Peer Review File

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Reviewer A

The authors intend to demonstrate the significance of presence of STK11 mutation in stage III NSCLC patients treated with concurrent chemoradiation (CCRT) with or without consolidation ICI. This study and he results represent only an incremental advancement in the field, and do not contribute significantly to the knowledge or data gaps in the NSCLC biomarker and prognosis space. The authors should address the very limitations they themselves acknowledge, especially the small
sample size of the study, and imbalance between STK mutant versus wild type patient numbers. Further univariable and multivariable analyses correlating Progression-Free Survival with clinical and genomic factors are difficult to reconcile as they appear to be extremely non-convergent for the same variables. The PFS plot showing statistically significant association with STK11 status is not depicted. I would recommend a major revision addressing these issues.

Comment 1: “The authors should address the very limitations they themselves acknowledge, especially the small sample size of the study, and imbalance between STK mutant versus wild type patient numbers.”

Reply: Agree with the comment. Changed the sentence on page 11 lines 15-18 to First, the retrospective nature, the small sample size, and the imbalance in the number of $TK11^{w}$ versus $TK11^{m}$ patients are recognized and expected as NGS is not always done in stage III NSCLC, and most patients will have $TK11^{w}$.

Comment 2: “Further univariable and multivariable analyses correlating Progression-Free Survival with clinical and genomic factors are difficult to reconcile as they appear to be extremely non-convergent for the same variables. The PFS plot showing statistically significant association with STK11 status is not depicted.”

Reply: In our multivariable model, we accounted for as many known and suspected indicators of survival in our evaluation of the effect of STK11 mutational status on PFS as the data would allow. Variable filtering and inclusion into a multivariable model based solely on those that are significant on univariable analysis may not always be reflective of the best model, since the effects of confounding can impact the results (https://onlinelibrary.wiley.com/doi/pdf/10.1111/tri.12895). We see one such example in the case of ECOG status and STK11 mutational status, which is a case of negative confounding or suppression. As seen in Table 1, ECOG status is not equally balanced by STK11 mutational status; all those with ECOG 2-3 do not have a STK11 mutation. ECOG 2-3 is linked to worse survival outcomes in general. The imbalance

of ECOG 2-3 will make survival appear worse for the non-mutated STK11 group, drawing them closer to the mutated STK11 group. Thus, it is impossible to know the independent effect STK11 mutational status has on PFS without adjusting for ECOG status. When accounting for ECOG status, we can see that the suppressor effect is eliminated and the effect of STK11 mutation can be evaluated free of this confounding.

The Kaplan Meier curves in Figures 1 and 2 are reflective of the univariable effect of STK11 mutational status on PFS and OS. Unfortunately, Kaplan Meier curves cannot be drawn for multivariable models. These curves are displayed as a graphical representation of the underlying survival rates between STK11 mutation groups to aid in interpretation of the final multivariable model which accounts for other clinical covariates known to be associated with survival.

No changes made in the text

Reviewer B

The present manuscript reports on the prognostic and predictive role of STK11 mutations on stage III NSCLC patients treated with concurrent chemoradiotherapy +/- consolidative immunotherapy.
This is a very hot topic and no previous studies have investigated the impact of STK11 mutations in these patients.

Some comments:
• In the methods, it should be included some additional details on methodologies for STK11 mutations determination: Which platform? Tissue and/or plasma?
• Recently, some studies have questioned the use of consolidative immunotherapy in stage III inoperable oncogene-addicted NSCLCs (Hellyer JA, et al. J Thorac Oncol. 2021; Aredo JV, et al. J Thorac Oncol. 2021). The results of these studies together with the present one might question the use of durvalumab in all stage III NSCLCs after concurrent chemo-radiotherapy, regardless of molecular status of the tumor.
A recent study (Krishnamurthy N, et al. Eur J Cancer 2021) showed that STK11 mutations correlate with a poor prognosis regardless of therapy. However, STK11 alterations alone did not associate with inferior immunotherapy outcome in the pan-cancer setting or in NSCLC. These results should be commented.
• Gene names should be italicized

Comment 1: “In the methods, it should be included some additional details on methodologies for STK11 mutations determination: Which platform? Tissue and/or plasma?”

Reply: Agree with the comment. Changes made as mentioned below

Changes in the text: Added "*STK11*^{m} was identified by next generation sequencing of the tumor tissue using the platform of choice available in the institutions participating in this study.," page 6, line 21-22.

Comment 2: "Recently, some studies have questioned the use of consolidative immunotherapy in stage III inoperable oncogene-addicted NSCLCs (Hellyer JA, et al. J Thorac Oncol. 2021; Aredo JV, et al. J Thorac Oncol. 2021). The results of these studies together with the present one might question the use of durvalumab in all stage III NSCLCs after concurrent chemo-radiotherapy, regardless of molecular status of the tumor."

Reply: Agree with the comment. Reviewed and added information from mentioned articles.

Changes in the text: Added "In another driver mutation, the *EGFR* rather than *STK11*, it was questioned on retrospective studies whether there is significant benefit from durvalumab after CCRT.[21,22] Page 10 lines 6-8

Comment 3: "A recent study (Krishnamurthy N, et al. Eur J Cancer 2021) showed that STK11 mutations correlate with a poor prognosis regardless of therapy. However, STK11 alterations alone did not associate with inferior immunotherapy outcome in the pan-cancer setting or in NSCLC. These results should be commented."

