Peer Review File

Article information: http://dx.doi.org/10.21037/atm-20-968

Response to Reviewers Comments:

Reviewer A

Comment 1：The analysis isolated PD expression, and made crude conclusion that they are related to the prognosis. Indeed they are as we all know, yet the prognosis is also influenced by tumor factors, and therapy factors. Therefore the study must include these factors before any conclusion can be made

Reply 1: Thank you for your serious consideration of our study and we would like to make an explanation to your question. In our paper, univariate and multivariate analyses including tumor factors were carried out to evaluating the independent prognostic factors. Finally, multivariate analysis of ADC subgroup showed that only TNM stage and PD-L1 expression were considered as independent poorer predictors. Meanwhile, in SCC subgroup, PD-1+ TILs and TNM stage were considered as independent poorer predictors. We try to exclude the influence of tumor factors to estimate the prognosis of PD expression.

The patients in our cohorts were stage I-III and R0 resection. They were all treatment-naive before surgery. After operation, they received standard therapy according to their TNM stage and NCCN guidelines, that is either platinum-based adjuvant chemotherapy or observation. We believe this part of the treatment is balanced. The limitation of this research was no data on the therapy in postoperative recurrence cases. However, in our paper, we focus on the prognosis that is not related to treatment and reflects the intrinsic factors of the tumor. Also, we have noticed that in some other researches (such as Thompson ED, Zahurak M, Murphy A, Cornish T, Cuka N, Abdelfatah E, et al. Patterns of PD-L1 expression and CD8 T cell infiltration in gastric adenocarcinomas and associated immune stroma. Gut. 2017 May;66(5):794-801), the prognosis of PD expression was discussed in this form.

Comment 2：The manuscript failed to mention any particularly therapy of the patients in their own database, nor in the other two database. How can one conclude on the relationship with OS and PFS?

Reply 2: Thank you for your serious consideration of our study and we would like to make an explanation to your question. Indeed, as mention above, the patients in our cohorts were stage I-III and R0 resection. They were all treatment-naive before surgery. After operation, they received standard therapy according to their TNM stage and NCCN guidelines, that is either platinum-based adjuvant chemotherapy or observation. We believe this part of the treatment is balanced. The limitation of this research was no data on the therapy in postoperative recurrence cases. In order to solve the problem, disease free survival time (DFS) was analyzed which was not affect by the therapy in postoperative recurrence. Therefore, our results directly reflect the prognostic role of PD-L1 and PD-1 for relapse in postoperative NSCLC and indirectly reflects prognostic role of PD-L1 and PD-1 for OS regardless of the treatment after recurrence.

Comment 3：Introduction and discussion are very focused and good, though the discussion failed to mention the major limitations of the study, and possible future use and next step. These would make the discussion more valuable and offer some perspective. It would be important to point out that other immunotherapies, such as ADC, CART, as well as small molecule inhibitors, which may impact OS and PFS also in addition to PD status.

Reply 3: Thanks for your comments on our paper. We have revised the discussion part according to your suggestions.

Changes in the text:

Page 17, line 362-371: The limitation of this research was no data on the therapy in postoperative recurrence cases which could influence the overall survival time of patients, especially with the boom of novel agents for lung cancer these years. In order to solve the problem to some extent, disease free survival time (DFS) was analyzed which was not affect by the therapy in postoperative recurrence. The clinical relevance

of treatment factors with PD-1/PD-L1 pathway and overall survival time needs to be elucidated in the future. Besides, these findings were established in a relatively smaller cohort, and so larger studies are warranted to confirm this in future. Last, although we have demonstrated the difference between the subtypes from the phenomenon, the specific molecular mechanism is still unclear. This will be emphasis of our next research.

Reviewer B

Comment 1：In several tables, adenocarcinomas are classified according to their differentiation (well, moderate-poor). In the last WHO classification, pulmonary adenocarcinomas are classified according to their morphological subtype (acinar, solid, papillary…) and no longer according to differentiation.

Reply 1: Thanks for your comments on our paper. However, Patients in our cohort were received surgical resection within 2010 or 2012-2014 and no such information of the classification were obtained from the archive of the institute of pathology. Thank you for your suggestions, we will redevise these patients according to your suggestions in the future.

Comment 2：The author could discuss pathophysiological hypotheses about the differences that they observed between adenocarcinomas and squamous cell carcinomas, e.g. the role of Neuropilin-1. (Nature Communications 2019; 10: 3345) or the role of CD8+CD103+ lymphocytes (Cancer Res. 2016 76: 1757-69).

Reply 2: Thanks for your comments on our paper. We have revised the discussion part according to your suggestions.

