Peer Review File
Article information: https://dx.doi.org/10.21037/tlcr-24-717

Reviewer A

I applaud the authors. This is a very well presented paper. This study adds to the understanding of Neoadjuvant chemoIO treatment benefit, especially for squamous cell lung cancers. This presented experience looking at biomarkers of neoadjuvant chemoIO treatment benefit is well presented. This study, albeit single institution, adds to this needed understanding to translate into clinical utility.

Reply: Thank you very much for your positive feedback and thoughtful comments on our manuscript. We are delighted that you found our study well-presented and valuable in contributing to the understanding of neoadjuvant chemoimmunotherapy treatment, particularly for squamous cell lung cancers. We appreciate your recognition of the importance of our findings, even within the context of a single-institution study, and we are encouraged that our work may help advance clinical utility.

Once again, we sincerely appreciate your time and valuable input.

Reviewer B

The authors conducted a retrospective analysis including 199 NSCLC patients who underwent neoadjuvant chemo-immunotherapy and showed higher pre-treatment SCC antigen level associated with improved EFS in this neoadjuvant cohort. They also investigated the patients without neoadjuvant treatment and showed higher SCC antigen level associated with poor prognosis, highlighting the neoadjuvant chemo-immunotherapy's effectiveness against NSCLC patients with high SCC antigen. The manuscript appears relatively well written, although there are several concerns that need to be addressed before publication.

1. As it is generally known, the authors show strong association with histology and SCC antigen levels in Table 3. On the other hand, there are several studies that show LUSC has better response to neoadjuvant chemo(-immuno)therapy than LUAD. The authors state these association between neoadjuvant efficacy, SCC antigen level and histology in discussion. There's an important question that should be addressed: The association between higher SCC antigen level and efficacy of neoadjuvant chemo-immunotherapy observed in the current study may be strongly biased by histology. If so, the finding has less clinical utility. Do you still see the similar association when the cohort is subset by histology?

Reply 1: Thank you for your valuable comment regarding the potential bias introduced by histology in the association between SCC antigen (SCCA) levels and the efficacy of neoadjuvant chemo-immunotherapy. As presented in Table 2, the multivariate Cox analysis demonstrated that SCCA is an independent prognostic factor for event-free survival (HR = 4.73, 95% CI = 1.09–20.47, P = 0.04), even after adjusting for histology and other clinical factors. This suggests that the prognostic value of SCCA is not solely

dependent on the histological subtype (LUSC vs. LUAD). Therefore, we believe that the association between higher SCCA levels and the efficacy of neoadjuvant chemo-immunotherapy is robust and not significantly biased by histology. Given these findings, we argue that SCCA remains a clinically useful biomarker for predicting treatment outcomes in patients undergoing neoadjuvant chemo-immunotherapy, regardless of their histological subtype. We have emphasized this in the discussion section to clarify that SCCA serves as an independent predictor, which strengthens its potential utility in clinical practice. We appreciate your consideration of this clarification.

2. The flow diagram starts with “lung cancer database”, but the nature of the database should be more clarified, e.g. NSCLC patients who underwent radical resection between Jan 2021 and Dec 2023. I am suggesting this because, according to the information provided in the Methods, this recruitment period (2021-2023) applies to neoadjuvant chemo-IO cohort, but obviously not for surgery-alone cohort. Figure 4 shows that surgery alone cohort patients had much longer follow-up period than neoadjuvant cohort, which means that these 2 cohorts derive from different database (possibly from same institution but from different study period). But, the Figure 1 starts from a single “lung cancer database”, which is misleading. The authors should provide flow diagram that truly represents the selection of patients in each study cohort.
Reply 2: Thank you for your comment. We have clarified in the manuscript that both the neoadjuvant cohort and the surgery cohort were derived from the same institutional database but from different recruitment periods and study designs. This clarification helps ensure the flow diagram is more accurate in representing the selection of patients.
Changes in the text: we have modified our text as advised (see Page 7, line 164-167).

3. In the tables and suppl tables that describe the results of regression analyses, reference factor should be clarified for each variables. For example, in Table 2, HR 0.57 (CI 0.13-2.6) is provided for “Gender (female vs male)”. Is this HR for female (vs male) or for male (vs female)? Please re-organise all tables to avoid confusions.
Reply 3: Thank you for your valuable suggestion. We have revised all tables and supplementary tables to clearly specify the reference factor for each variable.
Changes in the text: we have modified our text as advised (see Table 2, Table S4, Table S5, Table S8, and Table S9).

4. In Table S3, sensitivity and specificity are provided. What was the outcome of these specificity and sensitivity? MPR? Please clarify.
Reply 4: Thank you for pointing this out. We confirm that the outcome for the sensitivity and specificity analyses in Table S3 was Major Pathological Response (MPR). To clarify, we have revised the title of Table S3 to explicitly state that the sensitivity and specificity were calculated with MPR as the outcome.
Changes in the text: we have modified our text as advised (see Page 25-26, line 661-665).

