Article information: https://dx.doi.org/10.21037/tlcr-23-696

Reviewer A

Deng et al. retrospectively investigated the associations between systemic immune parameters and tumor-infiltrating lymphocyte (TIL) intensity, treatment response, and survival in a cohort of 191 Chinese patients with limited-stage, surgically resected SCLC tumors. The authors found that the prognostic nutritional index (PNI; 10 x serum albumin + 5×total lymphocytes count) showed the highest correlation with survivals. Furthermore, the authors combined the local immune score with the PNI and applied this integrated system in a cohort of extensive-stage SCLC patients treated with front-line chemo-immunotherapy. They found that the integrated scores had superior performance in predicting survival. The authors conclude that PNI is a promising biomarker for selecting SCLC patients for immunotherapy.
This study has several major issues.

Comment 1:
There is no validation cohort. The two cohorts the authors included in the study are distinct in tumor stages and treatments.
Reply 1:
Thanks for your careful review and constructive suggestion. We are sorry for not including any validation cohort, which mainly due to the scarcity of limited-stage SCLC samples. The cohort in this study was collected over 15 years and included the relatively largest sample size. Nevertheless, multi-center clinical trials are still needed for validation of the conclusions obtained in this study, and we have added this limitation at the end of the Discussion part.
Changes in the text:
We have added the discussion of the study limitations in the Discussion part, which included “no validation cohort was included in this study. The two cohorts were distinct in tumor stages and treatments, and these findings must be treated with caution and validated in future multi-center studies with larger sample size”. (see Page 17, line 364-368)

Comment 2:
Because immunotherapy is not standard-of-care for limited-stage SCLC, it is unclear why the patients with better PNI scores had a longer DFS and OS. Because the albumin level is one of the parameters in the PNI formula, it raises the question of whether the low PNI group represents those patients with cachexia. The analysis does not include other known biomarkers in SCLC prognosis, such as hyponatremia and LDH levels.
Reply 2:
Thanks for your constructive suggestions. Although there are no standardized indicators to represent cachexia status, we have evaluated other biomarkers such as hyponatremia and LDH as you suggested, to reflect the patients’ physical condition. As shown in revised Table 2, only eight and seven patients had hyponatremia and abnormally elevated LDH levels, respectively, and there was no difference in distribution between different PNI groups, which indicated no bias regarding the physical state of patients between groups. We have added these data in the Results part.
Changes in the text:
We have added these data in the Results part and revised the Table 2. (see Page 10, line 206-208).

Comment 3:
It was recently reported that clinical benefit from immunotherapy is associated with tumor capacity of antigen presentation in SCLC (PMID: 37210008). The authors should check MHC-I expression in their TMA and assess its correlations with PNI and LISS.
Reply 3:
Thanks for your constructive suggestion. Due to the insufficiency of TMA tissues, we performed RNA

sequencing on patients with available tumor samples and assessed the correlations between antigen presentation machinery related gene (HLA-A, HLA-B, HLA-C, B2M, TAP1, TAP2) expressions and PNI and LISS. No correlation was observed between PNI score and APM related gene expressions (Supplementary Figure S3A). However, high expression of APM related genes were associated with LISS-high groups (Supplementary Figure S3B). These results indicated that the association between PNI and immunotherapy benefit could not be fully explained by antigen presentation status. We have added these data in the Results part.

Changes in the text:

We have added these data in the Results part and added the new Supplementary Figure S3. (see Page 13, line 284-291).

Comment 4:

The PNI was calculated using the latest lab results within one month before the surgery or immunotherapy. This is a broad time range as the patient's nutritional status could change significantly over days. Have the authors compared the PNI scores in different weeks within one month in those patients with multiple blood drawn to assess how reproducible the results are? Using the lab results within one week before the surgery or immunotherapy would be more reasonable.

Reply 4:

Thanks for your detailed explanation and kind suggestions. We totally agreed with you that the patient's nutritional status could change significantly within one month, and we counted the specific intervals between each patient's blood test and surgery or immunotherapy. Most patients draw tests within 7 days before the surgery (164/191, 91.4%) or immunotherapy (77/91, 91.5%). And the longest interval did not exceed 15 days. Unfortunately the multiple blood tests data in different weeks was only available for few patients, and we have added this point in our Limitation section.

