Peer Review File

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Reviewer A

The authors retrospectively analyzed the outcomes of patients undergoing surgical resections for initially unresectable lung adenocarcinoma previously treated with TKI. They demonstrated the importance of detailed investigation of resected specimens, including prognostic factors PD-L1 expression and T790M mutation.

-> Thank you very much for your time and efforts reviewing this article. Your comments had been especially helpful, as they are essential for improvement of manuscript quality. We tried our best to correct the paper, so it can meet the standard of the Reviewers. Key changes we have made are as follows

1) Correction of the terms which can cause unnecessary confusion (minimize the overuse of term "salvage surgery") and defining the two groups of different tumor burden change in Methods sections

2) New results regarding PD-L1 change

3) Addition of new supplementary tables regarding comparison of post-resection outcomes between groups stratified by various factors found from resection samples.

4) Update in observation time after resection (changes in PFS and OS)

1. The discussion point is ambiguous. A negative correlation between PD-L1 expression with EGFR mutations has already been reported. If the changes of PD-L1 expressions could be shown before TKI treatment and at the timing of salvage surgery, it would be useful information.

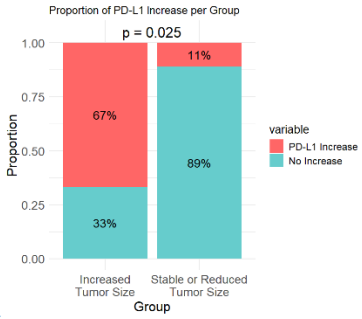
-> We appreciate your critical comment, and in the revised version, we rechecked data for changes in PD-L1 expression. There were 15 patients with paired data on 22C3 and 19 patients with paired data on Sp263. In our revised version, we have shown increase in PD-L1 expression between baseline and resected sample was correlated with increasing lung tumoral burden at time of resection. New paragraphs and two new figures were added in the results section of the revised version.

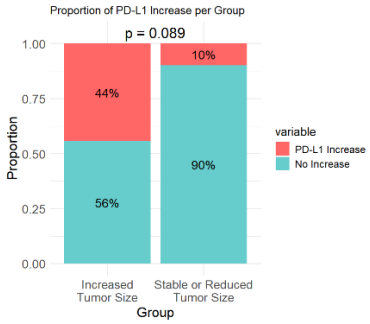
Change in Text (Results section) (Page 12, line 20-Page 13, line 3)

" *Comparison of Changes in PD-L1 Expression at Diagnosis and Resection Across Groups Stratified by Changes in Primary Tumor Burden*

There were 15 patients with paired data for the 22C3 assay and 19 patients with paired data for the SP263 assay, obtained both at the time of diagnosis and from the resected sample. In the disease progression group, where lung tumors showed regrowth or new lung tumor lesions developed at the time of resection, the proportion of patients with increase in PD-L1 (22C3) expression was significantly higher compared to the non-progressive disease group (67% vs 11%, P=0.025) as shown in Figure 8A.

Additionally, the disease progression group showed a higher proportion of patients with elevated PD-L1 (SP263) expression compared to the non-progressive disease group (44% vs 10%), although this difference was not statistically significant (P=0.089), as illustrated in Figure 8B.





Change in Text Discussion (Page 15 Line 18-26)
"Nevertheless, in our study, an increase in PD-L1 expression observed in paired samples was significantly associated with increased tumor burden at the time of resection. In our study, not only did the PD-L1 expression in the resection sample correlate with clinical outcomes, the change in PD-L1 expression relative to baseline also demonstrated a clinical correlation with the response to TKI treatment. Unfortunately, number of patients with paired PD-L1 expression was too small to conclude association with postoperative PFS or OS. The change in PD-L1 expression in EGFR-mutated patients undergoing initial TKI treatment requires validation through larger studies, and its association with other components of the tumor immune microenvironment needs further studies (24)."

