Peer Review File

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RESPONSE TO COMMENTS FROM REVIEWER A

This is an analysis of pre-treatment and on-treatment cell free DNA (cfDNA) among 50 patients with LS-SCLC to identify features predictive of disease recurrence. The authors identify on-treatment TP53 and RB1 mutations, specific fragmentome range, and fragmentome ratio as predictors of disease recurrence.

Main strength(s) of the manuscript: (#1) Addresses an important topic in the field - how can we identify high risk patients with LS-SCLC in order to potentially escalate therapy among these patients.

Main weaknesses of the manuscript: (#1) Minimal, fantastic work, excited for further work in this area.

COMMENT #1
Results: Consider adding a Table 1 with section 3.1 clinical characteristics.

RESPNSE #1
Thank you for suggesting the details. As recommended by the reviewer, we summarized the baseline characteristics of the study population in Table 1 and added them to the manuscript.

COMMENT #2
Results: Would favor changing language from "wild type" TP53 and RB1 to "non-detected" if the pre-treatment detected variant is cleared. I think this would be a more clinically oriented term.

RESPONSE #2
Thank you for the clarification. The main reason we chose the terminology "wild type" in the manuscript was to differentiate the patients with ctDNA detected but without genomic alteration from patients with ctDNA not detected. Hence, to avoid confusion, we maintain the word wild-type to indicate the patients with ctDNA detected but no mutation identified from *TP53* and *RB1* gene.

COMMENT #3
Results 3.4: I think the p values in the last sentence are reversed.

RESONSE #3
As pointed out by the reviewer, we identified the typo in the indicated section. Based on the

reviewer's suggestion, we made modifications to our manuscript as below.

Page 12: Exploratory analysis showed that patients with detectable *RB1* mutations in their on-treatment samples had significant shorter a shorter OS time (*P* = 0.002), whereas *TP53* mutations showed shorter but not significant OS difference (*P* = 0.083) (Supplementary Fig. 2).

COMMENT #4
Discussion, line 363-364: This is the second evaluation of ctDNA to predict disease recurrence in LS-SCLC - see PMID 34589931

RESPONSE #4
Thank you for providing the evidence. Based on the reviewer's information, we change the indicated sentence as below.

Page 17: However, to our knowledge, this study represents one of the early research outcomes evaluating disease recurrence based on ctDNA analysis for patients with LS-SCLC.

COMMENT #5
Minor English language fluency edits are needed throughout

RESPONSE #5
We conducted additional English proofreading on our revised manuscript. Thank you.

RESPONSE TO COMMENTS FROM REVIEWER B

This paper describes state of the arts experiments with ctDNA on ways to predict the further development of SCLC in patients with limited disease.
So far without clear successes.
The reason to publish this manuscript could be to let readers know that this work was carried out.
On the other hand there are some negative points that argue agiamst publication.
1. There is no clear and feasible path to clinical improvement based on the predictions.
2. It is now clear that there are various subtypes of SCLC differing in transcriptrion of genes. So mRNA typing seems more relevant than ctDNA typing.

RESPONSE to REVIEWER B
Thank you for recognizing the value of our study. We agree with the key points made by the reviewer B that our study has limitation in clinical adaptability using ctDNA based approach. At the same time, as the transcriptional factor expression based SCLC subtyping is comprehensively investigated to predict the clinical efficacy and response to certain therapy, ctDNA might only provide the limited information. However, as the liquid biopsy approach including fragmentomics, methylation data, circulating tumor RNA based approach is investigated in multiple cancer type, we think our data has value to the readers that some of the investigation has been also attepted in

SCLC using ctDNA.

RESPONSE TO COMMENTS FROM REVIEWER C

Park et al. demonstrated the utility of combining genomic and fragmentomic approaches to detect ctDNA in limited disease small cell lung cancer, and explored the relevance of these ctDNA parameters in prognosticating patients based on recurrence-free survival. The authors used a commercially available panel targeting 106 cancer-related genes to identify genetic alterations in plasma DNA. In parallel, fragment size distribution was also analyzed, given the rationale that the proportion of smaller fragments are enriched in tumor-derived DNA. The article is well written, with a straightforward message. However, addressing and clarifying the issues below must be carried out prior to acceptance.

COMMENT #1
Both cfDNA and leukocyte libraries were generated for targeted deep sequencing (section 2.3). However, use of data from leukocyte sequencing was not mentioned in the results. Did the authors check for clonal hematopoiesis? Did the authors use the blood cell data as control for mutation calling? Was this data used at all?

