metastases of Triple Negative Breast Cancer

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Author’s response to reviews:

Dear Editors,

Thank you for considering our manuscript entitled “Heterogeneity of PD-L1 Expression in Primary Tumors and Paired Lymph Node Metastases of Triple Negative Breast Cancer” (BCAN-D-17-00104) for revision. We are thankful to you and the reviewer for pointing out some important modifications, which are highly constructive to restructure the manuscript. We have thoughtfully taken into account these comments. The explanations of what we have changed in response to the reviewers’ concerns are given point by point in the following “Response to the Reviewers” part. A clear copy of revised manuscript according to the comments from reviewer is provided. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. About the English writing of the manuscript, we have asked for the American Journal Experts (http://bit.ly/AJE_BS) to revise the paper before it was resubmitted to the magazine.

We hope that all the changes in the article will fulfill the requirements to make the manuscript acceptable for publication in BMC Cancer. I am looking forward to receiving your feedback.

Yours Sincerely,

Wentao Yang on behalf of the authors.
Responses to reviewers:

For Reviewer:

Mohamed Mokhtar Desouki (Reviewer 1): This is very interesting paper, which investigated the expression of PD-L1 in primary and paired lymph node metastases in triple negative breast cancer, which carry the worst prognosis. The manuscript is well written with clear design, interesting findings and detailed analysis.

Authors: We thank the reviewer for the positive assessment of our study.

Few comments:

1. What the authors mean by infiltrating immune cells in the LN metastasis? Are those the native cells present in the LN? if so, please refer to the cells as nodal lymphocytes throughout the manuscript

Authors: We apologize for the lack of clarity. We mean the native cells present in the LN, and we have corrected it as nodal lymphocytes throughout the manuscript.

Changes:

Abstract:

Page 1, line 20:

“Results: PD-L1 expression exhibited spatial heterogeneity in both the tumor cells and the infiltrating immune cells or nodal lymphocytes of PTs and LNM. The PD-L1 expression levels were significantly higher in the lymphocytes and tumor cells of the lymph node metastases than in the primary tumors”

Materials and Methods:

Page 5, line 1:
Tumor cells and infiltrating immune cells or nodal lymphocytes were scored separately in the PTs and the associated LNMs.”

Results:

Page 5, line 19:

“PD-L1 is expressed in tumor cells and associated infiltrating immune cells or nodal lymphocytes”.

Page 6, line 3:

“Discordance of PD-L1 expression between tumor cells and lymphocytes in the PTs and LNMs”.

Page 6, line 10:

“Among these patients, 54 (53.46%) possessed positive nodal lymphocytes (range, 5-80%; median=20%)”

Discussion:

Page 8, line 19:

“This study revealed differences in PD-L1 expression between LNMs and paired PTs in both tumor cells and infiltrating immune cells or nodal lymphocytes in node-positive TNBC.”

Page 11, line 1:

“Furthermore, tumor infiltrating immune cells and nodal lymphocyte subsets within tumor microenvironments in PTs and LNMs should be analyzed.”

Figure:

we have amended all "LNM-infiltrating immune cell" as "LNM-nodal lymphocyte" in the figure 2,3,4 and figure s2.

2. How to explain the significant difference with the absence of LVSI. All the cases have LN met and theoretically speaking have LVSI which may not be detected or sampled.
Authors: lymphovascular invasion was historically identified through hematoxylin and eosin staining, so the LVSI may be not sampled or detected, which will induce the inaccuracy of related results. We thank the reviewer for highlighting this inaccuracy, and We have removed related data throughout the manuscript

Changes:

Abstract:

Page 2, line 2:

“PD-L1 expression was associated with high grade and more stromal tumor-infiltrating lymphocytes(TILs).”

Results:

Page 7, line 8:

Deleted the sentence “The presence of PD-L1-positive cells in both the PTs and LNMs was significantly associated with an absence of lymphovascular invasion(LVI).”

Page 7, line 18:

“In contrast, no significant clinicopathological differences were found among the three groups, except for differences in TIL score(p=0.028) (Table S1).”

Discussion:

Page 8, line 20:

“Furthermore, the presence of PD-L1-positive tumor cells was significantly associated with a high score of TIL.”

We also deleted related data in table 1 and supplementary tables.

3. What the authors mean by "metastasis" in the text and also in table 1. Is this the distant metastasis? If so, please indicate that.

Authors: We apologize for the lack of clarity. We have amended it as “distant metastasis”. we also amended it in the results section.
Changes:

Results:

Page 7, line 13:

“Lymphocytes PD-L1 expression in the LNMs was significantly associated with increased distant metastasis (p=0.033).”

Table 1:

We have changed the word “metastasis” to “Distant metastasis”.

4. What is the total number of positive cases for PD-L1 in primary tumors. Are they 39 or 41? Please fix the second paragraph in the results section accordingly

Authors: We thank the reviewer for highlighting this mistake, for which we profusely apologize. The total number of positive cases for PD-L1 in primary tumors is 39, and we have fixed it the second paragraph of the results section.

Changes:

Results:

Page 6, line 6:

“PD-L1 expression was identified in the PTs of 39 patients (38.61%). Among these 39 patients”.

