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Review Comments (Round 1)

Reviewer A

I congratulate the authors for a very well written manuscript on the important aspect of predictive immune biomarkers. Their data utilizing readily available cost-efficient real-world real-time laboratory tests and emphasizing the predictive aspect of early on treatment monitoring adds to the clinical utility of dynamic therapeutic monitoring.

I do think two areas warrant further clarification and discussion which would strengthen the understanding of the data and clinical utility impact of your study:

Comment 1. The line of receiving ICI Rx and whether ICI-alone or chemo-ICI Rx carry different ICI Rx benefits and may well reflect different results. Second line ICI-alone Rx may well be different than first-line Rx, as the prior chemotherapy becomes a potential tumor and host confounding factor. Also, inflammatory status of the host appears to have different effects on chemo-ICI compared to ICI-alone. There is a proteomic study (Rich et al J Immunother Cancer 2021;9:e002989) of inflammatory markers including CRP that indicates a high inflammatory state ICI benefit can be overcome with using chemo-ICI but not ICI-alone. The presented data may not apply to those RX with chemo-ICI (could that be another manuscript in the 798 patients?). A more clearly emphasized point that this data is with second line ICI-alone Rx and extending the limitation discussion emphasizing that this data may be different in chemo-ICI Rx patients would help the reader put this into clinical utility context.

Thank you for sharing this information. We evaluated only IO-alone therapy because the combined chemotherapy can mask the effect of the immunotherapy. Also, as reviewer's comment, it could be the former chemotherapy could influence the second line IO-only treatment. Therefore, we add the line of the IO therapy to evaluate the line of therapy affects to the overall survival in NSCLC

patients. It appears that the hazard ratio for second-line chemotherapy is 1.81 (95% confidence interval 1.06-3.09; p=0.029) compared to first-line chemotherapy. This suggests that there may be a higher risk of negative outcomes or reduced efficacy with second-line ICI-alone compared to first-line ICI-alone. In the manuscript, we added that we focused on the ICI-alone therapy in the introduction part.

Changes in text

Introduction section, 3rd paragraph line 92-93

not combined with conventional chemotherapy

Comment 2. It may be best to consider excluding EGFR mutated lung cancers given the known poor ICI benefit in that group (as reflected by 17.2% in the non-survival group and 8.3% in the survival group). That may not affect the conclusions, but it stands out as a potential confounding factor.

Thank you for your valuable feedback. We have included supplementary analysis comprising patients with only EGFR mutations (n = 251). We found that the results were consistent with the previous results with including without EGFR mutation, even in the subgroup with EGFR mutations, and we have added the corresponding results in Supplementary Figure 3 and Supplementary Table 6 with regards to Hazard Ratio. Supplementary Figure 3 shows a Kaplan-Meier plot which indicates similar results to those obtained when EGFR mutations were included in all cases ($p < 0.001$). Additionally, in Supplementary Table 6, we performed Cox proportional hazard modeling and found that the Hazard Ratio was higher in the group with high baseline NLR in both univariate (HR=2.22, 95% CI=1.58-3.13, p<0.001) and multivariate analysis (HR=3.10, 95% CI=1.08-8.90, p=0.036). This finding is consistent with the previous analysis in which high baseline NLR was associated with a similar pattern. We have updated the methodology and results sections to include these additional analyses.

Changes in text

Methods, Statistical Analysis 1st paragraph line 146-150

Since EGFR mutated NSCLC patients are known to show low benefit of ICI therapy, we conducted a subgroup analysis of patients who were identified as having EGFR mutation. For this population,

we checked the OS with Kaplan-Meier method and analyzed the correlation between markers and OS using the Cox proportional hazards model for variables that were used in the analysis above.

Results 7th paragraph line 217-221

The analysis of the EGFR mutated NSCLC patients showed similar to the previous analysis, the Kaplan Meire curve was distinguished between the groups, and the survival rate of patients with outliers detected in the early treatment period was relatively lower than that of other groups in all lab results (log-rank test, $p < 0.01$). Early treatment period LDH (HR = 4.26; 95% CI = 1.41-12.81; $p = 0.010$) was identified as a significant factor in the multivariable analysis (Supplementary Table 6).

