

Peer Review File

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Reviewer A

Comment 1: The patients' selection of each analysis should be reconsidered. Please show the characteristics of all the subgroups. If there is imbalance of important prognostic factors and/or predictive factors, such factors should be adjusted using analysis such as propensity score matching. There are many subgroups in this patient cohort (surgically resected ALK-rearranged NSCLC), such as cN0 and pN0, cN0 but pN1 or 2, cN1 or 2 but pN0, cN1 and pN1, etc. For comparison, please clearly choose appropriate control.

Reply1: Thank you for your valuable feedback regarding patient selection in our analysis. We have carefully reconsidered and redefined the analysis subgroups. Specifically, in the surgically resected ALK-rearranged NSCLC cohort, we have categorized OLNLM to represent cN0 but pN1 or 2, and NOLNM to represent cN0 and pN0 subgroups. We have added a new table 3 displaying the baseline characteristics of these two groups. Additionally, we have modified table 4 analyzing the predictive factors influencing OLNLM occurrence. Our goal is to compare baseline features between the OLNLM and NOLNM groups, without further analyzing the impact of the two groups on prognosis, so no propensity matching analysis was conducted.

Changes in the text: We have modified our text as advised in Section '3.2 Occult lymph node metastasis analysis', paragraph 1, sentence 1-5 (see Page 9-10, line 148-155; Table 3-4).

Comment 2: For comparison of post-operative treatment with chemotherapy vs ALK-TKI, please show the characteristics of each group (Fig.2 C, D, etc.). Are there any imbalances of background characteristics? If there are, please discuss true meaning of adjuvant treatment and other imbalanced factors.

Reply2: We have provided detailed characteristics in each group in Table 6. Upon examining the data, we did observe some imbalances in the background characteristics between the chemotherapy and ALK-TKI groups. Specifically, there are significant differences in age, radiotherapy, and the presence of STAS between the two treatment arms. Notably, patients receiving ALK-TKI tend to be older, which is consistent with clinical observations where elderly individuals may be less tolerant to chemotherapy. Additionally, radiotherapy is predominantly administered to patients undergoing chemotherapy.

Changes in the text: We added some data in Section '3.3 Recurrence-free survival and overall survival analysis', paragraph 3, sentence 1-4 (see Page 10, line 168-173; Table 6).

Comment 3: How the 'resected LN station' was determined before and during operation? Are there any systematic decision-tree how to handle LN station? The number of resected LN station may be up to the surgeons. This factor may not have biological (scientific) meaning. Please re-analyze without this factor. And/or please discuss the scientific meaning of 'number of resected LN station.'

Reply3: The 'resected LN station' is usually dependent on the tumor stage and surgeon. Our systematic decision tree on how to handle LN stations is based on the Chinese Medical

Association Clinical Guidelines for Lung Cancer (2018 edition), including: ① Lymph node dissection and sampling are deemed essential components of surgical procedures. Standard practice involves the removal or systematic sampling of three groups of mediastinal lymph nodes (Left: 4L, 5, 6, 7, 8, 9; Right: 2R, 4R, 7, 8, 9). The recommended minimum number of lymph nodes to be dissected or sampled is at least 12, including both mediastinal and intrapulmonary nodes. ② For Stage I to III lung cancer without preoperative evidence of mediastinal lymph node involvement (negative PET or EBUS, negative mediastinoscopy), extensive lymph node dissection does not show a significant upstaging or postoperative survival advantage. However, in cases where preoperative staging relies solely on imaging (without PET or EBUS, mediastinoscopy staging), lymph node dissection is still recommended (Class I Recommendation Evidence). ③ Preoperative imaging indicating predominantly ground-glass opacity (GGO) with a component >50%, and intraoperative confirmation of invasive adenocarcinoma with predominant adherent growth, suggests a very low probability of mediastinal lymph node metastasis. In such cases, selective sampling of 1-3 groups (Left: 5, 7, 9; Right: 2 or 4, 7, 9) of mediastinal lymph nodes is considered appropriate. We completely agree with your statement that this factor may not have biological (scientific) meaning. Therefore, we reanalyzed the relevant results without this factor. Changes in the text: We have modified our text as advised in Section ‘3.2 Occult lymph node metastasis analysis’, paragraph 1, sentence 4-5 (see Page 10, line 152-155; Table 4, 5, 7).

Comment 4: For accessing LN metastasis, FDG-PET is useful. Are there any data regarding PET-positive LN vs negative LN in this cohort?

Reply4: In our study, the majority of patients likely underwent ECT, abdominal ultrasound, cranial magnetic resonance imaging, and chest CT scans to evaluate the tumor metastatic status, instead of PET-CT examinations. Only 180 patients (29.9%) underwent PET-CT scans. We defined cN0 by integrating the results of PET and CT scans. Additionally, relevant PET-CT information was included in Table 1, 3. We added this point in the limitation section.