2. Actual salvage surgery was performed in only 45% of patients, and 55% of patients underwent resections for biopsy purposes.
-> Thank you for your critical comment. We acknowledge the distinction between salvage surgery and resections for biopsy purposes. We also agree that overuse of word "salvage surgery" may be misleading or overgeneralizing in some sentences. In revising the sentence, we have changed the regarding terms where necessary
Change in text
1) *Running title: Salvage surgery in NSCLC with targetable mutation
-> " Resection of lung tumor after TKI treatment"
2) Highlights section "*Prior research has highlighted the benefits of lung tumor resection post-targeted therapy in enhancing progression-free survival (PFS) for initially unresectable non-small cell lung cancer (NSCLC) patients.*"
3) Discussion section was corrected so no misleading terms are used.

3. In many parts of the results section, only p-values for prognostic comparisons are shown, with no graphs or tables. This is very confusing. The authors should show those graphic data.
-> Thank you for your important comment. In the revised version, we added 5 new supplementary tables showing comparative analysis of PFS and OS rate between groups stratified by tumor differentiation of resected sample, R status, T790M mutation, preoperative ECOG score, and degrees of pleural invasion. We wrote the Results section, so these results are well read to the potential readers. PFS and OS rates were all updated, because in the revised version, observation period was extended.

Changes in the text (Page 13 line 4- Page 15, line 5)
"Comparison of PFS and OS rates between groups stratified by various factors
Tumor differentiation level of resected sample
In the analysis of survival outcomes stratified by tumor differentiation level, there were a statistically significant difference in 6-month PFS rates and 2-year OS rates between patients with well and moderate differentiation compared to those with poor differentiation (P=0.046 and P=0.031, respectively). Specifically, patients with well and moderate differentiation exhibited a 6-month PFS rate of 85.7%, significantly higher than the 56.3% observed in patients with poor differentiation. Additionally, while the 2-year OS rate was 100% in the well and moderate differentiation group, it was significantly lower in the poor differentiation group at 55.6% (Supplementary Table 2).
Resection status (R0 vs R 1-2)
Further stratification by resection status (Supplementary Table 3) showed no statistically significant differences in PFS or OS between patients with no residual tumor (R0) and those with microscopic or gross residual disease (R1-2) at most time points. Group with R0 resection showed tendency of superior higher

PFS rates, but no statistical significance was present.
Impact of T790M Mutation Status
The analysis of lung cancer patients stratified by T790M mutation status (Supplementary Table 4) reveals differences in survival outcomes. Patients with T790M mutation from resected sample showed significantly better PFS rate at 12 months (90.0% vs. 48.0%, P=0.022) and 18 months (87.5% vs. 26.3%, P=0.003) compared to those without the mutation.
Survival Outcomes by ECOG Performance Status
When stratified by ECOG performance status prior to resection (Supplementary Table 5, patients with an ECOG score of 0 demonstrated better 12-month PFS (71.4% vs. 22.2% in ECOG 1 and 50% in ECOG 2, P=0.032) and superior 2-year OS (100% vs. 33.3% in ECOG 1 and 50% in ECOG 2, P=0.008) compared to those with higher ECOG scores.
Pleural Invasion and Survival
The degree of pleural invasion also significantly influenced survival outcomes (Supplementary Table 6). Patients with no pleural invasion (PL0) had a higher 6-month PFS rate of 84.05% and 2-year OS rate of 84.6% compared to groups with more extensive invasion (P=0.025 and P=0.042, respectively).

4. The follow-up time is too short to conclude the importance of PD-L1 and T790M mutation.
-> We agree that the follow-up period could impact the conclusions. The submitted manuscript had last follow up date of September 2023. Reflecting your opinion that the follow-up time was too short, we extended the observation period by 7 months (Updated in April 2024). Postoperative PFS and OS were updated. Updated survival outcomes were reflected (Table 1, PFS and OS rates, Table 5 and Table 6). All Kaplan-Meier graphs were redrawn (Figure 4-8)
*Change in Text (Results section)
"Post-resection PFS
In the univariate analysis, brain metastasis at diagnosis (P = 0.008, HR = 4.122 [95% CI, 1.450–11.721]), liver metastasis at diagnosis (P=0.043, HR=3.817 [95% CI 1.042-13.986]), differentiation level of cancer cells from the resected samples (poorly differentiated vs. others) (P = 0.024, HR = 2.834 [95% CI, 1.148-6.996]), lymphovascular invasion (P=0.047 for reference value), and PD-L1 expression (≥1% from 22C3 or SP263) (P = 0.025, HR = 4.239 [95% CI 1.200–14.972]) showed significant associations with post-resection PFS.
In the Model I multivariate analysis, which included age, gender, brain metastases, liver metastases at diagnosis, cancer differentiation level, presence of pleural invasion from the resected sample, lymphovascular invasion, and PD-L1 detection from the resected sample, none of the factors showed a significant association. In the Model II multivariate analysis, only PD-L1 detection from the resected sample demonstrated a significant association with PFS (P = 0.028, HR = 5.465 [95% CI 1.200–24.885]) (Table 5).

