Peer Review File
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Reviewer A

This study investigates the relation of PD-L1 expression and lung adenocarcinoma with solid component and several molecular alterations. The concept is not new as also stated by the authors, there have been several investigations that interrogate PD-L1 correlation with solid histologic component and molecular alterations, However the sample size is large, and information of molecular alterations is comprehensively assessed. Overall the manuscript is well written although there are some grammar issues that may be easily addressed.
There are major revisions that should be addressed specifically focused on statistical analysis that should consider application of correction or multivariate analysis due to several factors that may be influencing the results.
Also references may not be updated there are several references from 2022 that could be added such as the study of Miyazawa et al Thorac Cancer 2022 or the study of Cruz-Rico et al Pathol Onbcol Res 2021 to name a few.
Some minor comments include to clarify if the authors analyzed the expression of PD-L1 in the solid component separately to other patterns. Have the authors analyzed surgical tumors separately? since solid component may not be accurately assessed in biopsy specimens, I would recommend not to mix them together.

Reply: Thank you for your suggestion. As suggested by reviewer, we have added the suggested content to the manuscript on page.
1) We have changed and used multivariate logistic regression analysis in our manuscript (see Page 22-25, Table 2-4).
2) We have made the change and added new reference (see Page 16, line 316).
3) We thank the reviewer for pointing this out. We have revised. We didn't analyze the expression of PD-L1 in the solid component separately to other patterns.
We have analyzed surgical and biopsy tumors separately (see Page 21-24, Table 1-3).

Reviewer B

In the present study, the authors address an interesting topic, and include a large cohort of LUAD with solid pattern, complete data on PD-L1 and molecular alterations from a limited panel, and limitations are addressed.

I have some comments:

In the introduction (page 3, line 3) it is stated that adenocarcinoma subtypes "include the solid, adnexal, papillary, lepidic and alveolar types." Please change to "solid, micropapillary,

acinar, papillary, and lepidic predominant types for non-mucinous adenocarcinoma" in line with the IASLC/WHO terminology.
In the present study, it should be stated how mucinous adenocarcinomas were considered. Included if 5%+ solid (if any existed) or all excluded?
Also, regarding prognosis (page 3, line 4), solid pattern has not been shown to have worse prognosis than micropapillary (in addition to ref #3 and #4 also e.g. PMID: 32562873).

Reply: We thank the reviewer for pointing this out. We have revised the text to address your concerns and hope that it is now clearer (see Page 3, line 3-4; Page 5, line 59; Page 3, line 7-9).

Much of the introduction concerns immunotherapy and PD-L1 as a predictive marker. This is a very important topic, but the current manuscript does not address the predictive value of PD-L1 (incl. if PD-L1 in biopsies "can be as useful as those measured in resection specimens") or data on treatment response to immunotherapy. Hence, it would be appropriate to change some sentences to get the introduction in line with aims, collected data, and results.

Reply: Thank you for your suggestion. As suggested by reviewer, we have added the suggested content to the manuscript on page to get the introduction in line with aims, collected data, and results (see Page 3, line 12-36).

Solid predominant adenocarcinoma was defined (page 5, line 52) as a tumor with a solid component of at least 50%, whereas adenocarcinoma with solid component of 5%-45% was classified as solid minor adenocarcinoma. This is a clear definition. It is not exactly in line with the IASLC/WHO classification, as a predominant pattern may be below 50% (e.g. 40% solid, 30% acinar, 30% lepidic). Maybe it deserves a comment in the discussion, but it is of minor importance.

Reply: We appreciate the reviewer's insightful suggestion, and in our study, we define solid predominant and minor adenocarcinoma. Previous researches define this way[1,2]. The purpose of our study was to explore the effect of solid components' percentage on PD-L1 expression regardless of other histological patterns. Based on this classification, we found it important to evaluate solid components' percentage in both punctured and excised specimens (see Page 9, line 131-136).