Changes in the text: We added some data in Section ‘2.1 Patient selection’, paragraph 2, sentence 4 (see Page 7, line 100-103; Table 1, 3) and Section ‘4. Discussion’, paragraph 6, sentence 6 (see Page 13, line 236-237).

Comment 5: Results: ‘No patients discontinued medication’ > Is this true in real world situation with n>200 patients with adjuvant treatment? What is the definition of ‘discontinue’ (how many courses with chemotherapy and how many days with ALK-TKIs)?

Reply5: In our study, the term "discontinued medication" refers to the condition when patients ceased the prescribed treatment due to severe adverse reactions during the follow-up period. It does not include instances where discontinuation resulted from non-compliance due to patient-related issues. The sentence has been modified as follows ‘None of the patients discontinued medication due to severe drug-related side effects.’

Changes in the text: We have modified our text as advised in Section ‘3.1 Characteristics of pathologically nodal negative and positive patients’, paragraph 3, sentence 4 (see Page 9, line 142-143).

Comment 6: Discussions: ‘tailored LN dissection strategies’ > How? Please state more

specifically. What are the current strategies? What will be the future strategies?

Reply6: Current strategies based on the Chinese Medical Association Clinical Guidelines for Lung Cancer (2018 edition) include: ① Lymph node dissection and sampling are deemed essential components of surgical procedures. Standard practice involves the removal or systematic sampling of three groups of mediastinal lymph nodes (Left: 4L, 5, 6, 7, 8, 9; Right: 2R, 4R, 7, 8, 9). The recommended minimum number of lymph nodes to be dissected or sampled is at least 12, including both mediastinal and intrapulmonary nodes. ② For Stage I to III lung cancer without preoperative evidence of mediastinal lymph node involvement (negative PET or EBUS, negative mediastinoscopy), extensive lymph node dissection does not show a significant upstaging or postoperative survival advantage. However, in cases where preoperative staging relies solely on imaging (without PET or EBUS, mediastinoscopy staging), lymph node dissection is still recommended (Class I Recommendation Evidence). ③ Preoperative imaging indicating predominantly ground-glass opacity (GGO) with a component >50%, and intraoperative confirmation of invasive adenocarcinoma with predominant adherent growth, suggests a very low probability of mediastinal lymph node metastasis. In such cases, selective sampling of 1-3 groups (Left: 5, 7, 9; Right: 2 or 4, 7, 9) of mediastinal lymph nodes is considered appropriate. We have recognized the challenges in preoperatively determining mutation types, therefore, we cannot make modifications to the existing lymph node dissection strategy based on our current conclusions. Therefore, we have revised the sentence to 'ALK rearrangement was reported to be associated with a higher rate of OLNLM compared to ALK-negative adenocarcinomas'.

Changes in the text: We have modified our text as advised in Section '4. Discussion', paragraph 2, sentence 3 (see Page 11, line 193-194).

Comment 7: Limitations: One additional limitation of this study is only single center patients. Please add this limitation.

Reply7: We agree, and we have added it into the "limitations" section.

Changes in the text: We added some data in Section '4. Discussion', paragraph 6, sentence 7 (see Page 13, line 237-238).

Comment 8: Please show the background characteristics of OLNLM and NOLMN (Table3, etc.). Background characteristics is necessary to appropriately interpret the meaning of Table3.

Reply8: In response to your suggestion, we have added a new table (Table 3) to comprehensively compare the baseline characteristics between these two groups. We have also incorporated a detailed introduction in the article to appropriately interpret the meaning of Table 3.

Changes in the text: We added some data in Section '3.2 Occult lymph node metastasis analysis', paragraph 1, sentence 1-3 (see Page 9-10, line 148-152; Table 3).

Comment 9: Table4: Please reanalyze without the factor of 'adjuvant treatment'. This factor depends on the clinician's decision not from biological factor of NSCLC itself.

Reply9: We have reanalyzed the factors influencing the recurrence of pathologically nodal negative patients, and exclude the 'adjuvant treatment' factor. Upon reevaluation, we observed some changes in the results compared to our previous analysis. Notably, the histological

differentiation is no longer identified as an independent predictive factor [HR, 2.259; 95%CI, 0.903-5.649; P, 0.081].

Changes in the text: We have modified our text as advised in Section '3.3 Recurrence-free survival and overall survival analysis', paragraph 2, sentence 1-2 (see Page 10, line 163-167; Table 5).

Comment 10: Please clearly show N factor in each patients' characteristics, such as cN0, cN1, pN0, pN1 etc. Those are well known prognostic factors.

Reply10: We have made the necessary corrections to clearly delineate the N factor in each patient's characteristics, in both the tables and the text.

Changes in the text: We have modified our text as advised in Section '3.1 Characteristics of pathologically nodal negative and positive patients', paragraph 1, sentence 5 (see Page 9, line 134-135; Table 1, 3, 6, 7).

Comment 11: What kind of ALK-TLIs were used? Please specify.

