Reviewer's report

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

Version: 1 Date: 15 Jul 2018

Reviewer: Maria Lung

Reviewer's report:

Please include all comments for the authors in this box rather than uploading your report as an attachment. Please only upload as attachments annotated versions of manuscripts, graphs, supporting materials or other aspects of your report which cannot be included in a text format.

Please overwrite this text when adding your comments to the authors.

Although as non-invasive clinical specimen cfDNA has been studied in many cancer types, it is not well-characterized and studied in ESCC, as commented by the authors in the Introduction. So, this study has some novelty and potential clinical significance. However, the reviewer has many concerns about the quality of this study, as listed below.

1) The quality of sequencing data is unknown. Although the average coverage was described by the authors. The detailed NGS data statistics is missing in the manuscript

2) The robustness of their NGS benchwork and analysis pipeline for detecting mutations in cfDNA is not clear. What method is used for mutation calling? Can the authors catch the mutations with allele frequency as low as 0.5% accurately? Have the authors validated the mutations detected by cfDNA by other approach? There is a large variation in the sequencing depth in cfDNA. Some sample has average coverage as low as 154X. Six patients have no mutation detected in cfDNA before surgery. It is highly possible that it is caused by the low sequencing coverage in their study.

3) Suggest the authors to demonstrate the reproducibility of their workflow, and evaluate the sensitivity and specificity for detecting the mutations with low allele frequency by the spike-in experiments.

4) The information about how to define the harmful mutation should be further clarified in the manuscript

5) The sample size of the study is rather small. Increase of sample size with robust bench work and analysis workflow will strengthen the conclusion in the manuscript.
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

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
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

Quality of written English
Please indicate the quality of language in the manuscript:

Needs some language corrections before being published

Declaration of competing interests
Please complete a declaration of competing interests, considering the following questions:

1. Have you in the past five years received reimbursements, fees, funding, or salary from an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

2. Do you hold any stocks or shares in an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

3. Do you hold or are you currently applying for any patents relating to the content of the manuscript?

4. Have you received reimbursements, fees, funding, or salary from an organization that holds or has applied for patents relating to the content of the manuscript?

5. Do you have any other financial competing interests?

6. Do you have any non-financial competing interests in relation to this paper?
If you can answer no to all of the above, write 'I declare that I have no competing interests' below. If your reply is yes to any, please give details below.

I declare that I have no competing interests.

I agree to the open peer review policy of the journal. I understand that my name will be included on my report to the authors and, if the manuscript is accepted for publication, my named report including any attachments I upload will be posted on the website along with the authors' responses. I agree for my report to be made available under an Open Access Creative Commons CC-BY license (http://creativecommons.org/licenses/by/4.0/). I understand that any comments which I do not wish to be included in my named report can be included as confidential comments to the editors, which will not be published.

I agree to the open peer review policy of the journal.