Peer Review File

Article information: http://dx.doi.org/10.21037/tlcr-21-165

Reviewer A

Comment 1: The authors explored prognostic significance of PD-L1 expression on tumor among patients with SCLC, including limited and extensive disease SCLC. They also examined concordances between three different IHC assays in this population. This research would provide some hints for future research optimizing immunotherapy for SCLC based on tumor PD-L1 expression, however several revisions are warranted before considering for publication. Please see my comments below.

Reply 1: We thank the reviewer the positive comments on our study.

Major comments

Comment 2: The criteria for choosing variables for multivariate analysis seems to be unclear. The authors stated that they used forward sequential method and put variables irrespective of their significance in univariate analysis. Please explain the validity of this approach in this research. Considering the relatively small sample size, the number of variables in multivariable analysis may need to be reduced based on significance in univariate analysis for establishing more sensible multivariate model.

Reply 2: Thank you for raising this point. In general, forward selection for Cox models, a classical variable screening method, has been widely used for model building when the number of covariates is relatively low. The advantage of forward selection is that it starts with smaller models. Normally, the selection process should be guided by the investigator taking into account, among other things, the a priori prognostic value of each variable considered. (1-3). Considering the relatively small sample size of our study and inconsistent results of clinical impact of PD-L1 expression in SCLC, therefore, forward selection methods could be considered more valid for our study. This statistical method has also been used in our previous study (4).

Changes in the text: The following sentences and references were added to the 'Statistical analysis' (lines 153-154, page 7). "Variables selection method for Cox

regression models was used the forward sequential method, which has been widely used for smaller models (1,2)."

Comment 3: In this study both LD and ED-SCLC were included. They adjusted this clinical characteristic in multivariate analysis simply as LD and ED. However, 12 patients among 59 patients did not receive chemotherapy in their cohort. Considering the chemo-sensitivity of SCLC, the use of chemotherapy may need to be cared in prognostic model. At least, this point is needed to be mentioned as a limitation of this study.

Reply 3: We agree with the reviewer's comment. Limited-stage (LS) patients (n=30) of our study received concurrent platinum-etoposide (PE) chemo and radiation therapy (CCRT) (n=17), lobectomy and PE chemotherapy (n=2), PE chemo and sequential radiation therapy (n=2), PE chemotherapy (n=6), only chest radiation therapy (n=1), and chose to go to another hospital (n=2). The detailed treatment modality according to the stage were added to the 'Patient characteristics' (lines 172-177, page 8). As above, treatment modalities were not uniform in our study, which may have caused inherent heterogeneity in the retrospective study. Although the treatment modalities of our study were heterogeneous, mostly patients of LS and extensive-stage (ES) received active treatment (included CCRT, lobectomy and chemotherapy, or chemotherapy and sequential radiation therapy; 21/30) and chemotherapy (20/29), respectively. Therefore, we used the accurate staging (included T and N stage) based on several imaging studies instead of the heterogeneous treatment modalities as a variable. For accurate staging, enrollment was limited to patients staged with contrast-enhanced chest CT scans, PET/CT (and/or whole-body bone scan), and brain imaging to maintain the homogeneity of the population (lines 292-294, page 13).

Changes in the text: 1) We have revised 'Patient characteristic' of Results (lines 172-177, page 8) as follows:

[before revision]

17 patients received concurrent platinum-etoposide (PE) chemo and radiation therapy, 2 patients received lobectomy and PE chemotherapy, one patient

received PE chemo and sequential radiation therapy, 27 patients received PE chemotherapy, 2 patients received only radiation therapy, and the other 10 patients received supportive care or chose to go to another hospital.

[after revision]
17 patients of LS received concurrent platinum-etoposide (PE) chemo and radiation therapy, 2 patients of LS received lobectomy and/or PE chemotherapy, 2 patients of LS received PE chemo and sequential chest radiation therapy, 6 patients of LS and 20 patients of ES received PE chemotherapy, one patient of LS and one patient of ES received only chest radiation therapy, and the other 10 patients received supportive care or chose to go to another hospital.

2) And, the following sentences were added to the 'Discussion' (lines 298-301, page 13). "~ treatment modalities of our study were not uniform, which may have caused inherent heterogeneity in the retrospective study. Therefore, we used the accurate staging based on several imaging studies instead of the heterogeneous treatment modalities as a variable."