RESONSE #1
Thank you for the comment. For the analysis, 100ng of the genomic DNA were used for NGS. The target amount of sequencing was 5 Gb and the average of sequencing depth was 1,134x (from 300x to 1,560x) as a result. We gathered PBMC variant calls that had less than 20% VAF from all PBMC samples. These variants comprised a set of blacklist alterations which were assumed to repeatedly occur sequencing errors or noise variants, including clonal hematopoiesis. [PMID: 34381078]

COMMENT #2
To which depth was each library sequenced? What was the average depth achieved for each target region? These should be included as supplemental data.

RESPONSE #2
Our platform reached an average of approximately 1,979× for cfDNA samples. (median 2,102x, min 682x, max 2,615x. Please refer to the Supplementary Table1 for the details.

COMMENT #3
What were the "in-house filtering steps" applied during variant calling? Are these published? At the very least, the read-depth and allele frequency cut-offs should be indicated in the methods section.

RESPONSE #3
In case of cfDNA data, we took variants that have at least 1% of VAF and 10 variant reads, considering small amount of ctDNA in plasma cfDNA. We lastly filtered out additional errors or noise with visual inspection using the IGV browser18 and finalized the tumor-driven mutations from each cfDNA.

[PMID: 34381078]

COMMENT #4
The authors should also mention which genetic alterations could be identified by the panel (e.g., SNVs, copy number alterations, indels, structural variants, etc.).

RESPONSE #4
Thank you for the clarification. The panels we used were available for SNVs, copy number alteration, and structural variants. Please find the following site for details regarding the list of genes available for each genomic alteration.

COMMENT #5
What were the criteria used to identify ctDNA-positive samples? The detection of at least 1 variant targeted by the panel? Is there molecular data from tumor tissue to verify that the variants are tumor-specific?

RESPONSE #5
There weren't paired tissue NGS data. We defined "ctDNA-positive" as detecting at least 1 variant targeted by the panel. The alteration detected by AL100 is a cancer-originated variant. If the circulating tumor DNA were detected, the short fragment would be in the bloodstream.

COMMENT #6
Fragmentomics analysis is typically performed on whole genome sequencing data of cfDNA. In this case, if I understood correctly, the authors used the data from the panel sequencing which is enriched at targeted regions and therefore could bias the fragment sizes that are sequenced (that is, enrichment may not be equal for all target regions and affected by probe length/sequence). Could the authors comment more on this issue? How is this bias mitigated?

RESPONSE #6
Thank you for point out a very important issue. AL100 also includes copy number variation analysis, and is a kit developed that is difficult to prove because capture bias does not occur in all sequenced areas. So the AL100 capture set doesn't really have much of an impact, it just affects where it affects fragmentation.)

COMMENT #7
Did the authors perform downsampling of data to normalize coverage across samples? This was not explicitly described. This step is important to be certain that changes in size distribution and fragment size ratios are independent of sequencing depth.

RESPONSE #7
Although the downsampling did not proceed, the throughput of among samples was produced as the same amount. The sequencing depth of cfDNA samples was similar, and it would not affect the fragmentomics analysis.

COMMENT #8
Related to #7, what sequencing depth is sufficient to be able to reproduce the results presented in the manuscript?

RESPONSE #8
The median of cfDNA concentration was 20ng (from 8ng to 20ng) and the target of data generation was 20Gb(from 14Gb to 30Gb). Our platform reached an average of approximately 1,979× for cfDNA samples (median 2102x, min 682x, max 2615x). (Supplementary Table; Table S1)

COMMENT #9
Please mention the criteria or reference used in order to confirm radiological progression (e.g., RECIST guidelines).

RESPONSE #9
For the confirmation of radiologic response, we used RECIST v1.1 based measurement.

COMMENT #10
Please include the softwares and programs used for statistical analyses and data visualization in the methods section.

RESPONSE #10
Statistical tests were performed using R, version 4.0.3 (http://www.r-project.org). Kaplan-Meier curves were constructed to analyze overall survival, and subgroups were compared using log-rank tests. P-values <0.05 were considered statistically significant.

Based on the reviewer's comment, we added the following sentence to the method section.
Page 9: We conducted a statistical analysis using R, version 4.03 (http://www.r-project.org). P-values <0.05 were considered statistically significant.

COMMENT #11
Results
The authors should include a tabular summary of section 3.1. The distribution of male/female patients should be included.

RESPONSE #11
Thank you for the suggestion. The same suggestion has been made by the other reviewer, and we added baseline demographics as Table 1 in Section 3.1.

COMMENT #12-13
#12 On page 5, line 183: "non-PeR group" was mentioned twice and should be corrected.

#13. On page 5, line 193: ctDNA must be changed to cfDNA.