Comment 3. Figure 2 description states strata 3 is "baseline and early physiological" and not baseline aberrant and early physiological as the legend states. A clearer alignment of the strata 1-2-3-4 in the description paragraph would provide a clearer reading.

We changed the description of the figure 2 as you have mentioned. We changed the strata3 notation in the description. We aligned the strata as 1-2-3-4 as it is stated in the Figure 2 strata.

Changes in text

In the Figure 2 descriptions we changed as follows “baseline and early physiological” as “baseline aberrant laboratory physiological and early treatment period physiological”.

Comment 4. treatment response should not be in caps in the title

Thank you for your sincere comment. We changed the treatment response to a lower case

Changes in text

In the Title section we changed as below.

Title, line 1

Prognostic value of baseline and early treatment response of neutrophil-lymphocyte ratio, C-reactive protein, and lactate dehydrogenase in non-small cell lung cancer patients undergoing immunotherapy

Comment 5 : Line 165-166 states non-NSCLC in 1,309 patients...figure 1 total 1,309 with 107 non-NSCLC

Reply

In the first paragraph of the results section, we changed the typo that occurred. As in Figure 1, we wrote that the patients determined from the pathological reports as 107 patients. Not 1,309.

Changes in text

Results section, 1st paragraph line 160

We changed the numbers to 107 not 1,309

Comment 6 : Line 99 has a superscript 12

Reply

Thank you for your comment, we synchronized all the font size to 10.

Reviewer B

Comment 1. I suggest some changes to the verbiage of the introduction. For example, combination chemotherapy and checkpoint inhibitor therapy is a common standard of care for oncogene negative NSCLC, not the standard of care.

Thank you for your precious comment. We have changed the term to common standard of care in the last part of the limitation section. Additionally, we could not find the "standard of care" statement in the introduction section. Therefore, we checked the whole manuscript and changed the phrase or any implications that suggests that combination chemotherapy and checkpoint inhibitor therapy is a standard of care.

Changes in text

We changed the term "immunotherapy as the standard of care" → "immunotherapy as the common standard of care".

Limitations section, 1st paragraph line 311

This study had some limitations. As this was a retrospective study, the selection and information bias of the population comprising patients with diverse characteristics primarily treated based on protocol before the prescription of immunotherapy as the common standard of care for NSCLC must be considered.

Comment 2. Methods and patient selection - the selection of patients with survival one year selects for a particularly healthy group of patients and may undermine the results of the study.

Thank you for your valuable comment. We have included patients who had shorter observation days, including those who died before 1 year of follow-up, which increased the subgroup study population from 597 to 970 patients. We conducted a demographic analysis and added it as Supplementary Table 7. In addition, we observed the survival curve of the inflamed and non-inflamed group as Figure 2. Our findings were consistent with those in the main result, which showed that the group exhibiting outliers in the early period had a lower mortality rate ($p < 0.001$). Furthermore, we found that the addition of patients who died before 1 year of follow-up led to better differentiation of the cohorts, as you mentioned, particularly in LDH.

We also added the Hazard Ratio analysis as Supplementary Table 8. Our findings were consistent with those in the main study, showing that early treatment period CRP was significantly associated with increased HR in both univariate and multivariate analyses. In particular, the HR for early treatment period CRP was higher than that for baseline CRP. (univariate: 6.64 95% CI = 4.40–10.02, $p < 0.001$, multivariate: 3.88 95% CI = 1.55–9.72, $p = 0.004$). We found similar results, including those for NLR and LDH, when including patients who died before 1 year of follow-up. We have included these results in the method, result, and discussion sections of our paper.

Changes in text

Methods, statistical analysis 1st paragraph line 150-153

Since there could be a selection bias, we also analyzed the patients who has less than one-year window period. For the group including the patients who had less than 1 year of OS, we compared the demographic variables between the survival and non-survival groups. Also, we analyzed the correlation between the markers and OS with Cox proportional hazards models that were conducted above.

Results section, 8th paragraph line 223-226

The distribution of population was similar with the results that excluded the patients who had less than one-year observation window (Supplementary Table 7). We also performed HR modeling for patients who survived one year or less. Early treatment period CRP and LDH remained significant factors to predict the OS. (Supplementary Table 8)

Comment 3. The labeling of the two groups of patients as "survival" and "non-survival" is confusing - suggest using another label such as "inflamed" and "non-inflamed"

Reply

We thank you for your precious comment, however since we divided our groups based on their outcome, which is death, we find it more convincing to label the groups as “survival” and “non-survival” for the readers. We appreciate your comment.

