Peer Review File

Article information: https://dx.doi.org/10.21037/jtd-23-379

Reviewer A

Wang and co-authors study the utility ctDNA for determining immunotherapy efficacy in advanced non-small cell lung cancer. In particular, they determine a cut off to predict outcome. This topic has been an extensively studied over the last few years, and consensus seems that ctDNA is a helpful predictor. This report underpins earlier findings.

While this work is not particularly novel, its publication would be useful in providing further confirmation of the utility of ctDNA.

However, the paper requires major revisions before publication. In particular, the authors completely ignore all the relevant published works. It would be highly valuable if their findings (especially the cut-off) are put into perspective of earlier work.

In addition, large portions of the Methods sections are missing, which makes it impossible to reproduce the labwork.

Below, I indicate in more detail what clarifications and adjustments are required:

General:

Specify the values of p-values not just upper bounds (e.g., lines 47, 170, 194). This facilitates meta-analysis. When p-values are small (e.g., lines 46, 144, 148, 156), please use scientific notation, e.g., $p = x.x \cdot 10^{-4}$.

Reply: We have revised these issues accordingly.

Please attach the analysed dataset to the supplementary information.

Reply: Attached files please find the dataset.

Please upload the analysis notebook to github/gitlab/bitbucket, or attach as supplementary information.

Reply: Attached files please find the dataset.

To facilitate comparison with other studies, it would be useful to also compute ctDNA in terms of mutant molecules per mL and the variant allele frequency.

Reply: We failed to study the mutant molecules in this retrospective study, we have discussed this as a limitation in the discussion. Thanks.

Abstract

Specific figures are quoted (line 29) but no reference is given.

Reply: We have revised the sentence. Page 2, line 28-29.

On lines 34-35: patients were not allocated to groups (which implies a kind of intervention), they were split in groups. (Also on lines 99 and 100).

Reply: We have revised the sentence accordingly. Page 2, line 34-36.

Line 46:
Lines 49-51: I assume that the ROC corresponds to the regression model? If so, specify this.
Reply: We have added. Thanks. Page 2, line 48-52.

Introduction:
Relevant list of related literature is completely missing. There is already a large body of work where circulating tumor DNA was tested. A non-exhaustive list I have accompanied (I think the last couple of weeks a few new papers were published). The authors should discuss in detail how their work relates to earlier published papers, see especially the review of Wang et al.
Reply: We have revised the introduction accordingly. Page 4, line 90-92.

Methods:
In the efficacy evaluation I miss at what time points the RECIST 1.1 evaluations were carried out. Every 4 weeks? Or after a certain number of cycles?
Reply: Every 2 cycles. We have added. Page 4, line 102-104.

On lines 99-100, the authors indicate that they split the patients in two groups. Does the OR group refer to CR and PR at any time point in the treatment? Or at a specific time point? Please clarify.
Reply: We have added. Page 4, line 102-104.

Are potential mutations arising from clonal hematopoiesis being accounted for (such as, for example, in the papers of Nabet *at al*., Weber *et al*., and Zou *et al*)? If yes, please specify. If no, please elaborate in the discussion.
Reply: We failed to study the mutant molecules in this retrospective study, we have discussed this as a limitation in the discussion. Thanks.

In the statistical analysis subsection, the regression model is not mentioned at all.
Reply: We have added. Page 5, line 145-152.

Earlier work of Donker *et al*. (see reference list) showed that smoking history, type of pathology and (if available) the number of previous lines of therapy are also important predictors. These should also be included in the regression model.
Reply: We have added smoking and pathology type in the regression model. See table 2.

A section is missing that describes how the circulating tumor DNA was measured. What kind of assay was used, what kind of sequencing machine, etc.?
Reply: We have added. Page 5, line 134-137

Which mutations were selected for determining the ctDNA value?
Reply: We have added. Page 5, line 134-137

At what time points was blood drawn? And what was the typical time window between blood draw and RECIST measurement?
Reply: We have added. Page 5, line 134-137.

Indicate how the diagnostic cut off was determined. Using Youden J?
Reply: We have added. Page 5, line 145-152.

Results:
The text does not indicate to what extent the data was censored. Both in terms of response criteria and survival. Please specifiy.
For the two-year mortality rate (line 148), survival analysis should be conducted.
The confidence intervals of the sensitivity and specificity were not reported on lines 157-158 and 164-165.
Please report the regression coefficients of the model.
Reply: We have added accordingly. See page 6, line 177-184 and page 12, line 364-367.