Post-resection OS
In the univariate analysis, only liver metastasis at diagnosis showed a significant association (P = 0.010, HR = 10.445 [95% CI 1.736–62.86]). In both Model I and II multivariate analyses for OS, no factor showed an independently significant association with post-resection OS (Table 6)."

5. Native English proofreading is needed.
-> The manuscript has undergone thorough proofreading by a professional firm before submission. Attached is the screenshot of the certificate. In the revised version, we also looked for sentences which could confusion, and corrected if necessary.

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12/20/2023

To whom it may concern:

The purpose of this letter is to verify that eWorldEditing, Inc. provided the editing services for the following manuscript:

PD-L1 expression from surgically resected lung tumors is predictive of early progression in patients previously treated with targeted therapy for initially unresectable NSCLC

Corresponding Author: KYUNG SOO KIM

The edited document was returned to the writer on 12/20/2023. We are unaware of any changes or additions made to the manuscript after that time.

Sincerely,

Jerry Nairns
eWorldEditing, Inc.

Reviewer B

The authors conduct an interesting study in the factors that may help making treatment decisions especially timing of salvage surgery.

-> We appreciate your time and efforts reviewing this article. Your comments were critical for adding value to the study. We tried our best to respond to your comment as accurately as possible, so it can meet the standard of the Reviewers. Key changes we have made are as follows

1) Correction of the terms which can cause unnecessary confusion (minimize the overuse of term "salvage surgery") and defining the two groups of different tumor burden change in Methods sections, we also added two representative figures to visualize two groups stratified by change in tumor burden at the time of resection

2) New results regarding PD-L1 change

3) Addition of new supplementary tables regarding comparison of post-resection outcomes between groups stratified by various factors found from resection samples.

4) Update in observation time after resection (changes in PFS and OS)

It is confusing that tumours described as non-resectable are then apparently resected- even if they have clinically progressed? Or was the surgery simply a reduction of tumour burden, rather than resection? The use of the terms in that way is a little confusing.

->We agree to your opinion. For clarification, all of the patients had lung tumors which were unable to perform complete resection at the time of TKI initiation. There are two groups of patients enrolled in the study. As was mentioned in the table 3, first group include patients who showed regressing tumor burden or maintained certain period of time of stability before resection, and second group underwent resection of regrowing or newly developed tumor for biopsy purpose.

-> "Resection" in this manuscript does not necessarily refer to "complete resection" of all lung lesions, but rather resection of lesion of interest (whether it is primary or newly developed lung tumor lesion). We authors thought over better selection of term other than "resection" but we could not find one. Reduction of the lung tumor has an objective of reducing lung tumoral burden (more like tumor debulking), but some of our enrolled patients underwent lung tumor resection for biopsy purpose.

-> We assumed that confusion may be due to possible overuse of the word "salvage surgery". Only 45% of the patients underwent salvage surgery, so we erased word salvage surgery in the sentences that could possibly mislead potential readers.

Change in text

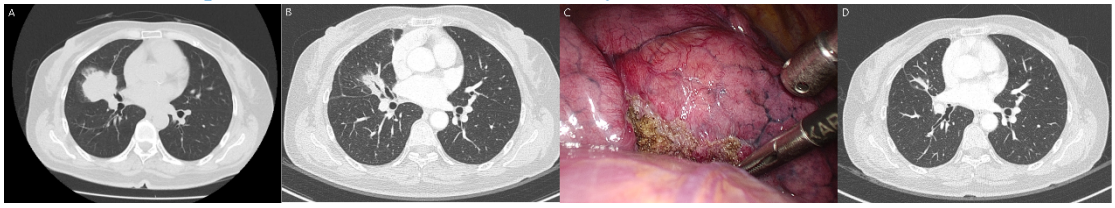
1) *Running title: Salvage surgery in NSCLC with targetable mutation