Changes in text

All the terms “the survival and non-survival groups” were kept as “survival and non-survival groups”

Comment 4. Characteristics long shown to be associated with improved OS, such as low ECOG, high BMI, and line of therapy for the selected checkpoint inhibitors were not included in Table 1 or analyzed.

Thank you for your comment. We also believe that ECOG, BMI, line of therapy, and clinical stage are important factors in our analysis. In response to comments from Commenter A and C requesting additional stage information, we have added cancer staging information and re-analyzed all data.

As suggested, we added the stage information to Table 1 and found that there was a significant difference in ECOG values between the deceased and surviving groups ($p < 0.001$). We also found that there were differences based on the initial stage of cancer. Furthermore, we found that there was a difference in cohorts based on the line of chemotherapy ($p < 0.001$). In particular, we observed that the inflamed group had more patients in the line 2 and line 3 or higher groups ($p < 0.001$). Multivariate Cox regression analysis showed that over having prescribed 3rd line of chemotherapy (hazard ratio [HR] = 3.19, 95% CI = 1.04 - 9.82, $p = 0.0430$) is an important

predictor than the baseline NLR status. However, this did not affect the effectiveness of early treatment period CRP and LDH levels.

We have updated our manuscript to reflect these findings in the methods, results, and discussion sections.

Changes in text

Methods section, Data collection 1st paragraph line 106-108

The demographic data included the age, sex, BMI (Body Mass Index), ECOG (European Cooperative Oncology Group) and line of chemotherapy of the patients when the immunotherapy was first prescribed. Also, the initial cancer stage value was collected.

Results section, 2nd paragraph line 170-174

Most of the patients were stage IV (n = 575, 96.3%). For line of chemotherapy, patients who had secondary line of treatment or more, were the majority in the non-survival group ($p < 0.001$). Also, for ECOG, the non-survival group 0.8 ± 0.9 were higher than the survival group 0.4 ± 0.6) ($p < 0.001$). Also, for line of chemotherapy, for groups that were prescribed with more than 2nd line, most of the patients were in the non-survival group ($p < 0.001$).

Comment 5. Characteristics associated with survival were not included in the univariate or multivariate models, thus it is unknown how these characteristics may have influenced the ultimate outcome

Thank you for your precious comment. We have added the variables you mentioned in Comment 4 and conducted Cox univariate and multivariate analysis. The results of this analysis are included in Table 3.

The analysis showed that BMI, ECOG, and line of chemotherapy were all significant in terms of HR. For BMI, the univariate HR was 0.93 (95% CI = 0.90–0.97, $p < 0.001$), and the multivariate HR was 1.01 (95% CI = 0.92–1.11, $p = 0.817$). For ECOG, the univariate HR was 1.68 (95% CI = 1.49–1.90, $p < 0.001$), and the multivariate HR was 1.48 (95% CI = 0.92–1.11, $p = 0.019$). For line of chemotherapy over stage 3, the univariate HR was 2.71 (95% CI = 1.83–4.01, $p < 0.001$), and the multivariate HR was 3.19 (95% CI = 1.04–9.82, $p = 0.043$).

When we added these variables to the analysis, the results were not significantly different from the analysis conducted without them. Early treatment period CRP and early treatment period LDH still

showed high HR values as from our initial analysis. In the previous analysis, baseline NLR status were significant predictors, however, this has changed by adding the additional variables. And the Multivariate Cox regression analysis showed that over having prescribed 3rd line of chemotherapy (hazard ratio [HR] = 3.19, 95% CI = 1.04 - 9.82, p = 0.0430) is an important predictor than the baseline NLR status. However, this did not affect the effectiveness of early treatment period CRP and LDH levels.