Changes in the text:

We have added the above limitations in the Discussion part, which included "Second, although most patients draw blood tests within 7 days before the surger or immunotherapy, the longest interval was up to 15 days and the patient's nutritional status could change significantly over days.". (see Page 18, line 382-384)

Comment 5:

Because this is a retrospective analysis, many inherent issues exist. However, no discussion of the limitations of this study could be found.

Reply 5:

Thanks for your kind reminding. We have added the discussion of several limitations of this study in the Discussion part.

Changes in the text:

We have added the limitations in the Discussion part, which included "Third, this was a retrospective, single-institution study. Thus, selection bias and time-trend bias are inevitable". (see Page 18, line 384-386)

Comment 6:

What is the cutoff of the 'H-score' used to assign a molecular subtype to a tumor? Significant intra-tumoral heterogeneity was reported in previous studies (e.g., Baine et al., JTO 2020 and Qu et al. 2022). I guess that the subgroups in Figure 2B may represent the predominant molecular subtype. In addition, SCLC-I was defined using the transcriptome data in Gay's study. Therefore, it is incorrect to call the fourth group "SCLC-I" in Figure 2B. I recommend including the 18 excluded tumors in the fourth group, which could be named 'all others'.

Reply 6:

Thanks for your questions and constructive suggestions. The molecular subtype for a tumor was assigned based on the highest H-score among subtyping markers. And the subgroups in Figure 2B did represent the predominant molecular subtype. We have added the above information in the Methods section and the Figure 2 legend, respectively.

We agreed that it was inappropriate to call the fourth group "SCLC-I", and we have revised it to "SCLC-Y" according to the YAP1 expression pattern. There were 46 excluded tumors (191-145) not available for subtype assessing with great inter-tumoral heterogeneity, which may not be suitable for merging with the

fourth group.
Changes in the text:
We have detailed the information in the Methods section and the Figure 2 legend regarding the molecular subtype assignation (see Page 7, line 137-138). And the Figure 2 was also revised.

Comment 7:
Figure 5A. Please use a table to compare demographic and clinical factors between the 'PNI-low' and 'PNO-high' groups. The color for 'Durva' on the label is wrong and could not be found in the figure.
Reply 7:
Thanks for your kind suggestions. We have added Supplementary Table S3 to compared demographic and clinical factors between groups. The color for 'Durva' on the label has been corrected.
Changes in the text:
We have added Supplementary Table S3 and corrected the color for 'Durva' in Figure 5.

Comment 8:
Minor issues:
1. Figure 2B and 2C. Please perform the statistical analyses between different subtypes.
2. Figure 2A. Please mark the boundary of the cancer. It appears that immune cells are mainly in the stroma tissues in the "PNI-high" tumor.
3. Figure 4A. What are the associations between these immune groups and tumor staging?
4. Figure 4B. What is the unit of the X-axis?
Reply 8:
Thanks for your questions and kind suggestions. We have performed the statistical analyses between different subtypes in Figure 2 and presented the significant P values in the figures.
We speculate that you are referring to the tumor boundary in Figure 3A and we marked the boundary in the H&E images. Immune cells only infiltrated the stroma in most of SCLCs.
We have added the correlation analyses between immune groups and tumor staging in Supplementary Figure S2A. High number of CD8+ T cells and high proportion of CTLA4+ cells were found in patients with pathological stage I tumors.
The unit of the X-axisin Figure 4B is the "importance score" calculated by the XGBoost algorithm.
Changes in the text:
We have added the above information in the corresponding sections and figures.

Reviewer B

I have thoroughly reviewed your manuscript entitled "Systemic immune index predicts tumor-infiltrating lymphocyte intensity and immunotherapy response in small cell lung cancer". The study aims to explore the prognostic value of the systemic immune index in the context of small cell lung cancer (SCLC) and its potential to predict responses to immunotherapy.

Comment 1:
The manuscript presents a comparative study of two distinct cohorts with different OS, which is challenging to interpret. It is imperative for the clarity and accuracy of the results that all figures explicitly indicate which cohort they pertain to.
Reply 1:
Thanks for your constructive suggestion. The second immunotherapy SCLC cohort (N=91) was only used in the last Results section of immunotherapy benefit analyses. We have added the corresponding cohort information in each figure legend for better understanding.
Changes in the text:
We have revised each figure legend with corresponding cohort information.

