Reviewer's report

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

Version: 2 Date: 31 Jan 2019

Reviewer: Maria Lung

Reviewer's report:

Reviewer's comments on revised BMC paper

The authors have provided much more information on analysis and sequencing quality details in their revised manuscript. More details have been added about bioinformatics analysis workflow scheme. Two additional figures were added to provide targeted sequencing coverage and ctDNA allele frequencies. Damaging mutations were given CADD scores to classify them.

However, there are still significant weaknesses in the manuscript because of small sample size, lack of specimens to validate mutations and determine overall sensitivity of mutation calling.

The authors can greatly improve this manuscript by adding more details that they should already have from their analysis.

Important to know the correlation between cfDNA mutations with matched tumor tissue mutations. An additional table is needed with details of variants detected in each patient in tumor/pre-surgery blood/post-surgery blood, so we can more easily see if there are any recurrent mutations detected amongst the mutations identified within the 16 patients. For example, is there any overlap between the top two mutated genes amongst the patients for TP53 (observed in 6 patients) and Notch (observed in 5 patients)?

The authors commented on one patient with recurrent disease having "6 of the 12 somatic mutations observed in the corresponding tumour DNA were detectable in cfDNA. For the other two patients, we only had post-surgery cfDNA, and in one of the two we detected a single mutation out of three somatic mutations detected in the tumor samples." Were all the mutations unique or were there recurrent mutations found? Any concordance for ESCC04 with 17/6 and ESCC09 with 10/8 in tumor/pre-surgery blood?

Explain or correct differences in numbers of mutations seen in Fig 4 vs Table 2 for ESCC09 (Table 2-8 vs Fig 4-7) and for ESCC10 (Table 2-16 vs Fig 4-15). Do some of the genes have more than one detected variant?
Add to discussion:

1) concordance of mutations identified in tissue vs blood (pre- and post-surgery)

2) difficulty to do screening assays using ctDNA for ESCC patients unless a panel of high frequency mutations are identified

3) results and conclusions limited by number of patients studied. For example, only 1 patient with recurrence showed 6 of 12 mutations in tumor DNA, same as cfDNA. For other patients with recurrence, for post-surgery cfDNAs there is only one mutation; no pre-surgery cfDNAs available. Cannot conclude.

Minor comments for clarification

Results - patient characteristics:

The text states this study includes a cohort of 17 patients. However, data presented in Table 2, Figures are for 16 patients. Why?

In text the age range is from 41-77, but Table 1 states this is 42-77. Need to correct.

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

Yes

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?
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No

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