Reviewer’s report

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

Version: 3 Date: 12 Apr 2019

Reviewer: Maria Lung

Reviewer's report:

1. I note that several additional files were added to address some of the comments on the earlier version of this manuscript. This is very useful.

I would suggest that the authors put some of this information in the main manuscript, in addition to keeping this in the additional materials. A simplified table can be extracted from Additional file 3 to highlight the top mutations and recurrent mutations observed, for example, for TP53, Notch2, and CDKN2A mutations, with information on specimen type (tumor/cfDNA), mutation observed, time point for taking specimen, etc. If needed, Table 1 and Figure 1 may be put into the Additional materials instead.

2. The authors stated that two mutations were detected post-surgery having MAF of 0.28% and 0.36%. However, from Additional File 3, mutations were also detected in WBCs in some of the patients (ESCC07 TNFAIP3) with similar MAF (0.31%). How can they ensure that the mutations detected in cfDNAs were not background germline mutations? Were any of the mutations detected by NGS validated by other methods such as Sanger sequencing or digital PCR? This needs to be done, as much as possible, and included in Methods section. There should be a statement of validation frequency included in the revised manuscript to provide assurance that the mutations identified are true. (This is the major revision needed now for this NGS study to be acceptable for publication.)

3. Please add statement on sensitivity and specificity of the NGS pipeline.

4. Figure 2 shows that overall the amount of plasma cfDNA before surgery was lower than post-surgery. Was the change in mutation load before and after surgery predictive of disease recurrence for individual patients? Was there any ctDNA analysis performed after disease recurrence? Is the mutational profile of the recurrence similar to the primary disease?

5. Overall limitation in study is due to few patients included in study with only two patients with sufficient cfDNAs to monitor for mutations after disease progression.
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.

Yes

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

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I am able to assess the statistics

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

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