TLCR TRANSLATIONAL LUNG CANCER RESEARCH
AN OPEN ACCESS JOURNAL FOCUSING ON CLOSING THE GAP BETWEEN "BENCH AND BEDSIDE"
IMPACT FACTOR 6.498

Peer Review File

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Reviewer A

The present work assesses the correlates of tumor volume in advanced NSCLC. Particularly, they study the interplay between tumor volume, tumor DNA detection on cfDNA using a small gene-panel with high sensitivity, and overall survival. Owing to the increasing importance of cfDNA determination/applications in advanced NSCLC, knowing the factors that influence it is of interest to the oncology community.
I have several questions/comments:

Comment 1: Since there is an association between total volume (as binary) and cfDNA detection, and also between presence of bone lesions and cfDNA detection, I was wondering about the association between total volume (as binary feature for instance, see table 1) and metastatic lesions in different organs (ie. bone, viscera, liver, …). They could provide with the data in the same table 1 and analyze possible differences between the groups.
Reply 1: We have updated Table 1 to include a comparison of the number of patients with metastases in bone, viscera, liver, and brain among patients with total tumor volume less than or equalt to 65 mL versus greater than 65 mL. We have included in the results section the findings that were significant or trending toward significance.
Changes in the text: Table 1; Results lines 231-234.

Comment 2: What is the number of patients with presence of both bone and liver metastases?
Reply 2: There are 12 such patients and the results have been updated to include this.
Changes in the text: Results lines 234-235.

Comment 3: In table 1, is there a statistical difference between "other histology" and "volume as binary"? Is there a statistical difference between KRAS mutations and "volume as binary"? Both features seem not evenly distributed between the two groups.
Reply 3: We have updated Table 1 to collapse adenocarcinoma and squamous cell carcinoma into a single group and compare that with "other" histology. There was not a statistically significant difference between the groups, although there were numerically more patients in the group with total tumor volume greater than 65 mL who had "other" histology, and we note this in the results section. We further updated Table 1 to compare the number of patients with mutations in KRAS as well as EGFR, ALK, and TP53 between total tumor volume groups and no differences were found that were significant or trending toward significance.
Changes in the text: Table 1, Results lines 235-238.

Comment 4: In the multivariate analysis of table 3, did they adjust for clinical features (i.e. including presence of certain lesions)? Could they analyze liver

metastases in the Table 3, separate from other visceral metastases?
Reply 4: In our original multivariate analysis for Table 3, we included only variables that were significant on univariate analysis (except for total tumor volume as a continuous variable, which was significant on univariate analysis but not included in multivariate analysis since total tumor volume as a binary variable was used instead). The univariate analysis did include presence of metastases in certain tissue sites, such as bone, viscera, and brain. Presence of tumor in bone and viscera were carried forward into the original multivariate analysis. We have updated the table 3 univariate analyses to include clinical features of age, sex, smoking status, histology, presence of tumor in liver, and liver tumor volume. Smoking status was found to be significant on univariate analysis and was carried forward into multivariate analysis, where there was a trend toward significance. This has been added to the results section

We further extended our analysis to include the above variables of age, sex, smoking status, histology, presence of liver tumor, and liver tumor volume in Table 4. This found age and presence of liver tumor to be significant predictors of survival on univariate analysis, which remained significant upon multivariate analysis. As a result of this additional analysis, total tumor volume as a binary variable is no longer statistically significantly associated with overall survival on multivariable analysis, however, we feel that this does not change our conclusions substantially and the results and discussion have been updated to include that total tumor volume has only a trend toward significance on multivariate analysis.
Changes in the text: Table 3, Table 4, Results lines 267-275, 286, 290-299, Discussion lines 315-316, 350-352, Conclusion line 400.

Comment 5: In the section "Predictors of Overall survival". "Total tumor 269 volume (HR: 1.71, 95% CI: 1.01-2.89, p = 0.046) and detection of cfDNA mutation (HR: 1.96, 95% 270 CI: 1.03-3.71, p = 0.040) remained significant predictors of overall survival (Table 4)." Bone metastases were really close to significance (p=0.056) and owing to the fact that the two significant variables are really close to 0.05, I would at least mention that bone were close to significant.
Reply 5: In the re-analysis we performed in response to comment 4, the results of the multivariate analysis changed and presence of bone metastasis is now significant. Total tumor volume and presence of liver metastasis trended toward significance. These changes have been noted in the results.
Changes in the text: Results lines 292-299.

Minor comments:

Comment 6: Please revised gene list provided on "2.3. Plasma NGS genotyping", lines 177 to 172. Note that not all names provided are gene names, see PD-L1, which is in fact encoded by CD274.
Reply 6: PD-L1 has been changed in the text to CD274. All other genes have been double checked and names are correct as listed in the text.
Changes in the text: line 192

Comment 7: To be consistent with how the cohort characteristics were provided from line 211 to 214, I would state the percentage of patients with ctDNA detection inn line 215, 78 (71%).
Reply 7: Text has been updated.
Changes in the text: Results line 238

Comment 8: Conclusions they do not discussed the fact that presence of bone metastases was associated with ctDNA but not their volume, could they discuss it?
Reply 8: Yes, this has been added to the discussion section.
Changes in the text: Discussion lines 329-333.

Comment 9: Line 289-290, "we were primarily interested in exploring factors that impact shedding dynamics of ctDNA", not sure the word dynamics is appropriate as they are assessing a single time point.
Reply 9: Text has been updated to remove the word "dynamics"
Changes in the text: Discussion line 317.