Discussion:
Since there is already such a large body of similar work, the cut-off determined by the authors should be compared to cut offs found in earlier work. If there is a large discrepancy, then this should be dicussed.
On lines 211-212 the authors incorrectly imply that no other ctDNA analyses of advanced NSCLC are carried out. As proven by the attached reference list, this is wrong.
On lines 214 the authors write that “ctDNA tests rarely show false positives”. However, clonal hematopoiesis can easily give many false positives.
On line 216-217 the authors write “Thus, ctDNA could be used to guide immunotherapy in advanced NSCLC”. However, this sentence does not logically follow from the previous sentence.
Reply: We have revised the discussion. See page 8, line 227-230.

Related works
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Donker, Hylke C., et al. "Decoding circulating tumor DNA to identify durable benefit from immunotherapy in lung cancer." Lung Cancer 170 (2022): 52-57.

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Reviewer B

Many aspects are missing in the manuscript. Especially, there is no information on how ctDNA was measured and validated, which is a crucial part of the further analysis and final findings. There is even no info on when and from where material for ctDNA was extracted. There were many attempts and still there are clinical trials validating the association between ctDNA level and treatment response rate, however, they were not discussed in the manuscript. The current IASCL treatment guidelines base on Keynote-189 and Keynote-407 and summarizing that “PD-L1 can no longer be used to screen the patients that would benefit from immunotherapy, as it cannot meet the urgent needs of precision immunotherapy in tumor patients” is overestimated and not supported by the obtained results.

Reply 1: We thanks for your kind suggestion. We have added the detection of ctDNA. see page 5, line 135-138. And in page 8, line 234-235, we did not dynamically monitor ctDNA levels. We have discussed it as a limitation.

Minor comment:
Lanes 74-84, 189-197; 211-217 are not confirmed by any references
Reply 2: We have added related references.

Lanes 115-116- please clarify which inclusion criteria for immunotherapy were expected, and which aim of treatment was applied – mono ICIs or ICIs+CTH
Reply 3: We have added. See page 4, line 106-109.

There is no information on how ctDNA was tested.
Reply 4: We have added, see page 5, line 135-138.

Reviewer C

In this study, the authors evaluated the value of ctDNA concentration in predicting the response to immunotherapy in advanced NSCLC. Blood ctDNA from 143 patients with advanced NSCLC receiving immunotherapy was analyzed. The authors established a nomogram model, which achieved 0.850 of AUC in the training set, and 0.732 of AUC in the validation set, respectively. The sensitivity and specificity of ctDNA levels for predicting therapeutic efficacy is not satisfactory. Moreover, some important information was missing including ctDNA collection time points, blood volume, and methods for ctDNA extraction, quantification and test. This study did not detect the dynamic changes of ctDNA during immune checkpoint inhibitors treatment. Compared to ctDNA test from a single time point, ctDNA dynamics could be better to predict treatment response.
Reply: We thanks for your suggestion and feedback. In page 5, line 135-138, the detection of ctDNA was added. And in page 8, line 234-235, we did not dynamically monitor ctDNA levels. We have discussed it as a limitation.

Reviewer D

How were these data presented in your Table 1? Please define them either inside the table or in table footnote.
Reply 1: We have revised the table 1 accordingly. See page 16, table 1.

Table 1 Comparison of the clinical features of the 2 groups

Category	Objective response group (n = 67)	Control group (n = 76)	t/χ^2 value	P value
Age (years)	58.82 ± 11.76	59.38 ± 12.61	0.274	0.785
Gender			2.538	0.111
Male	49 (73.13%)	46 (60.53%)		
Female	18 (26.87%)	30 (39.47%)		
Body mass index (kg/m^2)	25.45 ± 2.43	25.39 ± 2.65	0.118	0.906
History of smoking	26 (38.81%)	38 (50.00%)	1.805	0.179
History of alcoholism	21 (31.34%)	36 (47.37%)	3.815	0.051
Hypertension	16 (23.88%)	13 (17.11%)	1.011	0.315
Diabetes	7 (10.45%)	11 (14.47%)	0.525	0.469

2. Please define all abbreviations in Table 2 footnote.

Reply 2: We have defined the abbreviations in table s footnote. See page 17, table 2.