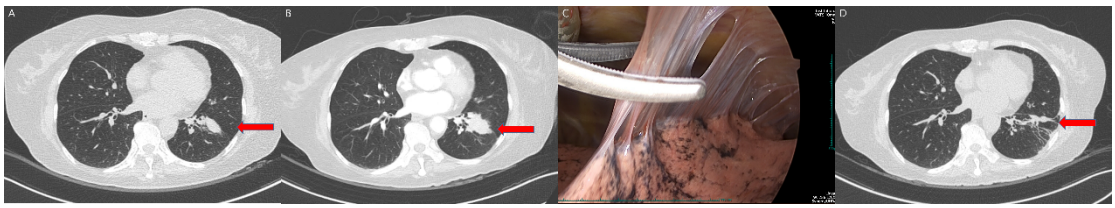
-> *" Resection of lung tumor after TKI treatment*"

2) Highlights section "Prior research has highlighted the benefits of lung tumor resection post-targeted therapy in enhancing progression-free survival (PFS) for initially unresectable non-small cell lung cancer (NSCLC) patients."

3) Discussion section was corrected so no misleading terms are used.-> "In patients with initially unresectable NSCLC with driver mutations, the lung tumor may regrow after a certain duration of sustained treatment response to targeted therapy, or new lung tumoral lesion may develop."

-> For better visualize the patients enrolled, we added two new figures showing change in key CT images of representative case per each group (groups shown in Table 3). I hope this effort helps potential readers to understand what patients were enrolled for the study.





Change in Text (Results section- Comparison between groups stratified by change in primary lung tumor at the resection)

"Figure 2 presents CT images at timepoints before and after resection for a patient whose primary lung tumor size regressed at the time of resection. Figure 3 displays CT images of a patient whose primary lung tumor showed regrowth after an initial period of response to TKI therapy."

- ➔ Furthermore, we renamed two groups stratified by change in tumor burden at the time of resection, so the meaning becomes more clear.
- ➔ Change in Text (Methods) (Page 6 Line13-20)
  "Patients were grouped according to change in lung tumors between initiation of prior targeted therapy and resection of pulmonary tumor. Non-progressive disease group comprises patients whose lung tumors have either regressed or remained stable before surgical resection. Tumor progression group includes patients who experienced tumor regrowth after an initial response to treatment or developed new tumoral lung lesions. Tumor regression was defined as when the primary lung tumor shrinks by more than 30% compared to its size before starting TKI treatment. Tumor regrowth is when the tumor grows by more than 20% compared to the CT scan just before the surgery, in comparison to the one taken right before that."

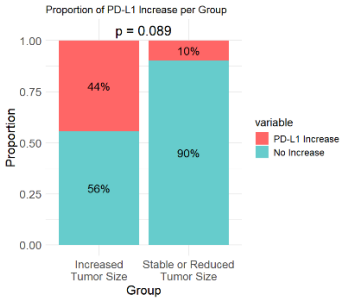
Completeness of resection was surely an important factor - how did that affect outcomes?

- ➔ We agree that the resection completeness is important. Thank you for your advice.
- ➔ The completeness of resection had some impacts. Patients who showed R0 resection were compared with those who showed R1-2. The later groups showed a tendency of worse 6-months, 12-months PFS, but statistical significance was not present. This result was shown as a **newly added supplementary table 3**, along with corresponding description in Results section
- ➔ Change in Text (Page 13, Line 14-18)

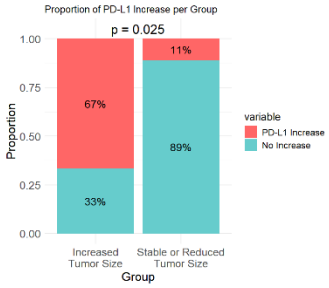
"*Resection status (R0 vs R 1-2)*
Further stratification by resection status (Supplementary Table 3) showed no statistically significant differences in PFS or OS between patients with no residual tumor (R0) and those with microscopic or gross residual disease (R1-2) at most time points. Group with R0 resection showed tendency of superior higher PFS rates, but no statistical significance was present."

