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Reviewer A

Thank you for the opportunity to review this interesting work.

Some general comments;

Interesting publication of your centres experience with STK11 mutant NSCLC. I feel the addition of the TCGA data analysis somewhat jarring with the rest of the study. Perhaps this could be excluded or published separately? Doing so may give the manuscript greater clarity. I also feel some of the conclusions you have made from your centres experience may be more hypothesis generating to guide future research.

Response: Our analysis of clinical data from our patients reports how STK11 co-mutations impact clinical outcomes and response to immunotherapy. We have included the TCGA analysis as it supports our main finding about the how STK11 co-mutations impact clinical outcomes and immune microenvironment. We agree with the reviewed that the conclusions are more hypothesis generating and we have re-worded our conclusions to clarify that.

Specifically;

Comment 1: Line 76 – It would be useful to include a reference for your stated frequency of STK11 mutations in NSCLC.

Reply: Reference citing frequency of STK11 mutations has been added (Skoulidis et al)

Changes in the text: page 4, line 84

Comment 2: Line 86 - It would also be worth mentioning the retrospective assessment of STK11 in KEYNOTE042 (doi: 10.1158/1538-7445.AM2020-CT084) and KEYNOTE189 (doi: 10.1158/1538-7445.AM2020-LB-397) which is quite relevant to your study.

Reply: KEYNOTE 189 discussion (Gadgeel et al) & KEYNOTE042 (Cho et al) has been added

Changes in the text: page 13, lines 309 to 315

Comment 3: Line 143 – it is not clear that any associations using Wilcox, Chi square or Fisher's exact test have been used in the study so I am not sure that this is mentioned. Outside of the TCGA analysis it is not clear that any formal statistical

significance testing has been performed (which is reasonable given the numbers used but this should be mentioned in your discussion).
Reply: Statistical significance testing has not been performed due to the sample size and this is now listed as a limitation in the discussion.
Changes in the text: page 15, line 362

Comment 4: Line 177 – would recurrence free survival (RFS) be a more relevant outcome after surgery?
Reply: For stage I-III tumors, PFS has been corrected to RFS. Definition for RFS as a measure has been added to 'variables' section of methods.
Changes in the text: page 6, lines 132-133 & Table 2

Comment 5: Line 192 – Durable clinical benefit (DCB) may be a relevant outcome but you have not mentioned capturing this in you "variables" section.
Reply: Definition for durable clinical benefit has been added to the 'variables' section in Methods.
Changes in the text: page 6, lines 133-135

Comment 6: Line 196-197 – Immunotherapy adverse events are mentioned here and this has not been brought up in the methods as a variable that was being captured.
Reply: We have added treatment-related adverse events as a variable that has been collected in the methods (variable) section.
Changes in the text: page 5, lines 122

Comment 7: Line 204 – I do not think you can really comment on the effect of PDL1 TPS on response based on 3 patients.
Reply: we have deleted the comment about effect of PDL1 TPS on response as PDL1 data was only available for 6 patients
Changes in the text: page 10, lines 235

Comment 8: Line 2-18-228 – I would include this section as a separate heading. It would also be helpful to the reader to include % of tumors in each respective subtype in the text.
Reply: TCGA immune gene expression signature analysis has been added as a separate heading.
Changes in the text: pages 11, line 254

Comment 9: Line 215-216 – What is the SKT11/TP53 co-mutated group compared to? STK11mutant/TP53wild type or STK11wildtype/TP53 mutant? Both?

