Peer Review File

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Reviewer A

Your manuscript promises with the title of molecular subtyping and associated assessment of the prognosis of LCNEC. Unfortunately, I can't find both at work. Unfortunately, no molecular testing was performed. They derive "molecular subtypes" of protein expression from immunohistochemical stains. Nor can a forecast assessment be made. This is done only via the peripheral blood LMR. Forecast factors for the DFS and OS were described here. However, this is independent of molecular subtyping. I believe that at least the title and the "literary focus" of the study should be revised. Better still would be the molecular sequencing you yourself announced. This would not only greatly enhance the work, it would also comply with the title promise. The approach is in principle very interesting and should be pursued accordingly. In my opinion, the number of cases in their centre would be more than sufficient.

Response: Thank you for your valuable suggestions.

1) The molecular subtypes of small cell lung cancer (SCLC) were defined by the relative expression of ASCL1, NEUROD1, POU2F3, and YAP1 transcription factors[1]. The expression of four transcription factors could be detected by RNA sequencing or immunohistochemistry method, since previous studies validated the molecular subtypes by RNA sequencing or immunohistochemistry in SCLC[1-3]. In our study, molecular subtyping was performed by immunohistochemistry method with reference to published study[1]. Thus, our study really explored the molecular subtypes of lung LCNEC.

2) The initial purpose of molecular subtyping was to identify patients with different prognosis for personalized treatment. However, we observed that there was no prognosis difference among different molecular subtypes, which was consistent with previous SCLC studies[4,5]. Therefore, we next explored the prognostic value of systematic inflammatory indicators,

aiming to identify patients with distinct prognosis and guide clinical treatment selection. We found that peripheral blood LMR was a prognostic factor for OS and DFS in patients with lung LCNEC. In our opinion, the title of our article is in line with our research content and exploration direction.

References:

1. Rudin CM, Poirier JT, Byers LA, et al. Molecular subtypes of small cell lung cancer: a synthesis of human and mouse model data. Nat Rev Cancer 2019;19: 289-97.

2. Baine MK, Hsieh MS, Lai WV, et al. SCLC Subtypes Defined by ASCL1, NEUROD1, POU2F3, and YAP1: A Comprehensive Immunohistochemical and Histopathologic Characterization. J Thorac Oncol 2020;15:1823-35.

3. Qu S, Fetsch P, Thomas A, et al. Molecular Subtypes of Primary SCLC Tumors and Their Associations With Neuroendocrine and Therapeutic Markers. J Thorac Oncol 2022;17:141-53.

4. Gay CM, Stewart CA, Park EM, et al. Patterns of transcription factor programs and immune pathway activation define four major subtypes of SCLC with distinct therapeutic vulnerabilities. Cancer Cell 2021;39:346-60.e7.

5. Ding XL, Su YG, Yu L, et al. Clinical characteristics and patient outcomes of molecular subtypes of small cell lung cancer (SCLC). World J Surg Oncol 2022;20:54.

Reviewer B

The authors retrospectively evaluated the molecular subtypes of LCNEC by immunohistochemistry. They also calculated blood LMR and evaluated the prognostic impact on LCNEC. They attempted to show the importance of the co-expression of major transcription factors, and the correlation between molecular subtypes and LMR. The following points need to be corrected.

1. This manuscript lacks novelty; a comprehensive analysis of transcription factors in LCNEC has already been reported. The sample size is also small compared to previous reports. It was already known that ASCL1 and NEUROD1 were both highly expressed in many LCNEC cases.

Response: Thank you for the comment.

1) Previous, there are two studies have systematically analyzed the four transcription factors

in lung LCNEC, one using RNA sequencing[1] and the other immunohistochemistry[2]. High- and co-expression of ASCL1 and NEUROD1 has been reported in above two studies, and our study confirmed this result. However, none of the studies have reported prognostic effect of different molecular subtypes in lung LCNEC, our results provide an answer to this question. We have changed the introduction part and used "relatively few studies have systematically analyzed the four transcription factors, the prognostic differences among different molecular subtypes were unclear" according the reviewer's comment. (See Page 5, line 57-59). And above two papers are also cited in the discussion section on the co-expression of different molecular subtypes (See Page 14, line 248).

2) In our study, we performed molecular subtyping in 64 patients, the sample size is slightly larger than previous two studies (48[1] and 49[2] patients). Due to the low incidence rate of LCNEC, studies with large sample size were very difficult. Although with limited sample size, there are still some novel findings in our study. We have added the discussion of sample size in the limitation part (See Page 16, line 295-297).

References:

1. Metovic J, La Salvia A, Rapa I, et al. Molecular Subtypes of Extra-pulmonary Neuroendocrine Carcinomas Identified by the Expression of Neuroendocrine Lineage-Specific Transcription Factors. Endocr Pathol 2022;33:388-99.

2. Popper H, Brcic L, Eidenhammer S. Does subtyping of high-grade pulmonary neuroendocrine carcinomas have an impact on therapy selection? Transl Lung Cancer Res 2023;12:2412-26.

2: Why did the authors focus on LMR rather than other inflammation markers such as LNR?

