Peer Review File

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Reviewer A

This is a well written letter to the editor that provides interesting and compelling data. The authors provide a useful analysis on KEAP1/NFE2L2/STK11 mutations by using larger databases. However, given the limits of these databases in which clinical data is sparse, the authors data while compelling is not confirmatory as they state. Nonetheless, this is helpful data and warrants publication.

Reply:
We thank Reviewer A for your time and careful consideration in reviewing our manuscript. We also thank you for your kind words. We have incorporated the suggested edits and believe these changes improve the scientific validity of our letter.

1. Page 1, line 12-13 authors write "there are no clinical biomarkers that predict response to radiotherapy". This is not 100% valid. There are biomarkers (i.e. HPV status and now data on de-intensified RT in this setting). I would suggest authors change to there are limited biomarkers to acknowledge that there are indeed biomarkers and that this is an active area of research.

Reply 1:
We thank the reviewer for bringing attention to this sentence, as we may not have been clear that we were referring specifically to NSCLC. There are multiple biomarkers that guide standard-of-care systemic therapies in NSCLC, including EGFR, KRAS, ALK, ROS, and PD-L1. These biomarkers do not guide radiotherapeutic strategies. We have changed the sentence to specify NSCLC (line 10-12):

"Although biomarkers routinely guide treatment decisions for systemic therapies in NSCLC, there are no clinical biomarkers that predict response to radiotherapy."

2. How do the authors definite "pathogenic mutations"?

Reply 2:
Putative driver (pathogenic) mutations versus variants of unknown significance were defined via OncoKB and Cancer Hotspots annotations in the cBioportal (cbioportal.org). This has now been defined in the text (lines 28-30):

"Putative driver (pathogenic) mutations versus variants of unknown significance were defined via OncoKB and Cancer Hotspots annotations in the cBioportal (cbioportal.org)."

3. Authors write that "here, we confirm that KEAP1/NFE2L2/STK11 mutations are common

and predictive", one would argue this retrospective study that has limited clinical information on patients does not confirm this finding, but provides further evidence to support this finding

Reply 3:
We agree that more definitive confirmation of predictive biomarkers requires prospective validation. We have changed the language to reflect this well-received critique (line 50-51):

"Here, we provide further evidence that KEAP1/NFE2L2/STK11 mutations are common and predictive of outcomes after radiotherapy in the TCGA database."

4. I believe that authors should discuss the limitations of this work within the manuscript.

Reply 4:
We agree and acknowledge the limitations of our retrospective review and we have now stated this more explicitly in our manuscript (line 56):

"Limitations of our study include retrospective analysis of datasets with limited clinical information available. Prospective validation of these findings is warranted."

Reviewer B

This is an interesting and well-written manuscript addressing alterations in the NRF2 pathway as potential biomarkers of radioresistance in NSCLC. Specifically, the authors provide evidence that KEAP1/NFE2L2/STK11 mutations are common and predictive of outcomes after radiotherapy in the TCGA database. My comments are minor.

Reply:
We thank Reviewer B for your time and consideration of our manuscript. We appreciate your kind words.

1) While the analyzed sample consisted of non-metastatic NSCLC patients it would be informative to provide stage and percentages.
Reply 1:
We agree that this information would be informative to the reader. It is now included in the table description.

2) Please indicate separately the sample size for each of the three prospective cohorts used in the analysis.

Reply 2:
This information is now provided throughout the text, as requested.

3) p53 mutations conferred enhanced radiosensitivity in a variety of cancers and Li-Fraumeni families tend to be at higher risk of secondary radio-induced malignancies. Is it known if

KEAP1/NFE2L2/STK11 mutations are similarly more common.

Reply 3:
The reviewer asks an interesting question relating to familial disorders that may be related to mutations in the KEAP1/NRF2 pathway. The most famous example is mutations in STK11 being associated with Peutz-Jeghers syndrome, which is "an autosomal dominant disorder characterized by the growth of polyps in the gastrointestinal tract, pigmented macules on the skin and mouth, and other neoplasms." STK11 is also associated with testicular germ cell tumor, and other cancers including familial pancreatic cancer and cutaneous malignant melanoma. KEAP1 mutations are similarly associated with multinodular goiter, Sertoloi-Leydig cell tumors, and squamous cell carcinoma. Interestingly, it is also associated with auto-immune disorders like multiple-sclerosis and Chron's disease in GWAS studies, suggesting that mutations lead to constituently active inflammatory and/or oxidative stress-response pathways. Finally, mutations in NFE2L2 may be activating or inactivating depending on if they involve the N-terminal Neh2 domain. NFE2L2 mutations have, therefore, been associated with lung squamous cell carcinomas but also immunodeficiency, developmental delay, hypohomocysteinemia, and abnormal height.

Because of the limited scope of our short research letter, we have excluded this topic from our discussion.

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