Peer Review File
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Reviewer A

Comment 1: Line60: PD-L1 expression on cancer cells determined by immunohistochemistry and the tumor mutational burden (TMB) assessed by next-generation sequencing (NGS) are the only validated predictive markers for ICIs response in randomized phase III trials 5. There is no consensus about the role of TMB as a predictive biomarker for survival.
Reply 1: Thank you for your comments. We deleted our text as advised.

Comment 2: line 132 and figure 1: Do you mean chemoimmunotherapy instead of the word chemotherapy.
Reply 2: Thank you for your comments. In accordance with the reviewer's comment, we changed this to chemotherapy or immunotherapy.
Changes in the text: Page 4, line 129, figure 1

Comment 3: The results should be reanalysed after taking in consideration lead time bias.
Reply 3: Thank you for your comments. We believe that lead-time bias should be considered in the Kaplan-Meier curve (Supplementary Figure 1) in the development of irAEs. The lead-time bias is affected by the longer dosing period of one arm, with irAE frequency being higher in the arm with the longer dosing period, in general, the incidence of irAEs has been reported to correlate with a favorable prognosis but in this study, the incidence of irAEs was not associated with a favorable prognosis. Therefore, we expect lead-time bias to have a small impact on our results.

Comment 4: Development of irAEs has been correlated with improved survival across many studies. How do you explain the difference in your results where high CRP associated with increase development of irAEs but worse OS and PFS? Do you think other factors contributed to worse OS due to ECOG or smoking status?
Reply 4: We appreciate the reviewer's comment on this point. In the present study, multivariate analysis on OS showed no statistically significant factors except high CRP and poor PS. Because occurrence of irAEs did not contribute to shorter OS, we consider that the influence of high CRP on OS is more important than the occurrence of irAEs.

Comment 5: Is the difference in demographics between the two groups (low vs high) is statistically significant? Do you want to check the P value?
Reply 5: We appreciate the reviewers' concerns in this regard. However, we believe that the omission of the P value for demographics is correct due to multiplicity issues.

Reviewer B

Comment 1: Regarding pneumonitis, are previously known risk factors such as prior radiotherapy, ILD, whether patients underwent double immunotherapy evaluated in this study? Does CRP have superiority in predicting pneumonitis compared to the above risk factors?
Reply 1: Thank you for your comments. As noted by the reviewers, interstitial pneumonia existing before treatment has been reported to be a risk factor for drug-induced pneumonitis (Shoji Kudoh et al. Interstitial lung disease in Japanese patients with lung cancer: a cohort and nested case-control study. Am J Respir Crit Care Med. 2008; 177(12):1348-1357). Therefore, in this study, a prior history of interstitial pneumonia was excluded. The following sentences were added to the Methods: exclusion criteria: history of severe interstitial pneumonia, including radiation pneumonia; Patients treated with double immunotherapy.
Changes in the text: Page 3, line 87-88.

Comment 2: A lot of other irAEs have reported risk factors such as autoimmune markers. All these risk factors considered?
Reply 2: Thank you for your comments. Previous studies have reported troponin and TSH as autoimmune markers related to irAEs (Adithya Chennamadhavuni et al. Risk Factors and Biomarkers for Immune-Related Adverse Events: A Practical Guide to Identifying High-Risk Patients and Rechallenging Immune Checkpoint Inhibitors. Front Immunol. 2022 26;13). However, the present study was retrospective and had a lot of missing data, which prevented a full analysis of autoimmune markers.

Comment 3: The percentage of severe irAEs (those likely to be grade III or more) is too high in this study. It is reported that the widely known percentage is less than 10%
Reply 3: Thank you for your comments. The frequency of irAEs in this study ≥Grade 3 was 21% in the Low CRP group and 26% in the High CRP group. This is a similar rate to the 26.6% of ≥Grade 3 irAEs in the KEYNOTE-024 study. We consider that our data of irAEs is not high frequency.

Comment 4: Authors should also consider if the correlation between CRP and high irAEs is a coincidence between disease burden and incidence of unfavorable outcomes.
Reply 4: Thank you for your comments. We determined the disease burden noted by the reviewer to be the clinical stage. We agree that this point requires clarification, and have added the Clinical stage to the Table 2.
Changes in the text: Table 2

Reviewer C

Major change comment 1: Results: a total of 12 patients had mutated EGFR. These patients are known to be resistant to immunotherapy. Consider performing the analyzes without these patients. The presence of these patients is an important confounding factor that has been able to bias the sample and therefore the results. It is important to perform a new analysis without these 12 patients.
Reply 1: We wish to thank the reviewer for this comment. We know that EGFR mutation is a favorable prognostic factor. In the present study, EGFR mutation was analyzed multivariate in the development of irAEs and OS to adjust for confounding factors, none of which were significant factors. Therefore, we consider it unnecessary to exclude patients with EGFR mutation.