Reference

1. Li J, You W, Zheng D, et al. A comprehensive evaluation of clinicopathologic characteristics, molecular features and prognosis in lung adenocarcinoma with solid component. J Cancer Res Clin Oncol. 2018;144: 725-734.
2. Zhang Y, Li J, Wang R, et al. The prognostic and predictive value of solid subtype in invasive lung adenocarcinoma. Sci Rep. 2014;4: 7163.

8% of the cases were biopsies. The proportion of solid component, as well as vascular and pleural invasion, cannot be adequately addressed in this group of patients. Also, this group

may differ significantly from resected cases for survival analysis (see below), and in my opinion, this group should be presented separately from the resected cases (also in tables and figures). However, there are some data of interest for the biopsies, so it may be of interest to keep them in the study.

Reply: We thank the reviewer for pointing this out. We have analyzed surgical and biopsy tumors separately (see Page 21-24, Table 1-3).

Concerning survival analysis, performance status is not included, which should preferably be mentioned as a limitation. Also, progression-free survival is not defined, which would be of value especially given the probable difference between resected and biopsied cases. Furthermore, last date of follow-up is not stated.

In Figure 4 the lines for PD-L1 low and high cross for both OS and PFS, and PFS is not statistically significant. This should be reflected in the results and discussion.

Also, biopsied cases (which are more likely to be stage IV) that are not treated with targeted therapy (exclusion criteria on page 5, line 54) in a population with 70% never smokers and 49% EGFR mutated cases makes the biopsied cases a really skewed group (e.g. very poor performance status may be a reason for not administering targeted therapy). Also, as mentioned above, not all histopathological characteristics here investigated may be appropriately addressed in biopsies. Hence, this group should be omitted in the survival analysis.

Reply: We thank the reviewer for pointing this out. We agree and have updated. We have omitted the biopsy specimens in the survival analysis. The new results can be seen Page 11, line 182-185 and figure3.

Please add percentages in Table 2 and 3 – it is easier for the reader if instead of just “EGFR mutation PD-L1 high 61, low 173, neg 350” the following data were given in Table 3 “EGFR mutation (584) PD-L1 high 61 (10%), low 173 (30%), neg 350 (60%)” etc.

Reply: This observation is correct. We have changed (See Table 2 and 3).

Figure 2. The figure may be more misleading than contributing. Here, EGFR contributes more than KRAS in the PD-L1 high group, but this is since the number of KRAS cases were few and the EGFR cases many. The correlation between PD-L1 and molecular findings is better presented in Table 3 and the text, and the figure can be omitted.

Reply: This observation is correct. We have deleted the figure 2.

Figure 3 and text (page 8, line 131). Why did the authors subdivide the cases based on PD-L1 neg/high and driver alteration pos/neg? It is difficult to see the clinical or biological gain (with grouping all different molecular alterations together) from this subdivision, and it does

not contribute to the results. The link of PD-L1 and mutation/fusion status to various histopathological factors may be investigated and presented without this subdivision. Also, it may be difficult to draw strong conclusions about a PD-L1 high/mutation positive group in general based on only cases with solid pattern.

Reply: We appreciate the reviewer's insightful suggestion and agree that it would be useful. We have removed the related content. Because we lack targeted therapy data, this classification seems redundant (see Page 10, line 165).

Minor comments:

Spell out TPS in the abstract and the first time in the text.
Reply: We've changed TPS to tumor proportion score (see Page 6, line 67).

PD-L1 1-49% is typically stated as "low" and not "moderate". Please consider revising.
Reply: We've changed moderate to low (see Page 6, line 70).

Table 3, "wild" or "wildtype" instead of "mild" for mutations.
Reply: We have fixed the error. We've changed mild to wild (see Page 23, Table 3).