Did PD-L1 status change in individual cases after targeted therapy?

- ➔ In our revised version, we have shown change in PD-L1 expression between baseline and resected sample. This was one of our main interests too, but we did not think to add the analysis result in the initial version. Thank you very much for your idea. In the revised version, increasing lung tumoral size or newly developed lesion at the time of resection showed significant association with increase in PD-L1 expression detected by 22C3 (P=0.025). New paragraphs and two new figures were added in the results section of the revised version.
  Comparison of Changes in PD-L1 Expression at Diagnosis and Resection Across Groups Stratified by Changes in Primary Tumor Burden
  There were 15 patients with paired data for the 22C3 assay and 19 patients with paired data for the SP263 assay, obtained both at the time of diagnosis and from the resected sample. In the disease progression group, where lung tumors showed regrowth or new lung tumor lesions developed at the time of resection, the proportion of patients with increase in PD-L1 (22C3) expression was significantly higher compared to the non-progressive disease group (67% vs 11%, P=0.025) as shown in Figure 8A.
  Additionally, the disease progression group showed a higher proportion of patients with elevated PD-L1 (SP263) expression compared to the non-progressive disease group (44% vs 10%), although



this difference was not statistically significant



(P=0.089), as illustrated in Figure 8B.

Change in Text Discussion (Page 15 Line 18-26)

"Nevertheless, in our study, an increase in PD-L1 expression observed in paired samples was significantly associated with increased tumor burden at the time of resection. In our study, not only did the PD-L1 expression in the resection sample correlate with clinical outcomes, the change in PD-L1 expression relative to baseline also demonstrated a clinical correlation with the response to TKI treatment. Unfortunately, number of patients with paired PD-L1 expression was too small to conclude association with postoperative PFS or OS. The change in PD-L1 expression in EGFR-mutated patients undergoing initial TKI treatment requires validation through larger studies, and its association with other components of the tumor immune microenvironment needs further studies (24)."

The timeframe of the study- how long did it take to recruit patients- is important.

- ➔ Thank you for your comment. To ensure uniformity, patient inclusion and exclusion criteria were applied at a single time point when acquiring data from the Clinical Data Warehouse (CDW). This approach was adopted to eliminate any potential bias associated with an extended recruitment period. Furthermore, in the revised version, updated data collection of PFS and OS were made at one day, so no discrepancy in patients' data were present between the study patients (this was possible because there were only 40 patients to be analyzed)
- ➔ The above information was incorporated in the Methods section
- ➔ Change in text (Materials and Methods- Patient selection and data collection)
  "To ensure uniformity, patient inclusion and exclusion criteria were applied at a single time point using the CDW in order to minimize potential bias associated with an extended recruitment period."

What staging was used- presumably AJGG 8th ed?
Histological type of tumour - need to state WHO grading was used?

- ➔ Yes, AJCC 8th edition was used. We will elaborate that in the Methods section of the revised manuscript.
- ➔ WHO guideline 2015 edition was used. We also noted that in the Methods section of the revised version.
- ➔ Change in Text (Page 6, Line 22-25)
  "TNM staging and pathologic typing
  The TNM staging of lung cancer followed the American Joint Committee on Cancer (AJCC) 8th edition guidelines (6). For pathological typing, the 2015 World Health Organization (WHO) classification was utilized (7, 8)."

Would it not be beneficial to look at the PRE surgery sample to help decide which patients to stratify to surgery?

- ➔ We appreciate your comment on pre surgery sample. We understand that pre-surgery samples, obtained at the initial diagnosis, could provide valuable insights for determining subsequent treatment strategies, and possibly optimal timing of delayed resection. However, in our study, the variability in pathologic parameters among these initial diagnostic samples hinder reliable analysis. We kindly request your understanding of this limitation. This issue, as it overlaps with the following comment, will be discussed in greater detail in the later sections of our response.

The blueprint study has shown that SP263 and 22C3 perform comparably in this space. With such small case numbers, should the scores not be combined as TPS regardless of antibody used?