Comment 10: Line 312. "which showed that total tumor volume greater than 65 mL was the only significant factor in predicting likelihood of mutation detection", is that referring to multivariate table 3? If that is the case, presence of bone lesions remained significant as well.
Reply 10: This was a mistake and has been updated to reflect that total tumor volume was that most significant factor and had the greatest effect size magnitude.
Changes in the text: Discussion lines 342-343.

Comment 11: As CHIP mutations have been described to be present in cfDNA, I would mention as a limitation that no germinal correction was applied on cfDNA, thus some mutations detected might be due to this process and not "tumor mutations", which could influence their findings.
Reply 11: Yes, this is a fair point and has been added to the discussion on limitations.
Changes in the text: Discussion lines 385-386.

Reviewer B

This study, "Tumor Volume as a Predictor of Cell Free DNA Mutation Detection in Advanced Non-Small Cell Lung Cancer", begins to answer an important question related to tumor size and feasibility of ctDNA-based genomic profiling. Some comments to consider are listed below:

Comment 1: The authors address the limitation of a small targeted NGS panel in the discussion - this deserves to be expanded to address the technical limitations of an assay that detected somatic alterations in 71% with 0.1% limit of detection and how it compares to other published cohorts of larger ctDNA panels with detection rates >80% in newly diagnosed advanced lung cancer and higher concordance rates with tissue.

Reply 1: The discussion of limitations has been expanded to include discussion of recent publications with higher rates of mutation detection using larger panels.
Changes in the text: Discussion lines 370-378.

Comment 2: How would the authors expect the data to change with larger, more sensitive assay? Please add to discussion.
Reply 2: Discussion has been expanded to include this.
Changes in the text: Discussion lines 380-384.

Comment 3: These data are presented in aggregate form - to improve the reader's understanding of individual patients, please provide a supplemental table for each patients tumor volume, sites of metastasis and corresponding tumor volumes, mutations identified in ctDNA, VAF, mutations found in tissue NGS
Reply 3: This is being provided as a supplementary excel spreadsheet table.
Changes in the text: Supplementary table, Results lines 252-253.

Comment 4: The authors allude to tumor metabolism being a major contributor of ctDNA shed in the circulation - can any analyses be performed to expand on metabolic activity and tumor shed? Are any trends seen that increase/decrease cfDNA/ctDNA quantity in the blood?
Reply 4: This is the subject of an ongoing analysis by another researcher in our group and is not yet published. We are not planning to include metabolic analyses in the present paper, but our unpublished data thus far indicate that this is not likely a meaningful predictor of mutation detection in a large patient cohort at our institution.
Changes in the text: No update in present text.

Reviewer C

This study evaluated the tumour volume from the lung in combination with other metastatic sites as a prognostic factor for advanced non-small cell lung cancer patients. The study also aimed to associate the tumour volume results with the detection of ctDNA, the overall survival of patients and variant allele frequency. This work brings the measurement of tumour volume from metastatic sites in addition to primary lung tumour volume for the total tumour volume. It is an interesting work done with 110 patients enrolled in the study. Although the authors claim that the present work differs from others due to the detailed tumour volume delineation, I am not convinced of the novelty factor of the manuscript. Different reports have associated tumour volume with ctDNA levels in NSCLC studies, suggesting that tumour volume is a determinant of the feasibility of mutation detection in plasma. Also, other reports, as mentioned in the discussion section, have associated ctDNA levels with metastases in bones and viscera of lung cancer patients.

Comment 1: If the measurement of tumour volume is better using the technique in the present manuscript, I suggest the authors to include a detailed comparison between their detailed measurement technique and the others that have been used.

Reply 1: We do not have data to compare our method of tumor volume assessment with methods using lesion measurements on axial imaging. As such, and since a methods comparison is outside the scope of this paper, we have removed any value judgement on which method is better from the discussion.
Changes in the text: Discussion lines 359-364.

Comment 2: In addition, discuss the feasibility of measuring the volume as stated in clinical practice. How does this approach of measuring complement what has been done in the literature and what data could be lost if you decide to do the less detailed and more common analysis (calculations on cross-sectional imaging tumour measurements)?
Reply 2: There are limitations in feasibility of implementing our measurement methods in clinical practice. Our research group is currently working on automated collection of volumetric data using these methods, but this is not yet ready for publication. As such, we have indicated that future work may allow automated volumetric data collection and that this could improve feasibility. We will avoid making a direct comparison of what data may be lost by using our methods versus imaging measurement methods for volume determination since we do not have data on this.
Changes in the text: Discussion line 394.

Comment 3: Another limitation is regarding the lack of germline controls for sequencing analyses. I would suggest the authors include the mutation gene list as a horizontal bar graph to make it easier for the visualization of mutations and frequency.
Reply 3: Yes, the lack of germline controls is an important limitation as well. Discussion of this has been added to the limitations portion of the discussion section. We are also including a horizontal bar graph of the gene mutations as a supplemental figure.
Changes in the text: Discussion lines 385-386. Supplemental figure.

Comment 4: Also, Introduction should explain the disease burden of NSCLC and the clinical need. These articles give an overview on the NSCLC stats and unmet need (A Kulasinghe, Cancers 11 (3), 380, 2019; A Kulasinghe, Cells 9 (6), 1465, 2020 and J Kapeleris, Frontiers in Oncology, 2022)
Reply 4: We have updated the introduction to include discussion of these points.
Changes in the text: Introduction lines 120-121.

Reviewer D

Comment 1: The article is written in a correct and understandable way and the topic is of current interest. The article is well structured, the number of tables and figures is adequate and they provide useful information.
Reply 1: Thank you for the review.
Changes in the text: none

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