Comment 4: They proposed that at least one positive among three IHC assay may be utilized as a prognostic factor for SCLC. Although this approach can be accepted due to small sample size of each IHC assay, 22C3 did not show prognostic significance. Please mention this point to help readers interpret the intent of the authors.
Reply 4: We thank the reviewer the good comment. Although the different PD-L1 IHC assays are approved or in development as companion or complementary diagnostics to different ICIs, targeting of PD-1/PD-L1 have the same pathway (5). In this sense, we assigned two cat¬egories according to positivity for 1/3 PD-L1 assays. As pointed out by the reviewer, 22C3 ($p=0.380$) and SP263 ($p=0.062$) assays did not show prognostic significance, but the positive group of these assays showed longer OS than the negative group (figure 3). Furthermore, a multivariate analysis revealed the only SP142 assay of all assay variables to be independent predictors of longer OS (Table 2). Although these outcomes may have caused a relatively small sample size in our study. our results showed the

clinical impacts of SP142 expression in SCLC.
Changes in the text: The following sentences were added to the 'Prevalence and correlation of PD-L1 expression' (lines 185-187, page 9). "Although the different assays are approved or in development as companion or complementary diagnostics to different ICIs agents, targeting of PD-1/PD-L1 have the same pathway."

Minor points

Comment 5: In abstract, please show some information on treatment (chemotherapy etc.).
Reply 5: As suggested by the reviewer, we added some information on treatment to the abstract due to the limitation of the number of words.
Changes in the text: The following sentences were added to the 'Abstract' (lines 42-43, page 3). "~47 patients received the active treatment beyond platinum-based chemotherapy at our institution."

Comment 6: Methods: The authors examined CEA as a tumor marker in their cohort. Why not ProGRP or NSE, and put SCLC's tumor marker in prognostic model? Please show the reasons if available.
Reply 6: Thank you for raising this point. In our institution, two or more tumor markers is not routinely applied for suspicious lung cancer patients because of their low sensitivity and medical insurance coverage problems. Unfortunately, the ProGRP test is not available at our institution.
Changes in the text: No changes in the text.

Comment 7: Please show the number at risk for each K-M curve.
Reply 7: As suggested by the reviewer, we added the number at risk for each K-M curve.
Changes in the text: Figures 3 and S2 have been changed to new figures with the number at risk.

Reviewer B
Comment 1: Well-written manuscript to determine the clinical impact of 3

validated PD-L1 immunohistochemistry assess as the prognostic factor and small cell lung cancer.

Please consider the following suggestions –

As of now immunotherapy in combination with chemotherapy is approved only in extended stage small cell lung cancer [SCLC], not in limited-stage lung cancer. As rightly pointed out by authors - the study is retrospective, single-center and the total number of patients with extensive-stage was only 29, which makes it hard to conclude.

Reply 1: We thank the reviewer the positive comments on our study. As the reviewer pointed out, our study has several limitations. These limitations have already been addressed in the discussion (lines 288-305, page 13). Our results indicate that the expression of each PD-L1 assay is associated with longer OS, and the positive result of the SP142 assay is a particularly significant independent prognostic factor in patients with SCLC. Furthermore, expression of three PD-L1 assays in patients with ES-SCLC is associated with better outcome than LS patients. The clinical impacts of SP142 expression will become more importance in a new era of atezolizumab in ES-SCLC. Because the SP142 assay is complementary diagnostic for patients who are being considered for treatment with atezolizumab. Although more research is needed in another independent group to generalize or validate our results, this information will benefit clinicians and patients in determining the immunotherapy for patients with ES-SCLC. Furthermore, our results may serve as the cornerstone of further research on the PD-L1 expression (especially, SP142) in SCLC in the future.

Changes in the text: No changes in the text. The above information has already been addressed in the discussion (lines 288-305, page 13) and conclusions (line 308-316, page 14)

Comment 2: Immunotherapy has recently been approved as a first-line agent in metastatic small cell lung cancer in combination with chemotherapy. It is also approved as a third-line agent in metastatic SCLC after the failure of two chemotherapy regimens. The FDA approved four drugs, two of them being PD-1 inhibitors [Pembrolizumab, Nivolumab], and two of them being PD-L1 inhibitor [Atezolizumab and Durvalumab] in SCLC.