RESPONSE #12-#13
We apologize for the typos. We made changes in indicated word in the manuscript

COMMENT #14
What was the reason for excluding on-treatment ctDNA-negative (presumably based on panel sequencing data) samples from fragmentomics analysis? Performing this analysis on these samples could actually be an opportunity to increase the relevance of this approach as potentially complementary to conventional genetic analysis. In samples which are RB1 wild-type, could fragmentomics data alone classify PeR vs. non-PeR patients?

RESPONSE #14
In this analysis, it was determined that the ND sample did not contain sufficient cancer-originated DNA for analysis. Please understand that this model is developed to distinguish the cancer-originated fragmentation pattern, not an individual characteristic.

COMMENT #15
In section 3.3, the authors mentioned clonality being calculated from maximum VAF value. How exactly was this done? The “clonality” term itself is somewhat confusing and maybe the more appropriate term is tumor fraction, which is more commonly used in liquid biopsy literature. How were very low/low/high clonality defined? If clonality is based on maximum VAF, Fig. 2C becomes redundant and could be removed since the correlations between fragment sizes and ratios with maximum VAF are shown more comprehensively in Fig. 2D.

RESPONSE #15
That’s correct, clonality representing max VAF. Although it is correct to repeat it by dividing it into three groups, one is to display the trend and the other is to divide the groups and display them significantly. [PMID: 33727690]

COMMENT #16
The p-values should be shown for each correlation graph in Fig. 2D.

RESPONSE #16
Thanks for the comment. The fixed figure (with P-value added) was attached.

COMMENT #17
On page 6, lines 256-257: there is a mix-up in which mutation showed significant effects (i.e., p=0.002 is significant, p=0.083 is not significant).

RESPONSE #17
Thank you for point out the details. We also noticed that result is mis-described in the indicated

section. We change the following sentence as below.

Page 12: Exploratory analysis showed that patients with detectable *RB1* mutations in their on-treatment samples had significant shorter a shorter OS time (*P* = 0.002), whereas *TP53* mutations showed shorter but not significant OS difference (*P* = 0.083) (Supplementary Fig. 2).

COMMENT #18
For Fig. 4A, AUC values should be shown on the plots.

RESPONSE #18
Thanks for the comment. The fixed figure is attached.

COMMENT #19
In section 3.5, how was the optimal cut-off identified from ROC plots? This must be mentioned in the methods section.

RESPONSE #19
Using multipleROC (https://github.com/cardiomoon/multipleROC), we calculated the optimal cut-off value where each value predicts the non-PeR group.

We added following sentence to method section
Page 9: Receiver operating characteristics (ROC) curves were drawn to evaluate the predictive values of variables using multiple ROC (https://github.com/cardiomoon/multipleROC), and optimal cut-off values were calculated to predict the non-PeR group.

COMMENT #20
Why did the authors label the patients with wild-type RB1 and low fragmentation ratio as "high-risk group", and patients with mutant RB1 and high fragmentation ratio as "low-risk group." Could this be an error? It is counterintuitive that there is survival benefit to the "high-risk group" and the "low-risk group" has shorter RFS.

RESPONSE #20
Thank you for very important question. It was typo. We change the manuscript as below.

Page 13: Combining the fragmentation ratio status with the *RB1*-mutation status revealed an enhanced survival benefit in the ctDNA-based low-risk group with wild-type *RB1* and a low fragmentation ratio (n = 10), as the median RFS was not achieved during a median follow-up duration of 20.1 months. This group comprised 10 patients with a PeR (100%) and 0 patients with a non-PeR (0%). In contrast, the shortest median RFS (5.0 months) was observed in the ctDNA-based high-risk group with mutant *RB1* and a high fragmentation ratio (n = 10). This group comprised 1 patient with a PeR (10.0%) and 9 patients with a non-PeR (90.0%). The HR between the ctDNA-based high- and low-risk groups was 7.55 (95% CI: 2.14–26.6, *P* = 0.002) (Fig. 4E).

COMMENT #21
Based on Fig. 4E, it appears that RB1 mutation alone is enough to classify groups based on short and long PFS, and I wonder if there is added benefit of performing fragmentation analysis. In other words, as long as there is a mutant RB1, it does not matter whether the fragmentation ratio is high or low, the clinical prognosis will be worse. This is further corroborated by Fig. S2B, wherein RB1 mutation alone is as good, if not a better prognostic classifier. The authors also would not be able to argue that there is added value in including the fragment ratio as a classifier because the cohort is statistically underpowered (Group C n=1; Group D n=10). This is why my comment #4 above is quite relevant in establishing the independent/complementary relevance of fragmentomic analysis from genetic analysis. If the authors perform the fragment size analysis on the remaining ctDNA-negative on-treatment samples, and could show that it is effective in such cases, this would increase the impact of the manuscript.