Changes in text

Abstract, Results, conclusion section line 50-57

Results: In the non-survival group, the NLR, CRP, and LDH levels at the early treatment period were higher than those at the baseline ($p < 0.001$). The survival curves stratified based on aberrant laboratory findings in each period varied (log-rank test $p < 0.001$). Multivariate Cox regression analysis revealed that over having prescribed 3rd line of chemotherapy (hazard ratio [HR] = 3.19, 95% confidence interval [CI] = 1.04 - 9.82, p = 0.043) and early treatment period CRP (HR = 3.88; 95% CI = 1.55 - 9.72; p = 0.004) and LDH (HR = 4.04; 95% CI = 2.01-8.12; $p < 0.001$) levels were significant predictors of one-year OS.

Conclusions: Early treatment period CRP and LDH levels were significant predictors of OS in patients with NSCLC undergoing immunotherapy.

Results section, 5th paragraph line 211-213

The line of chemotherapy were also significant factors for predicting of OS. For 3rd line of chemotherapy, (HR = 3.19; 95% CI = 1.04-9.82; p = 0.043). However, the HR for stage value.

Results section, 8th paragraph line 223-226

The distribution of population was similar with the results that excluded the patients who had less than one-year observation window (Supplementary Table 7). We also performed HR modeling for patients who survived one year or less. Early treatment period CRP and LDH remained significant factors to predict the OS. (Supplementary Table 8)

Discussion section, 6th paragraph line 273-278

This study did not find a significant association between baseline and early treatment period NLR and overall survival. The hazard ratio (HR) for baseline NLR was 1.84 (95% CI 0.92-3.68; p = 0.084), although the multivariate Cox regression for baseline NLR showed a p-value of 0.08, which was likely due to the small sample size. A sensitivity analysis including patients with less

than one-year windows found a HR of 1.64 (95% CI = 0.99-2.70; p = 0.053), which was still not a significant result, but the trend remained present.

Conclusion section, 1st paragraph line 322-323

In patients with NSCLC who were prescribed immunotherapy, the early treatment period CRP and LDH levels were significant predictors of OS after the first immunotherapy.

Comment 6. The justification for the short time window of 8 weeks for CRP given that "the survival rate of lung cancer is low" is inconsistent with the study population of patients which included only those who survived greater than 1 year.

Thank you for your sincere comment. As you have commented, we included patients who had observation period less than 1 year. Including the patients with less than 1 year observation, we set the short time window of 8 weeks. We also changed the phrase "the survival rate of lung cancer is low" to a moderate tone "the timely identification of non-responders can significantly contribute to the overall survival of patients."

Changes in text :

Discussion Section, 6th paragraph line 285-286

Early treatment period CRP levels were significant predictors of OS. This was consistent with the results of a previous study, which reported that the CRP responder in whom the serum CRP levels decreased by 30% after immunotherapy relative to the baseline exhibited a good prognosis (HR = 0.20, 95% CI = 0.10–0.42). (16) However, the time window for distinguishing between the responder and the non-responder was 12 weeks in the previous study, whereas it was 8 weeks in this study. The short period needed to distinguish between the responder and non-responder is important because the timely identification of non-responders can significantly contribute to the overall survival of patients. Elevated CRP levels can be explained by persistent enhanced inflammatory responses in tumors that suppress anti-tumor immunity and promote cancer progression through several mechanisms, (24) resulting in a poor prognosis of immunotherapy.

Reviewer C

Comment 1. Was there any correlation between NLR, CRP, LDH and PD-L1 levels, ie 50%?

Reply

Thank you for your comment, we analyzed the correlation between the variables (NLR, CRP, LDH and PD-L1) and added in the Supplementary Figure 2. We analyzed based on two different categories : categorical and continuous. Categorical is where we discretized the values based on the physiological cutoffs. And the continuous is where the original continuous values were used.

In the categorized analysis, we found that some positive correlations over 50%. Baseline CRP levels and early treatment period CRP levels showed high correlation. Also, early treatment period NLR and early treatment period LDH showed 0.547 of high correlation.

However, in the continuous analysis, there were no strong correlations found between the variables that exceeded 50%.

