Peer Review File

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Response to Reviewer A:

Comment 1: This is retrospective study. PFS may influenced by many factors, especially evaluation interval and adverse events leading to discontinuation of the treatment. Please add more detailed patient characteristics, including evaluation interval (median, range), AEs, rate of discontinuation due to AEs, etc. Please compare these factors in each group. Please add mutation information in patient characteristics. Are there any difference in different groups?

Reply 1: We have added some relevant patient characteristics according to the reviewer's suggestion, including evaluation interval, mutation information, adverse events (AEs), and rate of discontinuation due to AEs.

Changes in the text: We have modified our text as advised. (see Page 19-21, Table 1)

Comment 2: Why OS is not different? Why significant PFS different is not translated into OS difference?

Reply 2: There are some reasons that improvements in PFS may not be accompanied by a corresponding lengthening of OS. PFS and OS are regarded as proximal and distal endpoints, respectively. As more treatments become available for NSCLC, more patients are receiving later-line regimens. Accordingly, a longer later-line therapy period would potentially lead to a dilution of the PFS treatment effect. On the other hand, statistically significant improvements in PFS may not translate into detectable OS benefits if the trial is insufficiently powered. The correlation between PFS and OS will get clearer as more trials are published and a larger sample can be analyzed.

Comment 3: Please show more detailed treatment of each group. Are there any imbalance with certain drug, such as osimertinib?

Reply 3: Considering the reviewer's suggestion, we have added a new table about the number of patients treated with each TKI drug in the study population, stratified by both baseline CK level and CK change during TKI therapy, as shown in Table 2.

Changes in the text: We have modified our text as advised. (see Page 22-23, Table 2)

Comment 4: CK is easily influenced by physical activities, such as running, golfing, etc. CK elevation may reflect the improved PS (patient activity) during the treatment. How the authors distinguish such kind of problem in this retrospective study?

Reply 4: Considering the retrospective nature of our study, it is difficult to distinguish whether CK elevation was caused by TKIs or physical activities. However, in our

opinion, the significance of CK monitoring is to provide a prognostic and predictive factor of durable efficacy in patients treated with TKIs, and even aid in intervention and individualized treatment when necessary. Also, further detailed prospective analyses are currently in progress to address this point.

Comment 5: How was response rate? Was the PFS difference also seen in RR difference in each group? Are the CK levels (baseline and elevation) prognostic, or predictive? This is very important point. How can the authors distinguish? If not, please suggest future research addressing this point.
Reply 5: Results of the ORRs were concordant with the PFS ones. The overall ORR was 67.4%. Patients with higher baseline CK levels had better ORR than the lower baseline CK group, but there was no significant difference between the two groups (74.6% vs. 60.3%; P=0.076). Likewise, ORRs were higher among patients experiencing significant CK elevation compared with those without (77.6% vs. 59.7%; P=0.029). However, we cannot fully differentiate the prognostic and predictive impact of CK level on patients. ORRs have suggested a predictive effect on patients treated with TKIs while the lack of CK monitoring for patients receiving general radiotherapy and chemotherapy hampered exploring whether higher baseline CK level could be regarded as a prognostic indicator. Further detailed prospective analyses are currently in progress to address this point.
Changes in the text: We have modified our text as advised. (see Page 11-12, line 256-261 & Page 15, line 357-363)

Comment 6: Cutoff value of 70 U/L was used in the main analysis. How will the results change with different cutoff? The result may be by chance (or may be robust).
Reply 6: The optimal cutoff for a higher baseline CK level was determined via X-tile software. Also, we have changed the cut-point to 60 U/L and 80 U/L, identical conclusions were obtained from these two cutoff values. Nonetheless, whether the cutoff value calculated from limited participants could be applied to other TKI-treated NSCLC patients remains to be verified.

Comment 7: The authors mentioned connection between CK and immune response in the discussion. In their own data or previous report, how is the correlation between CK levels and effect of immune-checkpoint inhibitors in NSCLC?
Reply 7: To the best of our knowledge, CK elevation was mostly considered as the side-effects induced by immune-checkpoint inhibitors in previous studies. Regrettably, there are relatively few studies devoted to this issue and the correlation between CK levels and the effect of immune-checkpoint inhibitors in NSCLC has not yet been elucidated.

Comment 8: Minor points: Figure1; Please explain Group1, 2, 3 and 4.
Reply 8: We are sorry for forgetting to interpret Group 1, 2, 3, and 4 in Figure 1 and we have modified our text as advised. (see Page 25, Figure 1)

Response to Reviewer B:
Comment 1: Few patients are enrolled in retrospective studies. More patients need to draw this conclusion.
Reply 1: Given that CK monitoring is not a routine serum chemistry test for patients receiving general radiotherapy and chemotherapy, only a limited number of patients are eligible for our study. Nonetheless, we identified a trend that both higher baseline CK level and significant CK elevation after treatment were associated with improved PFS, suggesting that CK might be an auxiliary prognostic and predictive factor of durable efficacy in patients treated with TKIs. To draw definitive conclusions, a validation study with larger sample sizes and a more rigorous design are currently in progress.

Comment 2: It is necessary to display the consort diagram as Fig. 1.
Reply 2: To the best of our knowledge, the CONSORT Flow Diagram is for a parallel randomized trial and is not applicable to our retrospective cohort study

Comment 3: The TNM staging system for each group should be presented in Table 1, Figures S2, and S3. Staging is a factor that strongly influences the effect.
Reply 3: We are very sorry for our negligence of the TNM staging system for each group since all 135 participants included in our study were with stage IV NSCLC patients, and we have redefined the patients' TNM staging in our revised manuscript.
Changes in the text: We have modified our text as advised. (see Page 10, line 221)

Comment 4: In retrospective studies, it is difficult to obtain accurate PFS data due to irregular tumor evaluation by CT and MRI. Do all patients usually have tumor assessments at the same intervals?
Reply 4: We have checked electronic patient records for all 135 patients included in our study. Chest CT scans were performed bimonthly during TKI treatment, following the Response Evaluation Criteria for Solid Tumors (RECIST) ver. 1.1. Hence, all 135 patients shared a similar tumor assessment interval, namely, approximately 2 months. Therefore, the PFS data obtained in our research is relatively reliable.

Comment 5: Overall survival is solid marker comparing to PFS. Authors should show OS data among groups.

Reply 5: Regarding OS, there was no significant difference between these groups. Since significant PFS difference is not translated into OS difference, we displayed the Kaplan-Meier curves in Supplementary Figure S1.
Changes in the text: We have modified our text as advised. (see Page 27, Supplementary Figure S1.)

Comment 6: Elevated CK levels may be related to TKI levels. In that case, other adverse events can occur frequently in the high CK and increased CK groups. If possible, I would like to be able to check the TKI blood levels of patients with high CK.
Reply 6: Given that our study is a retrospective study, the TKI blood levels of patients with high CK were not available. Further detailed prospective analyses with larger sample sizes and a more rigorous design are currently in progress to address this point.

Comment 7: Line156: below sentence exist. “Collected CK data included the history of cardiovascular events, muscular disorders and renal abnormality, relevant myocardial enzymes status, history of surgery within 5 days before study enrollment,” This study is a retrospective study, not a prospective study. Enrollment date cannot be fixed in this study.
Reply 7: We accept the reviewer’s criticism and have changed the expression from “within 5 days before study enrollment” to “within 5 days before TKI treatment”.
Changes in the text: We have modified our text as advised. (see Page 8-9, line 175-178)