5. Because optimal cutoffs are selected within the cohort for each variable, ideally an validation cohort is needed to test the generalizability. Without such validation, the results cannot be generalized. However, some statements in discussion appear too strong in this context: e.g. lines 379-382 in page 12 “This study thoroughly evaluated … and established that the PLR is an effective biomarker”. You should not say “established” here.
Reply 5: Thank you for your insightful comment. We agree that the wording in the discussion was too strong given that our findings have not been validated in an independent cohort. We have revised the statement to clarify that while our study suggests that the PLR may be a potential biomarker, further validation is necessary before any conclusions can be generalized.
Changes in the text: we have modified our text as advised (see Page 14, line 412-413).

6. In Table S5 multivariable regression analysis, the number of variables appears too many considering the cohort size, and therefore the fitness and robustness of the model is questioned. I suggest appropriately selecting the variables and presenting a better model.
Reply 6: Thank you for your comment. We selected the variables for the multivariable regression analysis based on the univariate analysis results, specifically including only those with a p-value less than 0.05. Additionally, we conducted a multicollinearity analysis and found no evidence of multicollinearity among the variables, indicating that they are sufficiently independent of each other. Therefore, we believe that the model is robust and statistically sound.

Reviewer C

The article entitled “Blood biomarkers to predict the efficacy of neoadjuvant chemo-immunotherapy in non-small cell lung cancer patients” shows a retrospective study on the role of different blood biomarkers that can be used to predict the response to chemo-immunotherapy in localized or locally advanced stages in NSCLC. Undoubtedly, the article presents a high scientific quality and shows a series of results and conclusions that are really interesting for the scientific community due to the increasingly expanded use of neoadjuvant chemo-immunotherapy. The article is well structured and easy to read, and the conclusions are quite clear. In addition, it is important to highlight the large sample size with a total of 199 patients treated with neoadjuvant chemo-immunotherapy, which is noteworthy considering the three-year recruitment. The overall article needs few changes and I believe it is of great value for publication in the journal. Both the sections of the article and the different figures/tables, references or supplementary materials are very correct and do not need modifications. The main changes that I think are needed are at the level of methodology or explanations of the methodology. The following changes are proposed for the article:
Major changes

- Methods: I think it would be important to explain on the basis of what criteria the 129 patients in the control group were selected. Given that it is a retrospective study, the methodology used to select the patients should be more clearly indicated and compared with the experimental group in order to know that the characteristics between groups are similar.
Reply 1: Thank you for your valuable comment. The inclusion criteria for the 129 patients in the control group have been clearly specified in Figure 1 of the manuscript.

- Methods: the authors should explain more clearly the objectives of the article.
Reply 2: Thank you for your valuable comment. We have revised the introduction to more clearly explain the objectives of the study. The updated text emphasizes the investigation of whether specific biomarkers from blood routine tests and tumor marker detection can predict the efficacy of neoadjuvant chemo-immunotherapy, with the goal of aiding clinicians in optimizing treatment plans and improving patient outcomes.
Changes in the text: we have modified our text as advised (see Page 5-6, line 123-127).

Minor changes
- Methods (lines 131-134): the ethics statement should be included in a different point that could, for example, go into the statistical analysis or be a different point within the methodology.
Reply 3: Thank you for your thoughtful suggestion. We respect your perspective on the placement of the ethics statement. However, we believe that its current position is appropriate as it ensures that ethical considerations are prominently addressed within the methodology section, which is a common practice. This placement emphasizes the importance of ethical approval in the context of the study design and patient selection, ensuring clarity for the readers.

- Methods (line 127): the total number of patients should go in the results.
Reply 4: Thank you for your helpful suggestion. We have made the necessary revisions and moved the total number of patients to the results section, as recommended.
Changes in the text: we have modified our text as advised (see Page 11, line 321).

- Methods: I think the objectives of the study should be more clearly stated.
Reply 5: Thank you for your valuable comment. We have revised the introduction to more clearly explain the objectives of the study. The updated text emphasizes the investigation of whether specific biomarkers from blood routine tests and tumor marker detection can predict the efficacy of neoadjuvant chemo-immunotherapy, with the goal of aiding clinicians in optimizing treatment plans and improving patient outcomes.
Changes in the text: we have modified our text as advised (see Page 5-6, line 123-127).

- Results: most of the results could be expressed using Hazard Ratio to make them more understandable.
Reply 6: Thank you for your valuable feedback. We understand the suggestion to express more results using Hazard Ratios for clarity. However, after careful

consideration, we believe that the current format presents the findings in a way that aligns well with the study's design and objectives. The statistical methods employed were chosen to provide the most accurate and meaningful interpretation of the data within the context of this research. Therefore, we respectfully prefer to retain the original presentation of the results.

In summary, as previously indicated, I believe that this is a manuscript of high quality. If the authors revise and make the changes correctly I think it is publishable in the journal.