Peer Review File
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Reviewer A

Comment 1: Introduction lines 67-76 several evidences suggested that biologically LCNEC resembles Adenocarcinoma. Please try to make comparison not only with small cell carcinoma.

Reply 1: Thank you for your comment to include a comparison with adenocarcinoma.
We agree that this comparison would provide a more comprehensive understanding of LCNEC. We have revised the [Introduction] accordingly. "L-LCNEC is known to be more aggressive than non-small cell lung cancer (NSCLC) such as adenocarcinoma, and demonstrates a lower response rate to standard chemotherapy regimens used for small-cell lung cancer (SCLC)." (on Page 5, Line 70-75)
Change in the text: Page 5, Line 70-75.

Comment 2: Introduction lines 77-83: Please try to make comparison not only with small cell carcinoma but also with adenocarcinoma

Reply 2: Thank you again for your insightful comment. We have revised the [Introduction] accordingly.
Change in the text: Added comparisons introduction on Page 5, Line 70-75.

Comment 3: Methods lines 90-98 pathological diagnosis were revised only on medical files or also by new samples examination? In the period covered by our study two different WHO classification were available and in general LCNEC diagnosis rarely is well indicate. In conclusion a centralized pathological revision should be performed.

Reply 3: We appreciate your comment regarding the pathological diagnosis.
We revised the [Methods] section "In our study, the pathological diagnosis was based on the available medical files, and all patients included had clear and definitive diagnoses. We excluded cases with diagnostic controversies or uncertainties. For non-surgical cases, additional sampling was conducted after an initial biopsy to ensure accurate pathological assessment. The final diagnosis was established by two experienced pathologists." (on Page 7, Line 100-105)
We fully acknowledge the importance of centralized pathological revision, but it falls beyond the scope of our current study. However, we appreciate your suggestion and added a discussion of these limitations in the [Discussion] section, highlighting the need for these in future studies. (on Page 16, Line 297-299)
Change in the text: Page 7, Line 101-105. Page 16, Line 297-299.

Comment 4: Methods lines 101-105: what happened when the two medical professionals disagreed?

Reply 4: Thank you for your comment regarding the situation when two medical professionals disagreed during the data collection process.
We revised the [Methods] section “Disagreements among professionals were collaboratively resolved through information re-reviews and dialogues.” (on Page 8, Line 125-126)
Change in the text: Page 8, Line 125-126.

Comment 5: Results lines 142-145: did you collect also 7 combined-LCNEC? This percentage of combined LCNEC is expected according to literature? How the non neuroendocrine component were assed, only on morphology? Or with Himmunohistochemical evaluation?

Reply 5: We appreciate your comment regarding the presence of combined LCNEC.
We did collect7 cases of combined LCNEC. “The percentage of combined LCNEC (7/31, 22.6%) in our cohort aligns with the range (10-25%) reported in the literature.” (on Page 5, Line 61-62, on Page 14, Line 255-256)
For the assessment of the non-neuroendocrine components in combined LCNEC, both morphological evaluation and immunohistochemical techniques were conventional performed (on Page 7, Line 105).
Change in the text: Page 5, Line 61-62, on Page 14, Line 255-256. Page 7, Line105.

Comment 6: Results lines 142-144: median Ki67 is not useful

Reply 6: Thank you for your comment regarding the usefulness of median Ki-67.
We have removed this section in the Table 1 and text (on Page 10, Line 166).
Change in the text: Table 1. Page 10, Line 166

Comment 7: Results lines 150-153: Which method was used to test PDL-1? TPS? CPS?

Reply 7: We appreciate your comment regarding the method used to test PDL-1 expression.
We have revised the manuscript to provide clarity on the [Methods]. “The 22C3 pharmDx companion diagnostic assay (Agilent Technologies, Santa Clara, CA, USA) was utilized for IHC analysis of PD-L1 in formalin-fixed paraffin-embedded tissues. A tumor proportion score (TPS) of ≥ 1% was considered positive.” (on Page 7, Line 108-111)
Change in the text: Page 7, Line 108-111

Comment 8: Discussion lines 202-203: why in your opinion ICI treatment worked better in c-LCNEC?