RESPONSE #21
Thank you for the question. It was similar to previous question related to comment #14. It was determined that the ND sample which did not contain sufficient cancer-originated DNA for analysis. Please understand the limitation of this study.

COMMENT #22
The relevant p-values must be displayed on Fig. 4E.

RESPONSE #22
Thanks for the comment. The fixed figure was attached.

COMMENT #23
Discussion
The most prominent issue that the authors must address is how to mitigate the confounding effect of RB1 mutation to the utility of fragmentomics analysis. Addressing this would require expanding the cohort to include more wild-type RB1 on-treatment samples to further explore the independent value of fragment size analysis in LD-SCLC, but this is most likely unrealistic at this point. The authors should then discuss this limitation in the manuscript, and recognize that in this cohort, the combination of fragmentomics analysis and genetic ctDNA data (i.e., mutation analysis) provides little additional benefit compared to analysis of genetic ctDNA data alone.

RESPONSE #23
We agree with the reviewer's concern that RB1 mutation might have an unknown confounding effect on the burden of ctDNA. However, as pointed out by the reviewer, there are limitations to validate this issue at the moment. Hence, we added the sentence below to the limitation section of our manuscript.

Page 17: Lastly, due to an unidentified confounding effect between mutation analysis from genetic ctDNA and the burden of tumor DNA fragment, there could be a possibility that benefit of combining this two modality might have little benefit than our assumption.

COMMENT #24
The authors have already recommended the expansion of the cohort to include more longitudinal samples. Additionally, I suggest that increasing the number of patients should also be mentioned given the relatively small cohort investigated in this study.

RESPONSE #24
We agree with the suggestion of reviewer. As commented by the reviewer, we are expanding sample collection in more patients as well. We will report the subsequent outcomes from the larger cohort in the future.

COMMENT #25
On page 8, line 333: “conducted” should be changed to “conduct.”

RESPONSE #25
Thank you for the review. We conducted additional English proof leading on the revised version of manuscript as well.

RESPONSE TO COMMENTS FROM REVIEWER D

Summary:

In this article, the authors investigate the potential for metrics obtained from available ctDNA sequencing and fragmentomics assays to predict the likelihood of disease recurrence after definitive treatment for limited stage small cell lung cancer. I agree that it is compelling to try to use commercially available assays for prognostication and more adaptive/predictive clinical approaches, but in this case, in my estimation, the authors fall short of making a compelling case as currently written.

COMMENT #1
The overall organization of the study as written is somewhat confusing. There is an initial emphasis on total ng cfDNA extracted, VAF and comparisons of these metrics between groups. However, neither of these metrics in the currently commercially available assays are considered to be reliably quantifiable to the point of direct comparisons of absolute values between patients/groups. Certainly they are considered to generally correlate with plasma tumor DNA content and give some sense of clonality, but it’s not clear what the reader is supposed to learn from the detailed comparisons of these measures of tumor content between groups. Perhaps these metrics are best left to supportive information (e.g. supplemental tables).

RESPONSE #1
Thank you for the comment. We agree with the reviewer’s concern that our interpretation has limitation and might be confusing due to the reason that our analysis used various statistical methods. Based on the reviewer’s comment, we added more details of the data in the supplementary tables.

COMMENT #2
It's not clear which aspect – genes of interest or fragmentomics – is most clinically compelling to the authors. Does using both together work best? Whichever the approach, it would be best to validate this in a test cohort (can be retrospective).

RESPONSE #2
Thank you for the suggestion. As described in the manuscript, we looked into the representative genes first, such as *TP53* and *RB1*, and survival difference was observed as shown in Figure 3C and 3D. As fragmentomic outcome also showed meaningful difference, we combined these two results and merged them as a risk group, as shown in Figure 4E.

COMMENT #3
There is only a brief mention of the outcomes of patients with no detectable ctDNA at the on-treatment plasma sample. Conceptually, the event of total ctDNA clearance (below the assay limit of detection) is an important and clinically relevant comparison.
This point is the most interesting part of the paper and should be emphasized in any resubmission (or addition of more samples) – that among the patients with detectable ctDNA after two cycles of chemotherapy, there is still a prognostic difference among metrics that are identifiable via commercially-available ctDNA testing: "Surprisingly, early disease recurrence was observed in patients with persistent mutations identified in their samples even before completing the entire course of concurrent chemoradiotherapy treatment. As mounting data support the correlation of minimal residual disease (MRD) identified by somatic alterations after curative treatment and disease relapse (21), our results were somewhat unique in that they showed a correlation between MRD during the early treatment phase and the time to recurrence." However, as noted above, this should still be compared to cases with undetectable ctDNA. Is there a significant difference? If not, that is an argument for the authors' approach here – that there is rationale to try to find metrics within the commercially available assays that work better than just detection vs no detection. But it's not clear that this is the case.