Reply11: We have included information on the use of ALK-TKIs.

Changes in the text: We added some data in Section '3.1 Characteristics of pathologically nodal negative and positive patients', paragraph 3, sentence 2 (see Page 9, line 142).

Reviewer B

Comment 1: My main concern is the preoperative staging of patients. As far as I can see from the manuscript, the distinction between clinically positive and negative LNs was made by computed tomography. Since this distinction is very important for the later statement that patients with ALK translocation have a high rate of OLNLM, the determination based on CT alone seems questionable. Were PET-CTs and EBUS biopsies performed and can you provide these data?

Reply1: In our study, the majority of patients received ECT, abdominal ultrasound, cranial magnetic resonance imaging, and chest CT scans to evaluate the tumor stage, instead of PET-CT examinations. Only 180 patients (29.9%) underwent PET-CT scans. Only patients with suspected lymph node metastasis on PET undergo EBUS biopsy. In our cohort, only 14 patients (2.3%) underwent EBUS biopsy of lymph nodes. Therefore, we just integrated chest CT and PET-CT information for defining cN0. Additionally, relevant PET information was included in Table 1, 3. We added this point in the limitation section.

Changes in the text: We added some data in Section '2.1 Patient selection', paragraph 2, sentence 4 (see Page 7, line 100-103; Table 1, 3) and Section '4. Discussion', paragraph 6, sentence 6 (see Page 13, line 236-237).

Comment 2: Can you provide the N stage in Table 1? It would also be interesting to know where the upstaging occurs, in the N1 or N2 position.

Reply2: We have included the cN and pN stages in Table 1 to provide a clearer overview of the nodal status. Regarding your inquiry about the position of nodal upstaging, we further added Table 3 to specify upstaging predominantly occurs in the N2 position compared with the N1 position (59.1% vs 40.9%).

Changes in the text: We added some data Section '3.2 Occult lymph node metastasis analysis', paragraph 1, sentence 3 (see Page 9-10, line 150-152; Table 1, 3).

Comment 3: In addition to the number of stations involved, can you give the total number of lymph nodes removed and involved?

Reply3: The number of lymph node stations is not a biological factor influencing prognosis according to the comments of Reviewer A. Therefore, when reanalyzing, this factor was excluded.

Changes in the text: We have modified our text as advised in Section '3.2 Occult lymph node metastasis analysis', paragraph 1, sentence 4-5 (see Page 10, line 152-155; Table 4, 5, 7).

Comment 4: Were only R0 resections included? This should be reported.

Reply4: In our research, only patients who underwent R0 resections were included. This information was added to the revised manuscript.

Changes in the text: We added some data in Section '3.1 Characteristics of pathologically nodal negative and positive patients', paragraph 2, sentence 2 (see Page 9, line 137-138).

Comment 5: In Table 3, in the rows "Number of LN stations resected" and "Blood loss", the values appear to be incorrect. Although the values are the same in all columns, a significance of $p > 0.001$ is given.

Reply5: We have revised the values for "Number of LN stations resected" (5.3 ± 1.6 Vs 6.2 ± 1.3 $P < 0.001$) and "Blood loss" (63.3 ± 130.0 Vs 79.4 ± 102.0 $P = 0.110$).

Changes in the text: We have modified our text as advised (see Table 2).

Comment 6: In logistic regression analysis, the number of LN stations resected is one of the strongest predictors of OLNLM (OR 1.417), stronger than, for example, tumor diameter. Doesn't this mean that if you look more, you'll find more? So with that in mind, it would be interesting to know the number of lymph nodes resected, not just the number of stations. I would be interested in your interpretation of these results.

Reply6: We agree. After carefully reevaluating the clinical significance of the number of LN stations, we reconsidered that the number of lymph node stations is not a biological factor influencing prognosis according to the comments of Reviewer A. Therefore, when reanalyzing, this factor was excluded.

Changes in the text: We have modified our text as advised in Section '3.2 Occult lymph node metastasis analysis', paragraph 1, sentence 4-5 (see Page 10, line 152-155; Table 4, 5, 7).

Reviewer C

Comment 1: In the abstract, on line 61, it would be beneficial to provide an explanation for the abbreviation "RFS" to ensure clarity for readers.

Reply1: We have provided an explanation for the abbreviation "RFS" in the abstract.

Changes in the text: We added some data in Section 'Abstracts', paragraph 3, sentence 6 (see Page 3, line 45).

Comment 2: In the discussion section, it would be valuable to acknowledge the study's limitation regarding the absence of an analysis of ALK variants. Addressing this limitation would provide a more comprehensive understanding of the study's scope and its potential implications for the broader field of research.

Reply2: We have explicitly added this limitation of the absence of an analysis of ALK variants in the "limitations" section of the manuscript.

Changes in the text: We added some data in Section '4. Discussion', paragraph 6, sentence 5 (see Page 13, line 235-236).