5. Please include the initials of the 2 expert breast pathologists in the text. Also, delete the word "cancer" to read as "experienced breast pathologists"

Authors: We thank the reviewer for the suggestion, we have added the initials of the 2 expert breast pathologists, and deleted the word “cancer”.

Changes:

Materials and Methods:

Page 4, line 22:
“PD-L1 expression was independently assessed by two experienced breast pathologists, AQL and YX, who had no prior knowledge of the patients' clinical information.”

6. Mention the abbreviation the first time mentioned in the text, then use is consistently throughout the manuscript

Authors: We thank for the reviewer’s suggestion, and we have amended the abbreviation throughout the text.

Changes:

Abstract:

Page 1-2:

“Background: Programmed cell death ligand 1 (PD-L1) is a potential predictive biomarker of the response to anti-PD-L1/anti- programmed cell death 1 (PD-1) therapy in multiple cancers, including triple negative breast cancer(TNBC). The purpose of this study was to investigate whether PD-L1 expression is homogenous in primary tumors(PTs) and synchronous axillary lymph node metastases(LNMs) of TNBC.

Methods: PD-L1 expression was immunohistochemically evaluated in 101 TNBC patients' PTs and paired LNMs. PD-L1 expression in tumor cells and infiltrating immune cells or node lymphocytes in the PTs and associated LNMs was scored separately and was correlated with patients’ clinical parameters and prognoses.

Results: PD-L1 expression exhibited spatial heterogeneity in both the tumor cells and the infiltrating immune cells or node lymphocytes of PTs and LNMs. The PD-L1 expression levels were significantly higher in the lymphocytes and tumor cells of the LNMs than in the PTs. PD-L1 expression was also more frequent among the LNMs. PD-L1 expression was associated with high grade and more stromal tumor-infiltrating lymphocytes(TILs). Furthermore, the disease-free survival and overall survival were similar between the PT- negative/ LNM- positive and PT- positive/ LNM- positive patients, both of which exhibited worse disease-free survival(DFS) than PT- negative/ LNM -negative patients.

Conclusions: The differential expression of PD-L1 between the PTs and LNMs suggests that LNMs PD-L1 status may be used to indicate whether PD-1/PD-L1-targeted therapy would be suitable for a node-positive TNBC patient in the future.”

Abbreviations

Page 11, line 6-10:

7. Delete the negative cases from figure 2 (panel A and B)

Authors: The Figure 2 has been amended following the reviewer’s suggestion, and the legend of the figure 2 has been amended accordingly.

Changes:

Figure 2:

We have deleted the case 1 (panel A and B).

Figure legend:

Page 15, line 5-8:

“Figure 2 Differences in PD-L1 expression between PTs and LNMs. Case 1 showed negative PD-L1 expression in a PT (A) and positive expression in an LNM (B). Case 2 showed a PT exhibiting a low level of PD-L1 expression (C), and an LNM showing a moderate level of PD-L1 expression (D). Scale bar=50 µm.”

8. Move "The median age at diagnosis was 51 years (range, 27-74 years), and the median follow-up time was 49.03 months (range, 10.97-94.27 months)” to the result section

Authors: This sentence has been moved to the result section (page 8) as suggested by the reviewer. Please see page 8, line 2-4.

Changes:

In response to this comment, we have revised the third paragraph in the materials and methods section on Page 4 Line 3-4. We have moved sentence of “The median age at diagnosis was 51 years (range, 27-74 years), and the median follow-up time was 49.03 months (range, 10.97-94.27 months)” to the result section on page 4, line 2-4.
9. Delete "Taken together, these data confirm that PD-L1 expression has significantly negative effects on node-positive TNBC patients and that PT-/LNM+ and PT+/LNM+ patients have similar prognoses" from the result section. It is already mentioned in the discussion.

Authors: We thank the reviewer for this suggestion, and we have deleted this paragraph.

Changes:

Results:

Page 8, line 15-16:

We have deleted the paragraph "Taken together, these data confirm that PD-L1 expression has significantly negative effects on node-positive TNBC patients and that PT-/LNM+ and PT+/LNM+ patients have similar prognoses"

10. Change "might is not" to "may be not"

Authors: We thank the reviewer for the suggestion, and we have amended it.

Changes:

Discussion:

Page 7, line 7:

“Thus, PD-L1 negativity in a PT may be not sufficient to exclude a node-positive TNBC patient from receiving anti-PD-L1 therapy.”

11. Change "for the treatment of multiple different tumor types" in the introduction section to "for the treatment of different tumor types"

Authors: We thank the reviewer for the suggestion, and we have amended it.

Changes:

Introduction:

Page 2, line 16:
“Recently, PD-1 and PD-L1 have been shown to be promising targets for the treatment of different tumor types.”

12. Delete the spaces in (TNBC) to read as (TNBC) in the abstract

Authors: We thank the reviewer for the suggestion, and we have amended it.

Changes:

Abstract:

Page 1, line 14:

“Programmed cell death ligand 1 (PD-L1) is a potential predictive biomarker of the response to anti-PD-L1/anti-programmed cell death 1 (PD-1) therapy in multiple cancers, including triple negative breast cancer (TNBC).”