RESPONSE #3
Thank you for revisiting very important issue. At the moment, there is no method for detecting below the LOD due to the technical limit. As an alternative approach, the decrease in VAF according to treatment was shown to be statistically significant in Figure 3B. We agree with the reviewer that we have further plans for the validation of ctDNA clearance and prognosis with a larger cohort. For the last comment, the issue regarding the undetectable ctDNA, the same issue has been raised from the other reviewer. However, please understand that it was determined that the ND sample did not contain sufficient cancer-originated DNA for analysis.

COMMENT #4
How did the ctDNA metrics described correlate with patient response to treatment radiographically? Do the patients with deeper radiographic responses to treatment also have the better prognosis based on ctDNA findings?

RESPONSE #4
Unfortunately, we were not able to conduct a comparison between ctDNA and RECIST outcomes. Based on the nature of SCLC which showed a very dramatic response to concurrent chemoradiotherapy, we decided to make the comparison with duration of response rather than the depth of radiographic response.

COMMENT #5
How do these ctDNA findings correlate with tissue-based sequencing? Is there significance to the presence of p53 and RB1 mutations in the plasma other than the presence of ctDNA shed at that timepoint?

RESPONSE #5
In our analysis, we were not able to make the comparison due to no tissue based sequencing conducted. However, as a considered benefit of ctDNA, it is known that ctDNA basically reflects the patient's condition closest to real-time.

COMMENT #6
What would be the potential clinical implications of these findings? What would the authors propose doing if reliably able to detect low- versus high-risk group after two cycles of chemo?

RESPONSE #6
Thank you for suggesting a very important question. As the survival of LD-SCLC is still low, we think our approach, using a non-invasive method, might support the intensity of subsequent treatment after the current standard treatment. A possible considerable option could be a maintenance of immunotherapy or somewhat novel agent such as tarlatamab.

COMMENT #7
The range of time between pre-treatment and on-treatment samples seems long. For the patients with 118 days between diagnosis and completion of 2 cycles of chemo, can the authors explain clinical factors that caused this treatment delay? Do these clinical factors affect the overall prognosis (possibly confounding the correlation with plasma findings?).

RESPONSE #7
Most of the patients were collected for 2nd sample around 40 to 50 days after the first sampling. However, only one patient exceeded 60 days, which was a patient with the samples collected at 118 days. The reason for the delay in sample collection was that the patient hold the treatment due to the pneumonia and re-initiate the treatment after the completion of pneumonia treatment. To avoid the potential bias of selecting patients, we included this patient for the analysis.

RESPONSE TO COMMENTS FROM REVIEWER E

Park et al. assessed ctDNA based on genomic and fragmentomic analyses to assess the risk of recurrence in 50 patients of limited disease SCLC. Authors performed targeted sequencing of pre-treatment and on-treatment blood samples with AlphaLiquid® 100 target capture panel consisting of 106 cancer-related genes. The authors reported that patients with mutant RB1 and high fragmentation ratio showed worse survival. Results are compelling, yet there are some suggestions to improve the manuscript.

COMMENT #1
Authors should provide the table with baseline demographics information such as age, gender, smoking history etc. in the form of the Table.

RESPONSE #1
Thank you for the comment. We added the baseline demographic as Table 1 in the manuscript.

COMMENT #2
Authors report that targeted sequencing was performed using a 106 cancer-related gene panel. Authors should provide the list of genes in the panel in supplementary information. What was the size of the panel? Can the authors show an in-silico analysis of this panel to see how many SCLC patients harbor mutations within the panel space?

RESPONSE #2
The list of 106 genes used for AL100 was listed in supplementary table (Table S2). The coverage of SCLC samples was calculated using a database (cBioPortal) and filled in the supplementary table (Table S3).

COMMENT #3
It is not clear that fragmentomic analysis was performed across the genome or only in the genes included in the targeted panel? Did the authors use off-target reads too for the analysis? This should be clearly explained in the Methods.

RESPONSE #3
Thank you for the comment. The off-target region is only included in raw bam. Using filtered bam, only the target area was used. AL100 also includes cnv analysis, and is a kit developed after verification to ensure that capture bias does not occur in all areas. Therefore, in the AL100 capture set, the coverage of each gene is not significantly different and is not expected to affect the fragmentomics analysis.

COMMENT #4
Authors used the maximum VAF value from each patients to calculate the clonality but it is not clear what threshold was used to define the very low, low and high clonality. How were these

thresholds chosen?