Changes in text

Results section, 3rd paragraph line 184-188

In our correlation analysis in categorized variables, we found that early treatment period NLR, LDH were positively correlated to early treatment CRP levels, 0.468 and 0.619 respectively. Also, early treatment period CRP levels were positively correlated with baseline CRP levels with 0.78. Also, early treatment period NLR showed 0.547 of correlation with early treatment period LDH. However, with the continuous variables, there were no variables that showed correlation over 0.5. (Supplementary Figure 2A)

Comment 2. It would be important to provide the line of treatment for these patients, ie, first line, second line, etc as outcomes vary in recurrent and relapsed disease

Thank you for your comment, we added the number of line of treatment of the study population in the manuscript, Table 1, and included these information in our univariate and multivariate analysis (Table 3). Interestingly, we found that the line of chemotherapy was different in the survival and non-survival groups. There were more patients who were in their second line of treatment or more

in the survival group, which showed shorter survival ($p < 0.001$). However, the overall conclusion of the paper did not change by these effects.

Changes in text

Methods section, Data collection 1st paragraph line 106-108

The demographic data included the age, sex, BMI (Body Mass Index), ECOG (European Cooperative Oncology Group) and line of chemotherapy of the patients when the immunotherapy was first prescribed. Also, the initial cancer stage value was collected.

Results section, 5th paragraph line 201-202, line 211-213

The OS prediction model based on age, sex, BMI, ECOG, line of chemotherapy, initial stage and the baseline and early treatment period NLR, CRP, and LDH levels are shown in Table 3.

The line of chemotherapy were also significant factors for predicting of OS. For 3rd line of chemotherapy, (HR = 3.19; 95% CI = 1.04-9.82; p = 0.043). However, the HR for stage value.

Comment 3. Were all patients stage 4 or were there locally advanced NSCLC included also?

Thank you for your comment. We reviewed the electronic health record of the study population and identified the stage information of the patients. We acknowledge the fact that most of the study population were stage IV and this information was evaluated case-by-case by the medical doctor. (n = 575) However, there were some rare cases where the patient's initial stage was III. (n = 2) There were also patients whose initial stage were not acquirable. (n = 20)

We added the stage information in Table 1 and also included the information in our univariate and multivariate hazard risk analysis. However, there were no significant effects of the stage due to the similarity of clinical stage of the cohort.

Changes in text

Methods section, Data collection 1st paragraph line 108

Also, the initial cancer stage value was collected.

Results section, 2nd paragraph line 170-171

Most of the patients were stage IV (n = 575, 96.3%).

Comments 4.

Abstract

Line 1 – Would recommend changing the statement. We cannot generally say ORR to immunotherapies is low. Patients having high PD-L1 expression have good response to checkpoint therapy. A better way to phrase it would be as follows: Not all patients will benefit from immune checkpoint therapy and use of these medications carry serious autoimmune adverse effects. Therefore, biomarkers are needed to better identify patients who will benefit from its use.

Thank you for your comment. We changed the first sentence of the Abstract as you commented. "As the objective response rate of non-small cell lung cancer (NSCLC to immunotherapies is low" → "Not all non-small cell lung cancer (NSCLC) patients will benefit from immune checkpoint therapy and use of these medications carry serious autoimmune adverse effects. Therefore, biomarkers are needed to better identify patients who will benefit from its use."

Changes in text

Abstract, Introduction paragraph line 40-42

Introduction: Not all patients with non-small cell lung cancer (NSCLC) benefit from immune checkpoint therapy, and the use of these medications carries serious autoimmune adverse effects. Therefore, biomarkers are needed to better identify patients who will benefit from its use.

Comments 5.

Methods

Line 109- Since this is a retrospective study, I would not use the term "enrolled". Instead would use something like" identified " or any other term.

Thank you for your response. We changed the terms "enrolled" to "identified"

Changes in text

In the Abstract methods section and the discussions, we changed the "enrolled" terms to "identified" as you mentioned.

Review Comments (Round 2)

Line 53- "Multivariate Cox regression analysis revealed that over having prescribed" is not clear. Language needs to be corrected.

Response

we changed the text being ambiguous and having grammatical errors.

Changes

Multivariate Cox regression analysis revealed that having prescribed more than 3rd line of chemotherapy (hazard ratio [HR] = 3.19, 95% confidence interval [CI] = 1.04 - 9.82, p = 0.043) and early treatment period CRP (HR = 3.88; 95% CI = 1.55 - 9.72; p = 0.004) and LDH (HR = 4.04; 95% CI = 2.01-8.12; p < 0.001).