Reply: Comparison was made to STK11-wild/TP53-wild tumors. This has been specified in the text.
Changes in the text: page 12, line 277

Comment 10: Line 232 – 241 – I think your conclusions are too strong and in the absence of formal statistical significance testing not appropriate. It is confusing to the reader to state that "the presence of KRAS co-occurring mutations are negative predictors of response to immunotherapy" and "TP53 co-mutations may have a beneficial effect on responses to systemic therapies" when you talk of 5/6 KRAS with durable response and 5/6 with TP53 with DCB. I think this needs significant rewording to enhance clarity to the reader.
Reply: we have reworded these to say "both low TMB and the presence of *KRAS* co-occurring mutations may be associated with poor response to immunotherapy, while *TP53* co-mutations may be associated with improved responses to systemic therapies"
Changes in the text: page 12, lines 272-273

Comment 11: Line 239 – I don't think these results are similar to TCGA data analysis as there is no STK11 wild type comparator group when discussing your centres experience.
Reply: this has been corrected in the text to clarify that the improved survival is being reported for tumors with STK11/KRAS co-mutations using our center data
Changes in the text: page 12, line 277

Comment 12: Line 252 – I would not use the word "significant" here as this would imply significance testing has been performed (which it has not).
Reply: the word 'significant' has been deleted from the text
Changes in the text: page 12, line 288

Comment 13: Line 261-262 – I would argue that this study demonstrates that STK11 mutant tumours do worse with combination CPP than STK11 wildtype. There is no comparison to the addition or not of immunotherapy hence your statement is not appropriate.
Reply: this statement is for response to chemotherapy alone, we have ensured that the text specifies 'chemotherapy' and not combination chemoimmunotherapy.

Comment 14: Line 268 – it would be worth mentioning this finding (doi: 10.1016/j.ijrobp.2020.07.1187)
Reply: this study is now cited and reviewed in the Discussion section
Changes in the text: page 13, lines 320 to 322

Comment 15: Line 303-304 – It is unclear why KEAP1 mutation status would influence your ability to capture patient stage. This is confusing.
Reply: we have clarified this in the text that patient stage was not captured due to small sample size.
Changes in the text: page 15, line 367

Comment 16: Line 306-314 – Again I would argue that you haven't demonstrated that co-occuring mutations mediate the response to immunotherapy given the absence of formal statistical comparison.
Reply: we have modified text to say 'may be predictors' instead of 'are significant predictors' in Abstract as well as Discussion
Changes in the text: page 16, line 373 & page 3, line 58

Comment 17: Table 2 – I would omit the KEAP1 mutant column(s) given the small numbers involved. Also why is there a question mark in the 5th column for neoadjuvant chemotherapy (KEAP1-wt)?
Reply: We have presented data about KEAP1 co-mutated STK11 tumors as information about KEAP1 is not present in most of the papers on this subject and therefore our data provides additional information although the number of tumors with this co-mutation is small. The question mark in table 2 is a typo and has been deleted.
Changes in the text: table 2 on page 21

Comment 18: Figure 3 – Nice PFS graph. It would benefit from a formal test of significance and Log-rank test.
Reply: Statistical significance testing has not been performed due to the sample size and this is now listed as a limitation in the discussion.
Changes in the text: page 15, line 362

Reviewer B
The paper presents a small cohort of STK11 mutant NSCLC. The analysis requires major revision, but is scientifically sound. Main limitation is small sample size.

Comment 1 - you could provide the overall prevalence of STK11 mutations, i.e. from how many patients with available NGS data did you identify the 41? This also extends to table 1, i.e. are there relevant epidemiological differences compared to STK11wt or KRASmut/STK11wt tumors?

Reply: we have added that 323 patients had available NGS results. We agree with the reviewer that epidemiological factors may impact lung carcinogenesis but analysis f epidemiological data for more than 400 patients to analyzed this will be beyond the scope of this project.
Changes in the text: page 8, line 174-175

Comment 2 - l172 localized tumors would be st.I-II, while st. III is locally advanced
Reply: we have modified this to clarify that we are presenting results for stage I to III tumors and that loco-regional therapy includes surgery and radiation.
Changes in the text: Abstract, page 2, line 42; Results, page 9, line 196 and 199; page 9, line 208.