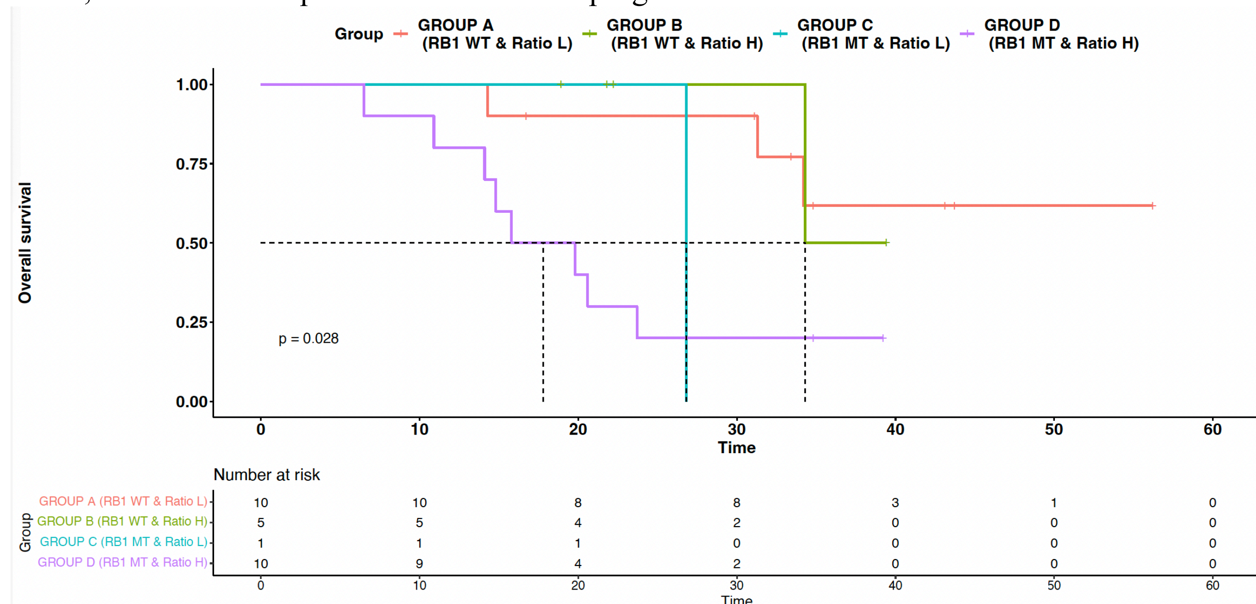
RESPONSE #4
The clonality was defined by using maximum VAF of each sample. The high, low, and very low clonality was defined as VAF over 40%, from 10% to 40%, and under 10%. [PMID: 33727690]

COMMENT #5
Did the authors try to check the predictive value of ctDNA level or TP53 or RB1 mutational levels in pre-treatment samples based on VAF? Authors should look at the paper of Nong et al., Nature Communications, 2018, Mohan et al J Thorac Oncol 2020 where they showed pre-treatment ctDNA levels were associated with poor prognosis in SCLC.

RESPONSE #5
Thank you for the suggestion. We looked into the baseline TP53 or RB1 mutation status but showed no difference in the ratio of TP53 or RB1 VAF high or low in PeR or non PeR group. The discrepancy between the journal suggested by the reviewer and our result might be derived from the difference in demographics of the population which include extensive disease SCLC in suggested journal.

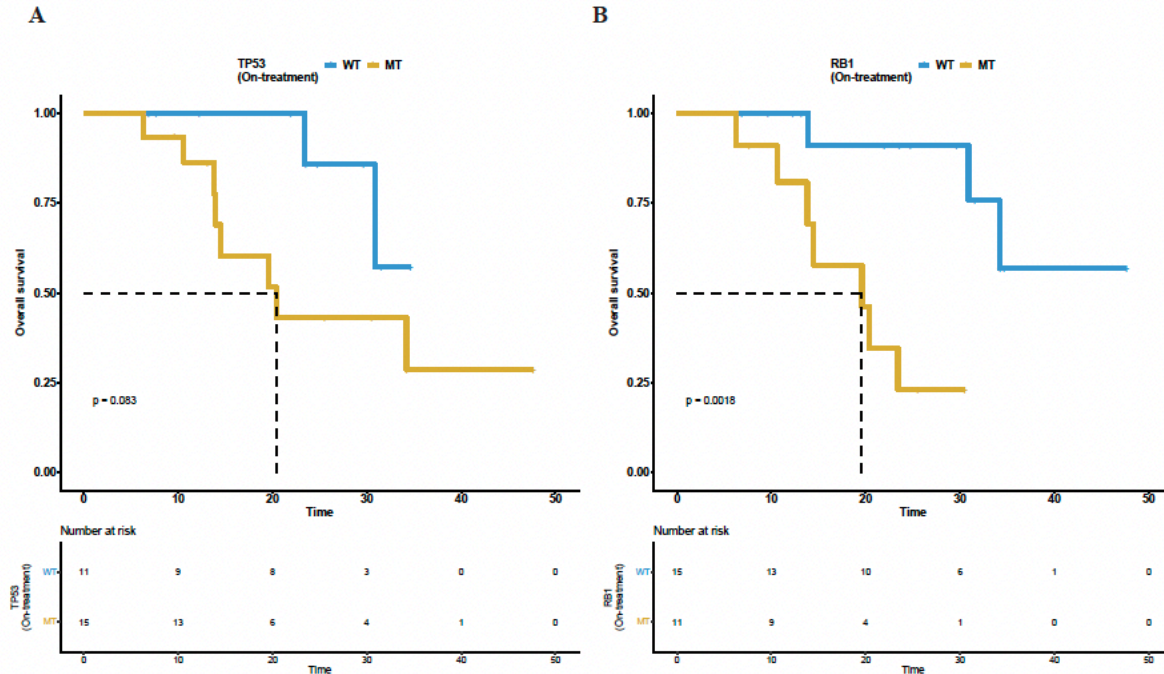
COMMENT #6
Did authors try to see the overall survival benefit (in addition to the shown RFS results) of combining the fraction of P1 with RB1-mutation status in on-treatment samples?