Comment 2:
Throughout the manuscript, there are instances where abbreviations are used without prior definition. This can lead to confusion and misinterpretation of the data presented. I strongly recommend providing a list of abbreviations with their full terms at the first instance of their use.

Reply 2:
Thanks for your kind suggestion and we have reviewed the entire manuscript and supplemented the full terms of each abbreviation at the first instance of their use.
Changes in the text:
We have supplemented the full terms of each abbreviation at the first instance of their use.

Comment 3:
In Figure 1, consideration should be given to performing a multivariate analysis including PNI as a variable. Such an analysis could potentially offer deeper insights into the independent prognostic value of PNI.
Reply 3:
Thanks for your kind suggestion and the multivariate analysis including PNI has been included in the Table 1, and PNI remained as an independent prognostic factor after adjusted for other variables.
Changes in the text:
No changes.

Comment 4:
The manuscript does not consistently report the number of at-risk patients in Kaplan-Meier curves. This information is critical for interpreting survival analysis since the number of patients at risk can significantly impact the survival probabilities at various time points.
Reply 4:
Thanks for your important suggestion and we have added the number of at-risk patients in KM curves for better interpreting.
Changes in the text:
The tables the number of at-risk patients have been added in the Figure 1, Figure 3 and Figure 5.

Comment 5:
Regarding the LD cohort, postoperative adjuvant chemotherapy and chemotherapy after recurrence have a significant impact on OS. The manuscript would benefit from an analysis that includes these treatments and a discussion of their results.
Reply 5:
Thanks for your constructive suggestions. We totally agree that chemotherapy treatment could significantly impact the survival of SCLC patients, therefore we performed stratification analysis in patients with or without adjuvant chemotherapy, as well as multivariate analysis including adjuvant chemotherapy as a variable to adjust the survival bias. It turned out that the PNI score still remained as an independent prognostic factor. We have added a discussion of postoperative adjuvant chemotherapy in the discussion section. Considering that many patients did not receive follow-up and treatment at our hospital after surgery or recurrence, the data of chemotherapy after recurrence was unfortunately not available for further analyses.
Changes in the text:
We have discussed the impact of postoperative ACT in the Discussion section, which included “Moreover, considering that postoperative adjuvant chemotherapy could significantly impact the suvival of limited-resected SCLC patients, the result that PNI remained as an independent prognostic factor regardless of ACT significantly increased its extensiveness of clinical applicability.” (see Page 16, line 347-351)

Reviewer C

This interesting original article may support predicting the efficacy of immunochemotherapy as first-line therapy in patients with SCLC. The authors showed a method to investigate the prognostic value of overall immune status by combining the PNI with local immune biomarkers in SCLC. The manuscript is well-written, and the results are clearly presented. However, I have a few major and minor comments, explained below.

Major Point
Comment 1:
・Was there Combined SCLC included in this study? If Combined SCLC was included, was there a

difference in SCLC subtype and immune microenvironment between SCLC and Combined SCLC?

Reply 1:

Thanks for your great question. No combined SCLC was included in this study but it is quite an interesting point which is warranted to explore.

Comment 2:

・The study states that the SCLC subtype was classified with an H-score; please indicate the Cut-off value of the H-score and the paper to which you refer. Please describe in detail the method of subtype classification of SCLC.

Reply 2:

Thanks for your constructive suggestions. The molecular subtype for a tumor was assigned based on the highest H-score among subtyping markers (PMID: 34534680). And the subgroups in Figure 2 represent the predominant molecular subtype. We have added the above information in the Methods section and the Figure 2 legend, respectively.

Changes in the text:

We have detailed the information in the Methods section and the Figure 2 legend regarding the molecular subtype assignation (see Page 7, line 137-138). And the Figure 2 was also revised.

Minor point

Comment 3:

・Please provide how many of the 191 cases in LS-SCLC and 91 in ES-SCLC were evaluated with TMA.

・In Figure 5, there is no color box for PNI; please add one.

・The color box in Figure 5, Durva color is wrong.

Reply 3:

Thanks for your kind suggestions. 129 cases in LS-SCLC cohort were evaluated with TMA and no cases in ES-SCLC cohort were evaluated with TMA. We have added this information in the Methods section.

We have added the color box for PNI as well as corrected the color for Durva group in Figure 5.

Changes in the text:

We have added the TMA information in the Methods section (see Page 6, line 116-118). The Figure 5 was corrected.