Response: Thank you for the question. NLR is widely used and represents the neutrophil to lymphocyte ratio. We explored the clinicopathological features and prognostic value of several systemic inflammation indicators, including peripheral blood lymphocyte-to-monocyte ratio (LMR), neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR) and systemic immune-inflammation index (SII). We found that LMR but not NLR, PLR or SII was prognostic indicator for DFS and OS in lung LCNEC. We have added this analysis in the introduction (See Page 5-6, line 62-70), methods (See Page 7, line 93-100) parts and in Table 1,2, Supplementary Table 4,6,7, and Supplementary Figure 2 of results part (See Page 11-13,

line 191-228).

SII was reported to be a new prognostic systemic inflammation indicator, patients with high SII score had a worse prognosis in NSCLC[1-2]. Previous two studies indicated that baseline high NLR or PLR was associated with poor prognosis in lung LCNEC patients[3-4], which is differ from our results. The differences may be related to different baseline characteristics of study population, small sample size and retrospective study design of these studies. Findings of the prognostic value of these inflammation indicators were needed to be interpreted with caution and validated in larger prospective cohorts. We added the discussion in Page 15, line 265-279.

References:

1. Fan R, Chen Y, Xu G, et al. Combined systemic immune-inflammatory index and prognostic nutritional index predict outcomes in advanced non-small cell lung cancer patients receiving platinum-doublet chemotherapy. Front Oncol 2023;13:996312.

2. Chen WH, Shao JJ, Yang Y, et al. Prognostic significance of systemic immune inflammatory index in NSCLC: a meta-analysis. Lung Cancer Manag 2024;13:LMT67.

3. Shirasawa M, Yoshida T, Horinouchi H, et al. Prognostic impact of peripheral blood neutrophil to lymphocyte ratio in advanced-stage pulmonary large cell neuroendocrine carcinoma and its association with the immune-related tumour microenvironment. Br J Cancer 2021;124:925-32.

4. Shi M, Zhao W, Zhou F, et al. Neutrophil or platelet-to-lymphocyte ratios in blood are associated with poor prognosis of pulmonary large cell neuroendocrine carcinoma. Transl Lung Cancer Res 2020;9:45-54.

3: The authors showed that CD68 correlated with LMR, however CD68 is generally known as a TAM marker. This result needs to be explained.

Response: Thank you for your suggestion. We found that high LMR was associated with a favorable prognosis in lung LCNEC patients, which was correlated with higher intra-tumoral CD3+, CD8+, and CD68+ immune cell infiltration. Previous studies showed that high CD3+ TIL, CD8+ TIL, and CD68+ TIM were associated with good prognosis in cancers[1-5], and high immune cell infiltration with anti-tumor effect in tumor tissue has been regarded as a hot tumor microenvironment[6,7]. LMR is the ratio of peripheral blood lymphocyte and monocyte. CD68 is a common marker of pan-macrophages in tumor tissue[8]. We can, therefore, speculate that the good prognosis of high LMR may be closely related to the hot tumor microenvironment as reflected by high CD3+, CD8+ TIL and CD68+ macrophages infiltration. Our study cannot

directly explain the relationship between LMR and CD68, which needs to be explored in further studies. We explained this results in our discussion part (See Page 15-16, line 280-294).

References:

1. Viveiros N, Flores BC, Lobo J, et al. Detailed bladder cancer immunoprofiling reveals new clues for immunotherapeutic strategies. Clin Transl Immunology 2022;11:e1402.

2. Yang F, Zeng Z, Li Y, et al. The prognostic value of a 4-factor neoimmunologic score system in non-small cell lung cancer. J Leukoc Biol 2022;112:1605-19.

3. Noma T, Makino T, Ohshima K, et al. Immunoscore Signatures in Surgical Specimens and Tumor-Infiltrating Lymphocytes in Pretreatment Biopsy Predict Treatment Efficacy and Survival in Esophageal Cancer. Ann Surg 2023;277:e528-e37.

4. Yi B, Cheng Y, Chang R, et al. Prognostic significance of tumor-associated macrophages polarization markers in lung cancer: a pooled analysis of 5105 patients. Biosci Rep 2023;43.

5. Wattenberg MM, Colby S, Garrido-Laguna I, et al. Intratumoral Cell Neighborhoods Coordinate Outcomes in Pancreatic Ductal Adenocarcinoma. Gastroenterology 2024;166:1114-29.

6. Zhang J, Huang D, Saw PE, et al. Turning cold tumors hot: from molecular mechanisms to clinical applications. Trends Immunol 2022;43:523-45.

7. Galon J, Bruni D. Approaches to treat immune hot, altered and cold tumours with combination immunotherapies. Nat Rev Drug Discov 2019;18:197-218.

8. Mellman I, Chen DS, Powles T, et al. The cancer-immunity cycle: Indication, genotype, and immunotype. Immunity 2023;56:2188-205.

4: The percentage of mixed LCNEC is incorrect on page 9.

Response: We were really sorry for our careless mistakes. The percentage of mixed LCNEC has been corrected on 34.0% (See Page 9, line 151).

5: Highlight box and STROBE Statement do not fit within the frame.

Response: Thank you for your suggestion. Due to discrepancies in the line numbers of the manuscript used for review and the original manuscript submitted, STROBE Statement did not fit within the frame. We updated the STROBE Statement on the basis of the revised article and attached it. We revised the Highlight box to better fix within the frame (See Page 4, line 35).