RESPONSE #6
Thank you for the comment; we conducted OS analysis using fragment ratio and RB1 mutation status, which showed quite a similar trend to progression-free survival.



COMMENT #7
Generally, there should be KM curves for OS for all analyses, in addition to the RFS results shown.

RESPONSE #7
Thank you for the comment. We included OS curve in the supplementary figure 2 as below.



Based on the reviewer's comment we modified Figure S3 also to include the overall survival data.

COMMENT #8
Authors showed that there was no difference in RFS in patients with ctDNA detectable compare to undetectable ctDNA (Figure 4A). This seems a bit paradoxical. Can the authors comment on why this may be?

RESPONSE #8
This is very important issue. There is no clear answer on this observation. However, since our method detects only 106 genes, there might be a chance that ctDNA could be under-detected if a mutation has occurred in genes other than our target gene.

COMMENT #9
Did authors perform multivariate Cox regression analyses to see if ctDNA (RB1 mut) combine with fragmentomics correlate with survival outcomes when taking into account other clinical covariates such as age, gender and smoking?

RESPONSE #9
Below are the Cox data using age and smoking history
Age: HR 1.079 (0.981 – 1.187; *P* = 0.116)
Smoking history (compared with current smoker)

- Ex smoker HR: 0.945 (0.276-3.24; *P* = 0.928)
- Never smoker HR: 4.959 (0.092-27.264; *P* = 0.066)

We did not conduct the analysis based on the gender.

COMMENT #10

The authors mistakenly state in the Discussion that their study is the first to evaluate disease recurrence based on ctDNA analysis for patients with LS-SCLC. The study by Chaudhuri et al. Cancer Discovery, 2017 included 3 patients with LS-SCLC and was the first to show that ctDNA MRD detection could be prognostic in these patients.

RESPONSE #10

Thank you for the comment. This issue has been also visited from the other reviewer as well and we modified our sentence as below.

Page 17: However, to our knowledge, this study represents the one of early research outcome evaluating disease recurrence based on ctDNA analysis for patients with LD-SCLC.

COMMENT #11

The authors also mistakenly state that their study was the first to explore the predictive value of fragmentomic analysis in terms of disease recurrence with soli tumors. This is incorrect; an example of a study to do this is Mathios et al., Nature Communications, 2021.

RESPONSE #11

Thank you for the comment. Based on the reviewer's suggestion, we also modified the indicated sentence as below.

Page 17: In addition, this study adds value to explore the predictive value of fragmentome analysis in terms of disease recurrence with solid tumors which has limited research outcome.

COMMENT #12

There is no validation (either held-out validation or internal cross-validation) which makes the survival analyses of high versus low in Figure 4 (and Fig S3) overfit to the Youden's index cutpoint derived from the same dataset.

RESPONSE #12

We understand that the validation issue is critical to increasing the value of our result. Please understand that we are currently exploring reproducibility through a larger cohort, which is not able to be included in this manuscript at the moment.

COMMENT #13

Sequencing metrics for each sample sequenced such as non-deduplicated sequencing depth, de-duplicated sequencing depth, and panel on-target rate should be provided in the supplementary

table.

RESPONSE #13
Please refer to the Supplementary Table1 for the details.

COMMENT #14
Line 183: 29 patients (58.0%) were assigned to the non-PeR group and 21 patients (42.0%) were assigned to the non-PeR group. 29 patients should be PeR group.