Major change comment 2: Results: it is necessary to clarify in the results the indications of the different types of immune checkpoint inhibitors that have been prescribed to patients. It must be specified whether they were scheduled in the first or second line. And after that, it should be analyzed if the results are maintained depending on which line the immunotherapy was prescribed and the expression of PD-L1.
Reply 2: Thank you for your comments. We agree with the reviewers and have added the treatment lines to Table 1. We also compared treatment lines by CRP and found a trend towards more first lines in the High CRP group. In general, the initial line of treatment correlates with prognosis, but as PFS/OS was shorter in the High CRP group, we do not believe that the line of treatment influenced the results of this study.
Changes in the text: Table 1

Major change comment 3: Discussion: a possible predictive biomarker of irAEs has been found in patients treated with immunotherapy. It is essential that an important part be added to the discussion on how we can apply this possible discovery in clinical practice.

It would be necessary to assess how patients can benefit from this finding and how we can apply it to oncology treatments.
Reply 3: Thank you for your comments. We agree that this point requires clarification, and have added the following text to the Discussion: The CRP has potential predictive biomarkers for irAEs, measurement of CRP prior to ICIs treatment can screen out and exclude those who are not suitable for immunotherapy. In future, the relevance of inflammatory markers such as CRP and IL-6, as well as routine blood parameters and other unidentified biomarkers, should be clarified in large prospective clinical trials. The best treatment option can then be offered within the ICIs treatment strategy.
Changes in the text: Page 7, line 220-224.

Major change comment 4: Discussion: The results in PFS are quite limited. It would be useful to explain the results of PFS so low in the discussion to understand the study sample well.
Reply 4: We agree that this point requires clarification, and have added the following text to the Discussion: PFS was very limited, with 3.3 months in the Low CRP group and 2.2 months in the High CRP group. Previous studies have shown a PFS of 3.5 months (95% CI: 2.1-4.9) in the nivolumab-treated group in the Checkmate-017 study in non-squamous cell carcinoma, which was similar to the results of this study.
Changes in the text: Page 6, line 200-203.

Minor change comment 1: Title: a more convenient title could be "High level of C-reactive protein as a predictive factor for Immune-related adverse events of immune checkpoint inhibitors in NSCLC: a retrospective study". It is necessary in my view that the article reflects that it is a retrospective study, and that NSCLC is carried out. Generalizing some results to the entire population with solid tumors that has only been performed in NSCLC I think is not convenient.
Reply 1: We wish to express our deep appreciation to the reviewer for his insightful comment on this point. In accordance with the reviewer's comment, we have changed this to Title.
Changes in the text: Title

Minor change comment 2: Abstract: indicate the country of realization in the methods to know in which population we carry out the study.
Reply 2: In accordance with the reviewer's comment, we have added this to abstract.
Changes in the text: Page 1, Line 36

Minor change comment 3: Abstract: in line 39 indicate the p of the result being explained.
Reply 3: Thank you for your comments. Statistical analyses on the severity of irAEs were not performed in this study due to multiplicity considerations.

Minor change comment 4: Keywords: add "biomarkers".
Reply 4: Thank you for your comments. In accordance with the reviewer's comment, we have added this to Key words.
Changes in the text: Keywords

Minor change comment 5: Introduction: in line 61-62 there is another biomarker that is known in immunotherapy which is general status (PS or ECOG). Consider adding.
Reply 5: We appreciate the reviewer's comment on this point. We have incorporated your comments by Introduction.
Changes in the text: Page 2, Line 61-62.

Minor change comment 6: Introduction: delete line 63.
Reply 6: Thank you for your comments. In accordance with the reviewer's comment, we removed this text.

Minor change comment 7: Introduction: line 77, delete "In the current observational single-center study". This part should go in methods.
Reply 7: Thank you for your comments. In accordance with the reviewer's comment, we have removed this text.

Minor change comment 8: Methods: No patient treated with CTLA-4? Please specify.
Reply 8: Thank you for your comments. This study includes NSCLC patients treated with anti-PD-1/PD-L1 antibodies among ICIs. Therefore, ICIs with anti-CTLA-4 antibodies were not included.