Durvalumab, an anti-PDL-1 antibody, in combination with chemotherapy vs. chemotherapy, showed improved OS in the front line setting for the treatment of extensive-stage small cell carcinoma, in phase III randomized controlled CASPIAN trial. Please include the approval of this agent as well in the manuscript.
Reply 2: Thank you for raising this point. As the reviewer suggested, we added a number of immunotherapy treatment options for SCLC.
Changes in the text: The following sentences and references were added to the 'Discussion' (lines 270-274, page 12). "The clinical studies of immunotherapy using ICIs have led to more treatment options in patients with SCLC (6). Based on the results of several clinical studies (7-10), PD-1/PD-L1 inhibitors, namely pembrolizumab, nivolumab, atezolizumab, and durvalumab, have been approved by the FDA as the treatment options of first or second and/or more lines in patients with recurrent or metastatic SCLC."

Comment 3: Advised authors to include an updated overall survival from the following article -
Liu SV, Reck M, Mansfield AS, Mok T, Scherpereel A, Reinmuth N, Garassino MC, De Castro Carpeno J, Califano R, Nishio M, Orlandi F, Alatorre-Alexander J, Leal T, Cheng Y, Lee JS, Lam S, McCleland M, Deng Y, Phan S, Horn L. Updated Overall Survival and PD-L1 Subgroup Analysis of Patients With Extensive-Stage Small-Cell Lung Cancer Treated With Atezolizumab, Carboplatin, and Etoposide (IMpower133). J Clin Oncol. 2021 Feb 20;39(6):619-630. doi: 10.1200/JCO.20.01055. Epub 2021 Jan 13. PMID: 33439693.
Reply 3: Thank you for raising this point. As the reviewer suggested, we added an update overall survival (OS) of IMpower133 study.
Changes in the text: We have revised 'Discussion' (lines 274-280, page 12) as follows:

[before revision]
Recently, the FDA has granted approval for the combination of atezolizumab, PD-L1 inhibitor, carboplatin, and etoposide for the frontline treatment of patients with ES-SCLC based on study findings, which resulted in significantly longer OS than chemotherapy alone.

[after revision]
Furthermore, the FDA has granted approval for the combination of atezolizumab, carboplatin, and etoposide for the frontline treatment of patients with ES-SCLC based on study findings, which resulted in significantly longer OS in the atezolizumab group than chemotherapy alone (median OS, 12.3 months vs 10.3 months, respectively; hazard ratio, 0.70; p=0.007) (8). Recently, updated results with a follow-up of 22.9 months continued to demonstrate an improvement in OS with atezolizumab group and a similar safety profile compared with chemotherapy alone group in patients with ES-SCLC (11).

Comment 4: There was a subgroup analysis done IMpower 133, the PDL1 expression in this trial was only reported and 34% of the study population and expression was more observed in immune cells rather than the tumor cells which is different from what was observed in non-small cell lung cancer. The patients with PDL1> 5% subgroup seem to have significant overall survival benefit however as mentioned above it was reported only in 34% of the study population there were other confounding factors as well.
PD-L1 testing was performed using the PD-L1 immunohistochemical (SP263) assay on a Ventana BenchMark ULTRA automated staining platform. The authors concluded that SP142 expression will become more important in the new area of atezolizumab - however that was not the assay that was used in small cell lung cancer. This is specifically [SP142 expression] used as companion diagnostic indications for urothelial carcinoma non-small cell lung cancer and triple-negative breast cancer.
Reply 4: Thank you for the reviewer's kind and detailed comments. As pointed out by the reviewer, IMpower133 study was investigated PD-L1 expression using the SP263 assay only in 34% (137/403) of the study population. In this study, PD-L1 expression (SP263) was more frequently observed on IC (50.4%, n=69) than on TC (5.8%, n=8) (11). Our study showed similar results that PD-L1 expression of the SP142 assay was more observed on IC (32.2%, n=19) than TC (8.5%, n=5). These results were added to the 'Prevalence and correlation of PD-L1 expression' (line 182, page 9). In Impower133 study, an OS benefit according

to PD-L1 expression was analyzed between atezolizumab group and placebo group. This study did not analyze PD-L1 positive group and negative group like our study. Therefore, caution is required to compare and analyze this study and our results.

As you know, SP263 assay has been registered by the FDA as complementary diagnostic assay for urothelial carcinoma and NSCLC who are being considered for treatment with durvalumab. However, the SP142 assay has been registered by the FDA as complementary (not companion) diagnostic assay for urothelial carcinoma, NSCLC and triple-negative breast cancer who are being considered for treatment with atezolizumab. We focused on that SP142 is complementary test for treatment with atezolizumab. Therefore, our results may serve as the cornerstone of further research on the PD-L1 expression (especially, SP142) in SCLC in the future. Please also see reply to your comment 1.