Reply 8: Thank you for your comment regarding the differential response in different subtypes.
We have added relevant [Discussion]. “We propose the potential for greater benefit of ICIs to c-LCNEC, which may be related to the high tumor mutational burden(33) and the distinct biological characteristics of combined components, but more clinical evidence is still needed.” (on Page 15, Line 276-282)
Change in the text: Page 15, Line 276-282

Reviewer B

In this case report, Shi, Song and their colleagues analyzed efficacy and safety of immune checkpoint inhibitors (ICIs) in lung large-cell neuroendocrine carcinoma (L-LCNEC). They retrospectively reviewed 34 patients of L-LCNEC treated with ICIs and reported that the objective response rate (ORR) was 29.4% and the disease control rate (DCR) was 82.4%. They also presented that the median progression-free survival (PFS) was 6.3 months and the median overall survival (OS) was 14.77 months. As an additional analysis, they compared the anti-tumor responses and survivals according to pathology, that is combined LCNEC (c-LCNEC) and pure LCNEC (p-LCNEC), and showed that the results were favorable for c-LCNEC. The observed incidence of immune-related adverse events (irAEs) was also reported and its grade 1/2 was 58.8% and grade 3 was 5.9%. Therefore, they concluded that ICI treatment showed efficacy and safety in advanced L-LCNEC especially in combined LCNEC. L-LCNEC is a rare histological type of lung cancer and the manuscript is worth publishing, however the reviewer is concerned that the authors should describe rationale for the number of patients or why they paid attention to combined LCNEC. In addition, some ICI agents described in the manuscript are unfamiliar with the reviewer. The reviewer wrote down the comments as below and hopes that they will address them correctly.

Reply: We sincerely appreciate the reviewer's comprehensive summary and feedback on our manuscript. We have taken their suggestions into careful consideration and have made the necessary revisions to enhance the clarity and scientific rigor of our study. We firmly believe that these improvements have significantly increased the manuscript's value for the readership.

Below are the comments:
Major comments:
Comment 1: The authors insisted importance of pathological types of c-LCNEC and p-LCNEC, however they did not describe the background of difference between both types at all. They did not clarify the reason why they paid attention to the pathological type, either. Please describe them.

Reply 1: We appreciate the reviewer's comment and recognize the importance of providing a clear background for the difference.
We have added the [Introduction] (on Page 5, Line 61-66) of c-LCNEC and reasons (on Page 5, Line 89-90) for focusing on this subtype.
Change in the text: Page 5, Line 61-66. Page 5, Line 89-90

Comment 2: The reviewer agrees with the description that L-LCNEC is a rare, but an aggressive pathological type of lung cancer. Almost all past retrospective analyses which examined the efficacy of ICIs for L-LCNEC included less than 100 patients with L-LCNEC. Nevertheless, the reviewer considers it necessary to clarify the basis for setting the number of cases because seven patients of c-LCNEC don’t seem enough number to discuss the efficacy of ICIs or any treatments. The reviewer recommends multivariable analysis with pathological type as one of variables. The authors should discuss them or try other approaches.

Reply 2: We appreciate the reviewer's concern regarding the sample size.
We acknowledge that the rarity of c-LCNEC imposes limitations on our study, resulting in a small number of cases (seven patients with c-LCNEC). However, we believe that despite this limitation, the inclusion of these cases still provides valuable insights into the efficacy of ICIs in c-LCNEC. In our revised manuscript, we explicitly acknowledge the limited sample size (on Page 15, Line 291-292) and have accordingly modified our [Conclusions] ("with the potential for greater benefits in the combined LCNEC subtype" rather than stating superior efficacy in c-LCNEC). (on Page 3, Line 47. Page 4 Highlight Box. Page 15, Line 279. Page 16, Line 305-306)
We have included the results of the multivariate analysis in Supplementary Figure 1 and in the [Discussion] section. "In COX regression multivariate analysis, pathological type (HR = 0.281, 95% CI = 0.091-0.765, P = 0.027) remained a significant factor affecting PFS." (on Page 15, Line 274-276)
Change in the text: Page 3, Line 47. Page 4 Highlight Box. Page 15, Line 279. Page 16, Line 305-306. Supplementary Figure 1. Page 15, Line 274-276

Comment 3: The reviewer acknowledges that past several reports (Wang 2022 Ann Surg Oncol, Zhang 2020 Lung Cancer or Handa 2022 Clin Lung Cancer) revealed that prognosis of c-LCNEC was equivalent to or worse than that of p-LCNEC, which gives the current manuscript novelty, however it seems quite hasty to propose efficacy of ICIs for c-LCNEC just because its PFS was better than those of p-LCNEC. Actually, ORR and DCR of both pathological types were not statistically different. Please consider and discuss them.