Reply: Agree with the comment. Reviewed the article and added a sentence as below.

Changes in the text: Added "In a cohort of 60 patients with *STK11*^{m} cancers that included NSCLC and others, *STK11*^{m} correlated with poor prognosis without specifically observing inferior outcomes associated with immunotherapy.[23]," page 10, lines 8-10.

Comment 4: "Gene names should be italicized"

Reply: Agreed. All gene names were reviewed and italicized.

Changes in the text: throughout the whole document.

Reviewer C

In my opinion, the manuscript soffers from different crucial limitations for the publication of this manuscript:

- In the text, the clinical issue is not well documented. I woudl uggest to better describe the clincial setting of this paper.

- In the text, the authros did not cosider any EGFR mutational status. In my opinion, this aspect is fundamental for the molecular stratification of NSCLC patients.

- Paptient cohort is very small for the identification of statistically relevant data.

- In the text, the authors did not classify STK11 mutations detected. Today, qualification of detected mutation is preordinant for molecualr approach.

- Histological classification for enrolled patient is not adequately discussed. In my opinion, this aspect should be better discussed.

Comment 1: “In the text, the clinical issue is not well documented. I would suggest to better describe the clinical setting of this paper.”

Reply: We agree. Changes as below.

Changes in the text: We changed / edited the following paragraph: “The effect of *STK11*^{m} on outcomes of stage III NSCLC treated with curative intent is unknown. It is unclear whether *STK11*^{m} affects prognosis of stage III NSCLC or predicts response to ICI consolidation after CCRT. In advanced stage NSCLC, *STK11*^{m} has been associated with poor response to chemotherapy and ICI, and inferior survival outcomes.[9-15] In this study, we sought to explore *STK11*^{m} as a prognostic genetic alteration in stage III NSCLC patients managed with definitive chemoradiation +/- consolidative ICI.

Comment 2: “In the text, the authors did not consider any EGFR mutational status. In my opinion, this aspect is fundamental for the molecular stratification of NSCLC patients.”

Reply: We agree this is important. We added the text mentioned below to the Methods. However, to further expand on the EGFR issue; it is accepted in the lung cancer literature that EGFR mutations are usually mutually exclusive with other driver mutations (STK11 in our paper’s case). So, it is expected the that the 11 patients with STK11 mutation in our study will be wt EGFR and ALK. However, acknowledging that EGFR status is not collected for the wt STK11 patients is important, and so, the text below was added to methods.

Changes in the text: “Information about other NSLCC driver mutations such as *EGFR* and *ALK* were not collected”. Page 7 lines 1-2

Comment 3: “Patient cohort is very small for the identification of statistically relevant data”

Reply: We agree the sample size is small; however, this is the largest we have in the

literature in terms of stage III NSCLC with STK11 mutation. We tried as much as possible not to exaggerate conclusions in this paper as we only mentioned that PFS was worse for the patients with STK11 mutation which corresponds with the literature about this mutation in stage IV NSCLC. We avoided commenting on OS in the abstract conclusions, we also clearly indicated larger studies are needed to confirm the findings in this study, and whether there is interaction between STK11 status and response to immunotherapy.
I am including again here the response from our statisticians about the validity of the statistical tests used and the interpretation of the results:
": In our multivariable model, we accounted for as many known and suspected indicators of survival in our evaluation of the effect of STK11 mutational status on PFS as the data would allow. Variable filtering and inclusion into a multivariable model based solely on those that are significant on univariable analysis may not always be reflective of the best model, since the effects of confounding can impact the results (https://onlinelibrary.wiley.com/doi/pdf/10.1111/tri.12895). We see one such example in the case of ECOG status and STK11 mutational status, which is a case of negative confounding or suppression. As seen in Table 1, ECOG status is not equally balanced by STK11 mutational status; all those with ECOG 2-3 do not have a STK11 mutation. ECOG 2-3 is linked to worse survival outcomes in general. The imbalance of ECOG 2-3 will make survival appear worse for the non-mutated STK11 group, drawing them closer to the mutated STK11 group. Thus, it is impossible to know the independent effect STK11 mutational status has on PFS without adjusting for ECOG status. When accounting for ECOG status, we can see that the suppressor effect is eliminated and the effect of STK11 mutation can be evaluated free of this confounding.

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Changes in the text: No changes made.

Comment 4: "In the text, the authors did not classify STK11 mutations detected. Today, qualification of detected mutation is preordinant for molecualr approach."

Reply: Agree that specific STK11 mutation may have significance. However, the small sample size included in this study impedes us from inferring significant correlation between even smaller subsets of STK11 specific mutations and outcomes. Like most of the other clinical literature published in stage IV NSCLC and STK11, the focus has been on "mutation versus no mutation" at this stage. This might change in the future when there is more robust database. We did not collect the specific

STK11 mutation specific subtypes.

Changes in the text: Added “Specific STK11 locus alteration information was not collected” on page 6 line 21 and page 7 line 1.

Comment 5: “Histological classification for enrolled patient is not adequately discussed. In my opinion, this aspect should be better discussed.”

Reply: We agree. Changes made as below.

Changes in the text: Added “Histology was divided into non-squamous and squamous NSCLC. Non-squamous histology was predominant in both the *STK11^{m} and STK11^{m}* groups comprising 62.7% and 72.7%, respectively, without noticing statistically significant difference (P = 0.73). page 8, lines 4-7.