Reviewer’s report

Title: Targeted sequencing of circulating cell-free DNA in stage II-III resectable oesophageal squamous cell carcinoma patients

Version: 4 Date: 20 Jun 2019

Reviewer: Reviewer 3

Reviewer's report:

"PEER REVIEWER ASSESSMENTS:

OBJECTIVE - Full research articles: is there a clear objective that addresses one or several testable research questions? (Brief or other article types: is there a clear objective?)

Yes - there is a clear objective

DESIGN - Is the current approach (including controls and analysis protocols) appropriate for the objective?

No - there are minor issues

EXECUTION - Are the experiments and analyses performed with sufficient technical rigor to allow confidence in the results?

No - there are minor issues

STATISTICS - Is the use of statistics in the manuscript appropriate?

Yes - appropriate statistical analyses have been used in the study

INTERPRETATION - Is the current interpretation/discussion of the results reasonable and not overstated?

Yes - the author's interpretation is reasonable
OVERALL MANUSCRIPT POTENTIAL - Has the author addressed your concerns sufficiently for you to now recommend the work as a technically sound contribution? If not, can further revisions be made to make the work technically sound?

Probably - with minor revisions

PEER REVIEWER COMMENTS:

GENERAL COMMENTS: The manuscript is sound although in my opinion is weak when it comes to the methodology employed which raises questions on the final results.

Nevertheless, the hypothesis and the logic is correct but it will be a stronger manuscript if more attention had been paid to the technical approach.

REQUESTED REVISIONS:

The problems with the manuscript arise with the technology and methodology employed. In my view it is not the correct way to properly address what the authors set out the address. This makes the manuscript weak and open to criticism.

ABSTRACT

- Line 57, "...all somatic mutations disappeared or decreased after surgery.": How can the authors be sure they disappeared rather than they were not detectible? I would change this statement in view that most patients post-surgery have very low levels of cfDNA; they used very low plasma volumes and had high yields of cfDNA.
METHODS

- Line 56, "DNA extraction, library preparation and sequencing" section: The technical aspect of the paper would have been strengthened by sequencing cfDNA from unrelated plasma controls to ascertain the error level of their whole sequencing approach.

- Lines 6-9, "Plasma cfDNA was extracted with the QIAamp circulating nucleic acid kit…": Although the authors state how much blood was collected, there is no indication of how much plasma they used for cfDNA extraction. That should be included as 2-5 ml of blood will yield a maximum of 1-2 ml of plasma which might become relevant later on.

- Line 14, "Nanodrop": This is a bit crude for purity assessment given the limitations of spectrophotometry. For cfDNA this is not good and it would be interesting to have done so by BioAnalyzer instead, mostly to check the purity of the cfDNA and potential contamination by WBC released gDNA. This becomes relevant when you take together the low plasma volume they have obtained and the high yields of cfDNA that went into NGS (about 30…)

"Data analysis" section

- Lines 48-51, "This indicates the sequencing error rate at this specific site": Have you done several runs using health controls to ascertain this? I fear this might only reflect the error for this run and not systematic error associated with the approach.

RESULTS

- Lines 40-46, "The median on-target coverage for the cfDNA samples was 613x (range 391x to 839x) pre-surgery and 752x (range 546x to 1932x) post-surgery": I am a bit concerned of the depth to which they sequence their cfDNA given the fact that the trend now is to sequence using error correction to a depth of 20000x minimum to be able to call mutations at AF of >0.3%. The becomes relevant when they claim mutations disappear, cannot it be they are not detecting them due to technical limitations? Authors should think about that alternative scenario.
DISCUSSION

- Lines 3-6, "Most of these mutations were not detected in cfDNA of blood samples obtained as early as 3-4 hours after surgery": It would be interesting for the authors to hypothesize why this is the case given that there is more cfDNA in post-surgery than pre-surgery (page 12, lines 48-51) and it might be expected a higher release of cfDNA following trauma (surgery) that could potentially mask cfDNA detection.

- Lines 26-29, "To overcome this shortcoming we monitored sequencing error rates for all positions for which we identified mutations in cfDNA using our predefined criteria and showed that these sequencing error did not pass our criteria": Again, unless this has been tested extensively on healthy plasma this might just reflect the error for this specific run.

- Lines 51-53, "In our study, a stage IIA patient with a mean MAF of 2.4% in pre-surgery cfDNA developed a recurrence five months after surgery": Did this patient have mutations detected in cfDNA post-surgery? Authors should mention that.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

No

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

No

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

No

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If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

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