Reply 3: We appreciate the reviewer's acknowledgment of the novelty provided by our study.
We have revisited the [Discussion] on several important aspects, including the median PFS, median OS, multivariate analysis, etc. (on Page 14-15, Line 270-282) We have carefully examined the data and provided a more comprehensive discussion in our revised manuscript.
Change in the text: Page 14-15, Line 270-282

Comment 4: The authors should analyzed the patient who underwent radical resection differently from those with unresectable tumors because their amounts of specimen used for pathological diagnosis were by far different. Some unresectable tumors which had been diagnosed as p-LCNEC might have been c-LCNEC. That should be described in limitation.

Reply 4: We appreciate the reviewers' valuable comments regarding the pathological diagnostic approach.
We agree that the difference in specimen amounts used for pathological diagnosis could potentially affect the accuracy of diagnosing c-LCNEC. In our revised manuscript, we have acknowledged this limitation in [Discussion]: "the absence of a centralized pathological revision that could have resulted in the inclusion of certain c-LCNEC cases within the p-LCNEC group." (on Page 16, line 297-299)
We have also restated the pathological criteria in the [Methods] section. "Cases with diagnostic uncertainties or controversies were excluded from the analysis. In non-surgical cases, additional

tissue sampling was often performed after the initial biopsy to ensure accurate pathological assessment. The final diagnosis was established by two experienced pathologists." (on Page 7, Line 101-105)
Change in the text: Page 16, line 297-299. Page 7, Line 101-105

Comment 5: If the authors focus on pathological type, please describe details of c-LCNEC, for example what pathological types (adenocarcinoma or squamous cell carcinoma?) were combined with each c-LCNEC tumor.

Reply 5: We appreciate the reviewer's suggestion to provide more details regarding the combined pathological types.
We have included the description in Supplementary Table 1 and discussed it in the [Discussion] section. Most patients combined LCNEC with AC (57.1%) or SCC (42.9%). (on Page 14, Line256-257) "Additionally, in three cases mixed with AC, with an evaluable composition ratio, AC accounted for 80%, 30%, and 15%. The corresponding PFS times were 9.47, 6.0, and 3.73 months." (on Page 15, Line 276-278)
Change in the text: Page 14, Line256-257. Page 15, Line 276-278

Comment 6: The authors described that they followed the NCCN guideline for treatment, however some ICI agents do not seem to belong to FDA approval. Please show the evidence that Tislelizumab, Sintilimab, Camrelizumab, Navulizumab or Toripalimab is recommended by NCCN guideline.

Reply 6: We apologize for the confusion caused by our previous statement.
Upon further review, we have decided to remove the mention of following the NCCN guideline for treatment as it may not be applicable in the context of the specific ICI agents mentioned.
Change in the text: Page 7-8, Line 119-120

Comment 7: Please discuss the reason why OS of 'Line1' was statistically worse than that of 'Line2 and more' although ORR of 'Line1' was statistically better than that of 'Line2 and more.'

Reply 7: Thank you for your comment regarding the difference between treatment lines.
We have revised the manuscript to provide [Discussion] on this section. We observed a shorter OS in the first-line compared to the latter, despite achieving a higher ORR in the first-line. We speculate that these results may be attributed to the inclusion of relatively small sample size and the potential biases introduced by baseline characteristics, such as adjuvant chemotherapy after surgery, PD-L1 expression, and subsequent treatment regimens, which could influence the OS outcome. (on Page 13, Line 243-240)
Change in the text: Page 13, Line 243-240

Minor comments:
Comment 1: Page 4, line 108; 'overall response rate' should be 'objective response rate.'

Reply 1: Thank you for pointing out the error in our manuscript.

We have made the correction “objective response rate” in the manuscript.
Change in the text: Page 8, Line 127

Comment 2: Figure 2; Scales of X axes of PFS and OS should be same, otherwise they might mislead readers.