"factor of poor prognosis" instead of "poor factor of prognosis" on e.g. page 9, line 152 and page 10, line 160.
"poor" instead of "bad" tumor differentiation on page 10, line 173.
Reply: We've fixed the error (see Page2, Abstract; Page 11, line 184; Page 11, line 189 and Page 11, line 197 and Page 12, line 212).

Figure 1. The histological images should be larger and preferably a bit brighter in the manuscript's final form.
Reply: We thank the reviewer for pointing this out. We have revised (see Figure1).

Row numbers 83 and 100: Avoid repeating, the same text in 83-85 and in 100-102!
Reply: We have fixed the error.

Row number 195: you wrote resultss. Correct the spelling!
Reply: We've changed resultss to results (see Page 13, line 236).

Reviewer C

Summary
The authors explored the correlation between PD-L1 expression and clinicopathological features and genetic correlation in 1186 lung adenocarcinoma patients with solid component (LUAD-SC). As a result, high expression level of PD-L1 was more observed in the group predominant with solid component. In addition, Expression level of PD-L1 was positively

related with KRAS mutation and ROS1 fusion and negative correlation with EGFR mutation. The patients were classified into four subgroups according to the mutation state of eight driver genes and PD-L1 expression, for potential treatment strategies. Subgroups with high PD-L1 levels and driver mutations had more advanced clinical stage and lymphovascular invasion. In conclusion, they suggest LUAD-SC with high expression level of PD-L1 is linked with unique clinicopathologic characteristics as well as driver mutations. The subgroup featuring driver mutations and high expression of PD-L1 can guide clinical practice.

Comment
This is an interesting paper and the contents are easily understandable. However, I have some questions and comments as below.

1. Page 3, line 3-4 (introduction): they describe five major histological patterns of non-mucinous lung adenocarcinoma as follow. “These include the solid, adnexal, papillary, lepidic and alveolar types.” However, lepidic, acinar, papillary, solid and micropapillary patterns are correct, I think.

Reply: We thank the reviewer for pointing this out. We have revised (see Page 3, line3-4).

2. Page 3, line 5-6 (introduction): they describe the solid-predominant pattern is more aggressive than the other four growth patterns. However, I think micropapillary pattern is also aggressive.

Reply: We thank the reviewer for pointing this out. We agree and have updated (see Page 3, line7-8).

3. Page 5, line 58-59 (materials and methods): PD-L1 expression’s IHC analysis was made by PD-L1 monoclonal 28-8 (Abcam, Cambridge, United 59 Kingdom) or E1L3N (Cell Signaling Technology, Danvers, United States) antibodies. Why do you use PD-L1 monoclonal 22c3 (DAKO)? This antibody is widely used as companion diagnostic tool in order to determine adaptation of immunocheckpoint inhibitor (pembrolizumab).

Reply: The study is a retrospective research. And our research group had limited funds and was not able to reuse PD-L1 22C3 for staining.
The Blueprint phase 1 study reported similar performances for the staining procedure of PD-L1 while using either 22C3, 28-8, and SP263 IHC assays[1]. Many studies have reported that there is a concordance between various PD-L1 antibodies, including 28-8 and E1L3N antibodies used in this study[2]. Despite the inconsistency in the PD-L1 antibody used, we found biological associations consistent with previous studies, which demonstrates the reliability of our results.

Reference:

1. Tsao MS, Kerr KM, Kockx M, et al. PD-L1 Immunohistochemistry Comparability Study in Real-Life Clinical Samples: Results of Blueprint Phase 2 Project. J Thorac Oncol. 2018;13: 1302-1311.

2. Gaule P, Smithy JW, Toki M, et al. A Quantitative Comparison of Antibodies to Programmed Cell Death 1 Ligand 1. JAMA Oncol. 2017;3: 256-259.

4. Page 9, line 137-139 (results): they describe “462 (57%) patients were classified into group 1, 81 (10%) into group 2, 134 (17%) into group 3, and 135 (17%) into group 4.” However, the number is different from Table 4.

Reply: We have fixed the error (see Page 10, line 171-173).