Minor change comment 9: Results: line 136, indicate lower and upper age limit.
Reply 9: Thank you for your comments. In accordance with the reviewer's comment, we have added this to Results.
Changes in the text: Page 4, Line 133

Minor change comment 10: Results: line 139, indicate smokers in % for greater

compression.
Reply 10: Thank you for your comments. In accordance with the reviewer's comment, we have added this to Results.
Changes in the text: Page 4, Line 135-136

Reviewer E

Comment 1: The impact of irAE on survival could be further evaluated: is there a survival difference when stratified by baseline CRP? May the higher baseline-CRP in patients experiencing irAE be a confounder and lead to worse survival?
Reply 1: We wish to thank the reviewer for this comment. In this study, patients experiencing irAEs were stratified by baseline CRP as shown in Supplementary Figure 2. In previous studies, it is also known that patients with higher baseline CRP have a worse prognosis in patients treated with ICIs. However, it has been reported that patients experiencing irAEs have a rather better prognosis.

Comment 2: Until what time after the start of treatment have irAE been included? Has CRP been measured in between? Have irAE been excluded, if CRP normalized between baseline and onset of irAE.
Reply 2: Thank you for your comments. The study selected irAEs that developed during the ICIs treatment period. CRP was also measured continuously, but was not excluded based on post-treatment CRP, as the patients were classified based on CRP measurements before ICIs treatment.

Comment 3: The different duration of ICI administration should be considered and discussed. Is it due to earlier progressive disease or discontinuation because of irAE. It should be stated how many patients stopped getting ICI because of irAE.
Reply 3: Thank you for your comments. We agree that this point requires clarification, and have added the following text to the Discussion: As for reasons for discontinuation of ICIs, 45 patients (53.6%) in the Low CRP group and 43 patients (54.4%) in the High CRP group discontinued due to disease progression. Discontinuations due to irAEs were 15 patients (17.9%) in the Low CRP group and 18 patients (22.8%) in the High CRP group.
Changes in the text: Changes in the text: Page 5, Line 160-162

Comment 4: Have irAE been included, if they appeared after the discontinuation of ICI

(which means presumably on a subsequent line of therapy)? It should be analyzed, whether the duration of ICI therapy had an impact on irAE frequency. As irAE can appear with considerable lag, it would be interesting to know not only the median but the range of the duration of ICI administration.
Reply 4: Thank you for your comments. As pointed out by the reviewers, the occurrence of irAEs after the completion of ICIs administration is very important. However, the investigation of the development of irAEs in this study covered the incidence of irAEs during the period of ICIs administration, and we were not able to confirm the occurrence of irAEs after the discontinuation of ICIs.

Comment 5: Test statistics (p-values) may be added to figure 3.
Reply 5: Thank you for your comments. Statistical analyses on the severity of irAEs were not performed in this study due to multiplicity considerations.

Comment 6: Cutoffs for p-values should be consistent throughout the manuscript (currently a mix of 0.01 and 0.001).
Reply 6: Thank you for your comments. We agree with you and have incorporated this suggestion throughout our paper.

Comment 7: Table 3 stats a CRP cutoff of 1 mg/L, is this correct and if yes, why is a different cutoff used?
Reply 7: Thank you for your comments. This error has been corrected in accordance with the reviewer's comment.
Changes in the table: Table 3

Comment 8: The impact of different histologies on the incidence of irAE could be further discussed.
Reply 8: Thank you for your comments. In accordance with the reviewer's comment, we added Histology to Table 2.
Changes in the table: Table 2

Comment 9: The authors may want to discuss the fact that treatment with anti-PD-L1 antibodies as compared to anti-PD-1 was more than twice as common in low-CRP patients than in high-CRP patients. Is there an explanation for this phenomenon and how might it influence the frequency of irAE?
Reply 9: In accordance with the reviewer's comments, an entry for anti-PD-1/PD-L1

antibody was added to the explanatory variables in Table 2.
Changes in the text: Table 2

Comment 10: The fact that irAE did not correlate with survial in this study should be discussed, as it stands in contrast to most findings in literature.
Reply 10: We appreciate the reviewer's comment on this point. In the present study, multivariate analysis on OS showed no significant difference in the occurrence of irAEs, which contributed to shorter OS at higher CRP. Therefore, we consider that the influence of high CRP on OS is more important than the occurrence of irAEs. The content up to this point is discussed in the textual discussion (Page 6, Lines 204-208).