Changes in the text:

Page 17, line 356-361: Some studies in recent years may provide possible molecular evidence that involved in suppressing immune responses in SCC patients. Leclerc et al (36) have reported neuropilin-1 (Nrp-1) defines a subset of CD8+ T cells displaying PD-1hi status and negatively influence CD8+ T cells immunity. Another report has

identified CD103 and its ligand E-cadherin which were important adhesion molecules promoting TILs antitumor functions in human lung tumors (37).

Reviewer C

Comment 1：Any reference/prior studies supporting 20% as the cut off to define high or low CD8 staining?

Reply 1: Thanks for your comments on our paper. In our paper, immunohistochemistry and multiplex immunohistochemistry performed on two independent cohorts. When CD8 was analysis by using multiplex immunohistochemistry which is a quantitative analysis，CD8 were classified as positive/negative using the median as cutoff. In immunohistochemistry cohort, 20% also the median value. So, in order to make balance between the cohorts, we choose 20% as the cut off to define high or low CD8 staining. Besides, in our prior study (Wen T#, Wang Z#, Li Y, Li Z, Che X, Fan Y, Wang S, Qu J, Yang X, Hou K, Zhou W, Xu L, Li C, Wang J, Liu J, Chen L, Zhang J, Qu X*, Liu Y*. A Four-Factor Immunoscore System That Predicts Clinical Outcome for Stage II/III Gastric Cancer. Cancer Immunol Res. 2017 Jul;5(7):524-534), 20% was also as the cut off to define high or low CD8 staining and the result with clinical significance, so we continued to use this definition in this paper.

Changes in the text:

Page 7, line 145: infiltration <20% or ≥20% in tumor site as the median value.

Comment 2：Please address the discrepancy in the definition of positive vs negative TC PD-L1 expression (line 70 and 89). Similarly, please address the discrepancy in the definition of positive vs negative IC staining for PD-L1, PD-1 and CD8 (lines 70-74 vs 89)

Reply 2： Thanks for your comments on our paper. For IHC cohort, definition of positive vs negative TC PD-L1 expression was according to the reference and actual staining (reference 1 and 2). For mIHC cohort, there is no unified and clearly definition, in order to make balance, a preliminary analysis of several patients were performed.

Through comparative analysis, we believed that 1% of mIHC equate to 5% in IHC cohort. Besides, in our prior study (Cao L#, Che X#, Qiu X, LiZ, Yang B, Wang S, Hou K, Fan Y, Qu X*, Liu Y*. M2 macrophage infiltration into tumor islets leads to poor prognosis in non-small-cell lung cancer. Cancer Management and Research.2019;11:6125-6138) 1% was also as the cut off to define high or low TC PD-L1 staining and the result with clinical significance, so we continued to use this definition in this paper. It is the similar situation for IC PD-L1, PD-1 and CD8.

Reviewer D

Comment 1： However, I found it particularly interesting that in SCC patients, there was no association of PD-L1 on immune cells with poor survival. What is the possible explanation for this, especially given that the PD-1 on CD8 TILs in these patients is associated with poor survival?

Reply 1: Thanks for your comments on our paper. From the KM curve, we found a tendency of association of PD-L1 on immune cells with survival, particularly in mIHC cohort although the differences were not significant (expect for OS in IHC cohort). Interestingly, this association was opposite to PD-1 in SCC patients. The possible explanation for this is that in SCC, PD-1 expression, which is especially expressed on TILs, reflected the dysfunction state of T cells, so PD-1 expression is associated with poor survival. For PD-L1 on immune cells, we considered them as reactive which were positively correlated with CD8. In some related studies about the prognosis of PD-L1 in squamous cell carcinoma also demonstrated PD-L1 positive expression in stromal lymphocytes, rather than in tumor cells, is associated with a longer survival in patients with esophageal squamous cell carcinoma (Ito N, Tsujimoto H, Horiguchi H, Shimazaki H, Miyazaki H, Saitoh D, Kishi Y, Ueno H. Clinical Significance of Programmed Death Ligand-1 Expression in Esophageal Squamous Cell Carcinoma. J Surg Res 2020; 251:321-8).

Comment 2： The authors may add a paragraph or two in the discussion to discuss the possible mechanisms that may be involved in suppressing immune responses in SCC patients (that involve the CD8+ PD-1+ TILs).

Reply 2: Thanks for your comments on our paper. We have revised the discussion part according to your suggestions.

Changes in the text:

Page 17, line 356-361: Some studies in recent years may provide possible molecular evidence that involved in suppressing immune responses in SCC patients. Leclerc et al (36) have reported neuropilin-1 (Nrp-1) defines a subset of CD8+ T cells displaying PD-1hi status and negatively influence CD8+ T cells immunity. Another report has identified CD103 and its ligand E-cadherin which were important adhesion molecules promoting TILs antitumor functions in human lung tumors (37).