Reply 2: We appreciate your attention to detail.
To address this issue, we have revised Figures and uploaded again.
Change in the text: Figure 2A-D

Reviewer C

This is a valuable study investigating the efficacy and safety of ICI in rare L-LCNEC, specifically c-LCNEC and p-LCNEC. It is interesting and provides additional insight into the treatment of LCNEC. Some points are presented below for your consideration for modification.
Reply: Thank you once again for your insightful comments, which have helped us improve the quality of our study. We appreciate your positive assessment of the study's value and its contribution to the understanding of LCNEC treatment. We have carefully reviewed the points you have raised and have made the necessary modifications.

Comment 1:
●Fewer resection specimens
Table 1. Diagnosis method：Surgery 15/34(44.1%)

cf.
[many pathologists feel they are unable to make a definitive LCNEC diagnosis without examination of a resection specimen. Indeed, existing WHO LCNEC classification cautions against definitive diagnosis unless a very substantial excision biopsy or resection is available.] (Br J Cancer. 2021 Oct 26; 125(9): 1211.)

I think this report is valuable because it is a rare cancer, and the number of cases is inevitably small and research is not easy. However, due to the small number of resection specimens, I have doubts about the authenticity of the LCNEC histology. The diagnostic criteria for pathology in [study design] should be described in more detail. For example, authors should indicate how many pathologists diagnosed the cases, or whether only a sufficient volume of specimens, if not a resection specimen, was evaluated.
Reply 1: We appreciate the reviewers' valuable comments regarding the pathological diagnostic approach.
We agree that the difference in specimen amounts used for pathological diagnosis could potentially affect the accuracy of diagnosing c-LCNEC. In our revised manuscript, we have acknowledged this limitation in [Discussion]: the absence of a centralized pathological revision that could have resulted in the inclusion of certain c-LCNEC cases within the p-LCNEC group. (on Page 16, line 297-299)

We have also restated the pathological criteria in the [Methods] section. Cases with diagnostic uncertainties or controversies were excluded from the analysis. In non-surgical cases, additional tissue sampling was often performed after the initial biopsy to ensure accurate pathological assessment. The final diagnosis was established by two experienced pathologists. (on Page 7, Line 101-105)
Change in the text: Page 16, line 297-299. Page 7, Line 101-105

Comment 2:
●Need detailed description of c-LCNEC
Table 1. Combined LCNEC N = 7/34(20.6%)

Can the author state that c-LCNEC was diagnosed from the resection specimen?
Also, what is the histologic component of each of the c-LCNECs? If there are more adenocarcinomas in the component, the prognosis may inevitably be better. The author should describe this in as much detail as possible.
Furthermore, since the author mentions the prognosis of c-LCNEC, the previously reported prognosis of c-LCNEC should also be mentioned in [Discussion].

Reply 2: We appreciate the reviewer's valuable comment regarding the detailed description of c-LCNEC.
To address this concern, we have re-evaluated our data and now provide a more detailed description of the histologic components of the c-LCNEC cases in Supplementary Table 1. In most cases (85.7%), the diagnosis of c-LCNEC was made through surgical specimens. Among the c-LCNEC cases, 57.1% were combined with AC, and 42.9% with SCC (on Page 14, Lines 256-257).
Furthermore, in three cases where the composition ratio of the combined components was evaluable, AC accounted for 80%, 30%, and 15%. The corresponding PFS times were 9.47, 6.0, and 3.73 months. (on Page 15, Lines 276-282).
In the revised [Discussion] section, we have included a discussion on the previously reported prognosis of c-LCNEC. (on Page 14, Lines 258-262).
Change in the text: Page 14, Lines 256-257. Page 14, Lines 276-282. Page 14, Lines 258-262

Comment 3:
●Many cases are not evaluated for PD-L1.
Any PD-L1 expression bias in c-LCNEC and p-LCNEC cases may have prognostic implications.

Reply 3: We appreciate the reviewer's comment regarding the evaluation of PD-L1 expression. We acknowledge the limitation of our study regarding the evaluation of PD-L1 expression in c-LCNEC and p-LCNEC cases. As a retrospective study, the availability of PD-L1 expression data was limited. PD-L1 expression assessment was not routinely performed for LCNEC cases during the treatment. In our revised manuscript, we have explicitly acknowledged this limitation in [Discussion]. (on Page 16, Line 299-301) We emphasize the need for future studies with larger sample sizes and standardized PD-L1 evaluation to further investigate the role of

PD-L1 expression in LCNEC.