Changes in the text: The following sentences were added to the 'Prevalence and correlation of PD-L1 expression'. (line 182, page 9). "(19 cases (32.2%) on IC and 5 cases (8.5%) on TC)"

Comment 5: Immunotherapy does not work in all patients. Research is ongoing to identify markers, which will help us to choose patients who will respond well to immunotherapy. PDL1 levels, tumor proportion scores (TPS), combined positive scores (CPS), and the number of mutations identified in the tumor (Tumor mutational burden -TMB) is being used as markers. For example, in the CheckMate 032 clinical trial - in a separate pooled analysis, patients with higher TMB had a response rate of 21.3% compared to 4.8% with low TMB. Even overall survival was better in patients with high TMB compared to low TMB. However, in the same clinical trial, PD-L1 tumor expression did not appear to predict response. In contrast, in KEYNOTE 158, the response rate was higher in PD-L1 positive tumors compared to PD-L1 negative tumors at 35.7% and 6% respectively. Overall survival was remarkably 14.6 months in PD-L1 positive tumors compared to 7.7 months in PD-L1 negative tumors.

Reply 5: As the reviewer noted, the CheckMate 032 and KEYNOTE 158 studies showed contradictory results on OS according to PD-L1 expression. Unfortunately, the clinical benefits of PD-1/PD-L1 inhibitors only occurs in a

minor subset of certain cancers. Therefore, it is of importance to identify patients who may potentially benefit from PD-1/PD-L1 inhibitors. Although PD-L1 expression is not a perfect biomarker, PD-L1 expression on TC (or IC) constitutes a logical biomarker for the prediction of treatment response to the PD-1/PD-L1 inhibitors so far. Furthermore, little is known about predictive biomarkers of PD-L1 expression in SCLC. Therefore, our results may serve as the cornerstone of further research on the PD-L1 expression (especially, SP142) in SCLC in the future.

Changes in the text: No changes in the text. The above information has already been addressed in the discussion (lines 227-233, pages 10-11).

[References]

1. Chowdhury MZI, Turin TC. Variable selection strategies and its importance in clinical prediction modelling. Fam Med Community Health 2020;8:e000262.
2. Hong HG, Zheng Q, Li Y. Forward regression for Cox models with high-dimensional covariates. J Multivar Anal 2019;173:268-90.
3. Christensen E. Multivariate survival analysis using Cox's regression model. Hepatology 1987;7:1346-58.
4. Lee YS, Nam HS, Lim JH, et al. Prognostic impact of a new score using neutrophil-to-lymphocyte ratios in the serum and malignant pleural effusion in lung cancer patients. BMC Cancer 2017;17:557.
5. Hendry S, Byrne DJ, Wright GM, et al. Comparison of Four PD-L1 Immunohistochemical Assays in Lung Cancer. J Thorac Oncol 2018;13:367-76.
6. Network. NCC. NCCN clinical practice guidelines in oncology (NCCN guidelines). Small cell lung cancer (Version 3.2021). https://www.nccn.org/guidelines/guidelines-detail?category=1&id=1462. Accessed March 23, 2021.
7. Paz-Ares L, Dvorkin M, Chen Y, et al. Durvalumab plus platinum-etoposide versus platinum-etoposide in first-line treatment of extensive-stage small-cell lung cancer (CASPIAN): a randomised, controlled, open-label, phase 3 trial. Lancet 2019;394:1929-39.
8. Horn L, Mansfield AS, Szczesna A, et al. First-Line Atezolizumab plus Chemotherapy in Extensive-Stage Small-Cell Lung Cancer. N Engl J Med

2018;379:2220-9.
9. Chung HC, Lopez-Martin JA, Kao SC-H, et al. Phase 2 study of pembrolizumab in advanced small-cell lung cancer (SCLC): KEYNOTE-158. J Clin Oncol 2018;36:8506.
10. Ott PA, Elez E, Hiret S, et al. Pembrolizumab in Patients With Extensive-Stage Small-Cell Lung Cancer: Results From the Phase Ib KEYNOTE-028 Study. J Clin Oncol 2017;35:3823-9.
11. Liu SV, Reck M, Mansfield AS, et al. Updated Overall Survival and PD-L1 Subgroup Analysis of Patients With Extensive-Stage Small-Cell Lung Cancer Treated With Atezolizumab, Carboplatin, and Etoposide (IMpower133). J Clin Oncol 2021;39:619-30.