RESPONSE #14
Thank you for the comment we change the typo.

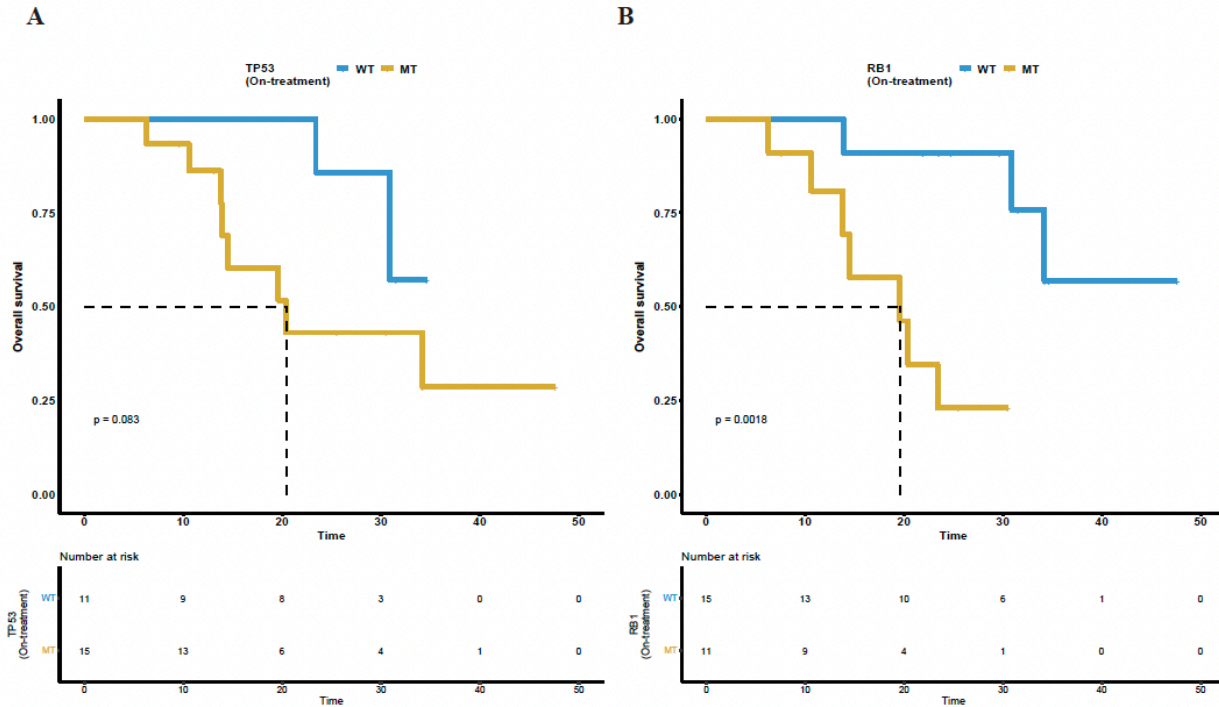
COMMENT #15
Line 192: It seems strange that 76.2% of patients in the PeR group had ctDNA detectable, while only 34.4% were detectable in the non-PER group. This is the opposite of what we would expect.

RESPONSE #15
The number is collect that among ctDNA detected patients, 76.2% were from PeR group and 34.4% were from non-PeR group.

COMMENT #16
Line 256-257: p-value for the overall survival plot for RB1 is significant while for TP53 is insignificant as wrongly mentioned in the text.

RESPONSE #16
We double checked below sentence and which matches with the Supplementary figure 2.

Page 12: Exploratory analysis showed that patients with detectable RB1 mutations in their on-treatment samples had significantly shorter OS time (P = 0.002), whereas TP53 mutations showed shorter but not significant OS difference (P = 0.083) (Supplementary Fig. 2).



COMMENT #17

Line 277: "low-risk group" seems to be referencing a high-risk group. This should be updated for clarity.

RESPONSE #17

Thank you for the correction. We revise the typo.

COMMENT #18

Line 364: evaluation of disease recurrence based on ctDNA analysis 364 for patients with LS-SCLC — It should be LD-SCLC to be consistent with rest of text.

RESPONSE #18

We apologize for the inconsistency in the description of data. We made change LS to LD throughout the manuscript.

COMMENT #19

All KM curves should be displayed as landmark analyses (where time zero is the time of the ctDNA blood draw) to reduce risk of guarantee-time bias.

RESPONSE #19

All the KM curve was drawn from the date of ctDNA blood draw which is close to the date of initial treatment. We added this information to the method section.

COMMENT #20
Authors should reference Sivapalan et al. Clinical Cancer Research, 2023, which is relevant to this study.

RESPONSE #20
Thank you for the suggestion. We added this reference to the manuscript.