Reviewer F

Comment 1: Abstract; “retrospective enrolled all adult patients…” sounds like you included ALL NSCLC patients at this hospital. Please include the dates. Using the term “enrolled” sounds like you consented patients prospectively for enrollment, so I would suggest stating that it was simply a retrospective study of adult patients etc.
Reply 1: Thank you for providing these insights. We agree that this point requires clarification, and have added the following text to the Abstract: Between December 1, 2015 to December 31, 2019, we retrospectively collected all adult patients with NSCLC who received at least one dose of an ICIs targeting the PD-1/PD-L1 axis at the Iwate Medical University Hospital in Japan.
Changes in the text: Page 2, Line 46-48

Comment 2: Abstract; Line 38-39 needs to be re-written as the latter part does not make sense: “which tended to be more frequent in the high CRP group incidence of entire irAEs was significantly.”
Reply 2: The reviewer's comment is correct. To clarify, we have changed the following text to the Abstract: The secondary endpoints were the relationship of progression-free survival (PFS) and OS.
Changes in the text: Page 2, Line 38-39

Comment 3: Introduction; Clarify the last sentence of first paragraph: TMB as a protective factor?
Reply 3: Thank you for providing these insights. TMB was removed from the text because of insufficient evidence as a protective factor.

Comment 4: Introduction; Please clarify if CRP has been associated with NSCLC prognosis regardless of treatment (in prior literature).
Reply 4: The reviewer's comment is correct. We agree with the relevance of this reference, and have added it to the Introduction and References.
Changes in the text: Page 2, Line 71, References 9),10)

Comment 5: Methods; Need more details about the “database” used for patient identification. Is CRP routinely done in all NSCLC at baseline?
Reply 5: Thank you for providing these insights. CRP was collected routinely before the administration of ICIs.

Comment 6: Methods; What were the dates for inclusion (earliest date)? Include in the methods.
Reply 6: The reviewer's comment is correct. In accordance with the reviewer's comment, we have added the dates for inclusion to Methods.
Changes in the text: Page 3, Line 91

Comment 7: Methods; It is not correct to say that all adult patients with NSCLC who received at least one ICI were included, but rather all those meeting inclusion criteria during the defined study period were included.
Reply 7: Thank you for providing these insights. We agree that this point requires clarification, and have added the following text to the Methods: Between December 1, 2015 to December 31, 2019, we retrospectively included adult patients (≥20 years old) with NSCLC patients met all those meeting inclusion criteria during the defined study period were included.
Changes in the text: Page 3, Line 91-93

Comment 8: Methods; Why 20 years old and not 18, which is typically considered “adult”?
Reply 8: We appreciate the reviewer's comment on this point. The age of adulthood in Japan was defined as 20 years old in 2021, when this study was initiated.

Comment 9: Methods; Provide citation for cutoff of 10 mg/L. Was CRP also readily available in the medical record?
Reply 9: Thank you for providing these insights. We agree with the relevance of this

reference, and have added it to the Methods. All CRP values were read from the electronic medical record.
Changes in the text: Page 3, Line 94
Comment 10: Methods; How were ICI toxicities confirmed? Were ICD codes used?
Reply 10: We appreciate the reviewer's comment on this point. The irAEs in this study were determined by a doctor's diagnosis.

Comment 11: Methods; How long was each patient followed for toxicities? Median duration of f/u?
Reply 11: Thank you for providing these insights. We agree that this point requires clarification, and have added the following text to the Methods: The date of the follow-up cutoff was December 31, 2021.
Changes in the text: Page 3, Line 100

Comment 12: Results; Please include how many received first line therapy, 2nd line, 3rd line or beyond etc. in table 1.
Reply 12: Thank you for providing these insights. In accordance with the reviewer's comment, we have added this to Table1.
Changes in the text: Table 1

Comment 13: Results; The median duration of ICI administration seems low (2.6 months) – why?
Reply 13: We consider PFS and duration of ICIs administration to be equivalent. We have therefore replaced the duration of ICIs administration with PFS and added the following text to the discussion: PFS was very limited, with 3.3 months in the Low CRP group and 2.2 months in the High CRP group. Previous studies have shown a PFS of 3.5 months (95% CI: 2.1-4.9) in the nivolumab-treated group in the Checkmate-017 study in non-squamous cell carcinoma, which was similar to the results of this study.
Changes in the text: Page 6, line 200-203

Comment 14: Results; Was PFS and OS analysis adjusted for line of therapy? This must be done since survival will obviously depend on line of treatment.
Reply 14: Thank you for providing these insights. As for whether treatment made a difference, it is difficult to adjust for this due to the small number of cases. It was subjected to univariate analysis, but it was not significantly a factor.