Comment 3 - l177: low sample size does not influence outcome; rather, small sample size does not allow interpretation of numerical differences in OS
Reply: the statement about low sample size influencing outcome has been deleted to avoid any confusion
Changes in the text: page 9, line 201

Comment 4 -l184 the BSC rate of 20% seems high, which may be of interest. Is this significantly different to the above mentioned STK11wt cohorts at your center?
Reply: About 20-25% of patients with stage 4 NSCLC do not receive treatment and this is similar to our patient population included in this study (David et al, Journal of Thoracic Oncology, 2017)

Comment 5 -l186 should be able to quantify objective response (ORR)
Reply: our ability to quantify the ORR is limited as we don't have access to the radiographic images for some patients' due to multiple reasons: patient did not get scan at progression, or scan was done at a hospital outside our health network.

Comment 6 -l183-200 swimmer plots would be suitable to visualize this case series. When you say OS was better/worse than, please provide HR and p-value or state it was numerically different with no statistical confirmation due to low sample size
Reply: Statistical significance testing has not been performed due to the sample size and this is now listed as a limitation in the discussion.
Changes in the text: page 15, line 362

Comment 7 -table 3-5 think how to express the key messages from the tables visually with figures and move all tables into the supplement.
Reply: As tables 3 to 5 have a lot of data that is being presented, it will be difficult to

translate them into figures.

Comment 8 -l232-241 avoid reiterating results. In so far as you find it necessary, say "is" if you have statistical significance confirmed in a second, independent cohort. That is, never in your paper.
Reply: this has been corrected in the text to clarify that the improved survival is being reported for tumors with STK11/KRAS co-mutations using our center data
Changes in the text: page 12, line 277

Comment 9 -l243-255 Be very cautious with the Skoulidis paper. There have subsequently several post-hoc analyses of large trials which must be discussed alongside. After the Skoulidis paper, STK11 was in all datasets prognostic but not predictive for ITx
Reply: Additional references to support that STK11 is associated with poor prognosis with immunotherapy for lung cancer have been added (Fleur et al, Papillon-Cavanagh et al, Krishnamurthy et al)
Changes in the text: page 12, line 285

Reviewer C
The authors have presented an excellent analysis of STK11 mutated NSCLC from both clinical outcomes and TCGA datasets. The data, statistical analysis and interpretation of results is very relevant and valid, and provide a significant advancement in knowledge about STK11 mutated NSCLC and associated therapy response predictions based on co-occuring mutations and TMB status.

Reviewer D
This manuscript described the co-mutation effects in STK11 mutant NSCLC from a retrospective analysis of clinical samples and TCGA data. STK11 mutant NSCLC has been reported to have dramatic impact on clinical outcomes of patients treated with immune therapy. The information described in this manuscript is interesting and clinically relevant. Nevertheless, the following should be addressed:
Comment 1) The authors mentioned "localized therapy" in Abstract and in Results. It is not clear what type of therapy described in the manuscript should be defined as localized therapy.
Reply: we have modified this to clarify that we are presenting results for stage I to III tumors and that loco-regional therapy includes surgery and radiation.
Changes in the text: Abstract, page 2, line 42; Results, page 9, line 196 and 199; page 9, line 208.

Comment 2) it will be helpful to provide information about number of patients with STK11 mutations identified in TCGA data analysis.
Reply: The number of patients in TCGA analysis by STK11 mutation status has been added to the text
Changes in the text: page 10, line 244

Comment 3) Is there any difference in clinical outcomes among different types of STK11 mutations (frameshift, truncating, and missense)?
Reply: we did not analyze the difference in outcomes by type of STK11 mutation due to the limited sample size.

Comment 4) The authors didn't mention about allele frequencies of STK11 mutations and possible effect from intratumoral heterogeneity or subclonal effects, which may also impact on clinical outcomes.
Reply: We did not have data on allele frequency and this has been added as a limitation in the Discussion section.
Changes in the text: page 15, lines 364 to 365.