- ➔ We agree that incorporation of both 22C3 and SP263 can be a good way to show association between PD-L1 expression of the resected sample with postoperative outcomes. In fact, in cox regression analysis for postoperative PFS and OS, resected sample PD-L1 expression incorporated both antibodies, and the combined PD-L1 expression was analyzed for association with the outcomes *(PD-L1 expression (≥1% from 22C3 or SP263) (P = 0.025, HR = 4.239 [95% CI 1.200–14.972]) showed significant associations with post-resection PFS.*
- ➔ We understand it was your considerate suggestion to make the best out of rather limited data, however, since analysis of paired data of PD-L1 expression was possible from the each platform (22C3 and SP263), we prefer to insist on the results of the revised manuscript.

The study collected a lot of data. Inclusion of histological pre-treatment factors and information if they changed may be ultimately clinically more informative

- ➔ We fully agree to the idea of analyzing pre-surgery samples can provide valuable insights for subsequent treatment strategies. We have provided in the response to previous comment, change in PD-L1 expression (separately for 22C3 and SP263) was associated with pattern of change in tumor burden at the time of resection. However, for other detailed histologic data from the presurgery samples, limitation exists in analyzing them.
- ➔ We understand that these pre-surgery samples are typically collected at the initial diagnosis of lung cancer. However, for the 40 patients enrolled in our study, the initial cancer diagnoses date range from year 2014 to year 2020. Moreover, the pathologic parameters examined, the immunohistochemistry techniques used, the analytical platforms, and the pathologists involved have varied depending on the time point and the center. Despite the potential value of pre-surgery samples, we believe that the variability in their collection and analysis introduces too much inconsistency for them to be reliably used for analysis. Consequently, we focused on information acquired from resected samples after prior TKI treatment, which were collected over a relatively narrower time range and under more consistent conditions.
- ➔ In the revised version, we added 5 new supplementary tables and regarding descriptions on association between **pathologic factors from the resected samples** with postoperative outcomes.
- ➔ Change in Text: (Results section- *Comparison of PFS and OS rates between groups stratified by various factors Page 13, Line 5- Page 14 Line 7)*

" *Tumor differentiation level of resected sample*

In the analysis of survival outcomes stratified by tumor differentiation level, there were a statistically significant difference in 6-month PFS rates and 2-year OS rates between patients with well and moderate differentiation compared to those with poor differentiation (P=0.046 and P=0.031, respectively). Specifically, patients with well and moderate differentiation exhibited a 6-month PFS rate of 85.7%, significantly higher than the 56.3% observed in patients with poor differentiation. Additionally, while the 2-year OS rate was 100% in the well and moderate differentiation group, it was significantly lower in the poor differentiation group at 55.6% (Supplementary Table 2).

Resection status (R0 vs R 1-2)

Further stratification by resection status (Supplementary Table 3) showed no statistically significant differences in PFS or OS between patients with no residual tumor (R0) and those with microscopic or gross residual disease (R1-2) at most time points. Group with R0 resection showed tendency of superior higher PFS rates, but no statistical significance was present.

Impact of T790M Mutation Status

The analysis of lung cancer patients stratified by T790M mutation status (Supplementary Table 4) reveals notable differences in survival outcomes. Patients with T790M mutation from resected sample exhibited significantly better PFS rate at 12 months (90.0% vs. 48.0%, P=0.022) and 18 months (87.5% vs. 26.3%, P=0.003) compared to those without the mutation.

Survival Outcomes by ECOG Performance Status

When stratified by ECOG performance status prior to resection (Supplementary Table 5, patients with an ECOG score of 0 demonstrated better 12-month PFS (71.4% vs. 22.2% in ECOG 1 and 50% in ECOG 2, P=0.032) and superior 2-year OS (100% vs. 33.3% in ECOG 1 and 50% in ECOG 2, P=0.008) compared to those with higher ECOG scores.

Pleural Invasion and Survival

The degree of pleural invasion also significantly influenced survival outcomes (Supplementary Table 6). Patients with no pleural invasion (PL0) had a higher 6-month PFS rate of 84.05% and 2-year OS rate of 84.6% compared to those with more extensive invasion (P=0.025 and P=0.042, respectively).”