Comment 15: Results; The CRP cutoff is 10 but Table 3 states 1.
Reply 15: Thank you for providing these insights. This error has been corrected in accordance with the reviewer's comment.
Changes in the table: Table 3

Comment 16: Results; What was the median duration of follow up for PFS/OS?
Reply 16: Thank you for providing these insights. In accordance with the reviewer's comment, we have added this to Methods.
Changes in the text: Page 5, line 163.

Comment 17: Results; Why not include PDL1 status as a covariate in the multivariate analysis for OS/PFS?
Reply 17: Thank you for providing these insights. We agree that this point requires clarification, and have added the PDL1 status to the multivariate analysis for OS.
Changes in the table: Table 3

Comment 18: Results; Please include the actual p-value unless less than 0.001.
Reply 18: Thank you for providing these insights. We agree with you and have incorporated this suggestion throughout our paper. We believe that the omission of the P value for demographics is correct due to multiplicity issues.

Comment 19: Results; There was a small difference in PFS but a major difference in OS. Can the authors speculate why?
Reply 19: We agree that this point requires clarification, and have added the following text to the Discussion: PFS was very limited, with 3.3 months in the Low CRP group and 2.2 months in the High CRP group. Previous studies have shown a PFS of 3.5 months (95% CI: 2.1-4.9) in the nivolumab-treated group in the Checkmate-017 study in non-squamous cell carcinoma, which was similar to the results of this study.
Changes in the text: Page 6, line 200-203

Comment 20: Results; Recommend Table 3 show both univariate and multivariate analysis like Table 2.
Reply 20: We thank the reviewer for this comment. In accordance with the reviewer's comment, we have changed this to Table 3.
Changes in the text: Table 3

Comment 21: Discussion; Include in the first paragraph the findings regarding OS/PFS too.
Reply 21: Thank you for your comments. We agree that this point requires clarification, and have added the following text to the Discussion: These data contribute to the prediction of the development of irAEs and the survival of patients treated with ICIs, and CRP may be one of the candidate biomarkers for predicting the development of irAEs and treatment response to ICIs treatment.
Changes in the text: Page 5, line 175-178

Comment 22: Discussion; It would be helpful to include some specific information from prior studies on the impact of CRP on irAEs and survival/prognosis, in NSCLC or other cancers. The authors should compare and contrast their results with prior findings.
Reply 22: Thank you for your comments. We agree with the relevance of this reference, and have added it to the Discussion (Page. 6, Line 190-192, Page 6, Line 196-199) and References.
Changes in the text: Page. 6, Line 190-192, Page 6, Line 196-199, References 19)

Comment 23: Discussion; Please include a paragraph on the potential clinical implications. If prospectively validated, then how could pre-treatment CRP be used in the clinic? Also discuss how CRP could be used along with other clinical, histology, and biomarker factors to determine prognosis and treatment selection.
Reply 23: Thank you for your comments. We agree that this point requires clarification, and have added the following text to the Discussion: The CRP has potential predictive biomarkers for irAEs, measurement of CRP prior to ICIs treatment can screen out and exclude those who are not suitable for immunotherapy. In future, the relevance of inflammatory markers such as CRP and IL-6, as well as routine blood parameters and other unidentified biomarkers, should be clarified in large prospective clinical trials. The best treatment option can then be offered within the ICIs treatment strategy.
Changes in the text: Page 7, line 220-224

Comment 24: Discussion; Why does this hospital collect CRP in the first place since this does not seem to be standard of care?
Reply 24: This is an interesting perspective. CRP is regarded as a meaningful inflammation marker in Japan and routine measurement of CRP before and during chemotherapy and immunotherapy treatment is standard practice.

Comment 25: Conclusion; Conclusion statement that “Identification of patients at high risk of irAEs would be of great help” is weak. What does “Great help” mean? How exactly?

Reply 25: Thank you for your comments. We agree that this point requires clarification, and have added the following text to the Conclusion: This study has shown that CRP should be measured prior to ICI treatment and that patients with high CRP can’t benefit enough from ICI treatment. CRP is a key factor in the choice of treatment for NSCLC patients.

Changes in the text: Page 7, line 227-229