Reviewer’s report

Title: Karyotyping of circulating tumor cells for predicting chemotherapeutic sensitivity and efficacy in patients with esophageal cancer

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Reviewer: Jason Chia-Hsun Hsieh

Reviewer's report:

The authors attempted to investigate the ploidy of CTCs and claimed that the triploidy of CTCs could correlate to the response of chemotherapy. Similar issues have been discussed for many years. Some problems exist which requires further clarification before it is considered to be published in BMC Cancer..

Major concerns:
1. The manuscript lacks a clear introduction of physiology and pathophysiology of multiploidy of CTCs. That greatly weakens the rationale of why the authors read the multiploidy of CTCs instead of cell counts. Please add a brief and essential description in the INTRODUCTION as the background knowledge to support (1) checking the ploidy of chromosome 8, not 9 or 10; (2) checking the ploidy of CTCs, not CTC counts or specific subtype markers of CTCs.

2. The clinical information, such as the primary site of the tumor, TNM stages, metastatic sites, radiation dose, chemotherapeutic agents, with or without surgery, and the definition of "benefit number" in the table of major finding were missing. Do the authors mean those patients with PR (n=31) or those who can continue to the second cycle of chemotherapy (n=58)? If the answer is the latter description, the patients who can "tolerate" 2 rounds of chemotherapy can be misclassified as "benefit." Besides, the same question will be raised for the definition of "chemotherapeutic sensitivity" in the manuscript title. These two problems have nearly ruined the major finding of this work.

3. The description of the critical methodology was missing.
   In Lines 88-90 in METHOD. The description of the methodology of CTC identification seems to be too brief. Although the methodology has been published, the authors should essentially describe the essential methodology in their words for readers who were not familiar with the device. For that purpose, I recommend the authors to add information containing:
   (3-a) The antibodies, kits with their manufacturing information and concentration are all required to describe.
   (3-b) The positive and negative controls of experiments. (clinical cases are much better)
   (3-c) The definition description of triploidy, tetraploidy or pentaploidy and multiploidy.
   (3-d) The definition of CTCs (by marker?) in this study.

4. Some critical references seemed to be misplaced, lost and inadequately cited.
   (4-a) For example, the author cited reference 1 and 2 to support their description of "the 5-year
survival rates of esophageal cancer patients remained as low as only 20-30% due to either chemo-sensitivity and/or acquired chemoresistance." Reference 1 concluded that CTC could provide important information for patients with metastatic breast cancer and reference 2 was relapse patterns of "early" esophageal cancer who underwent surgical resection. Moreover, the present study was done for patients who received chemotherapy, which means locally advanced stage and unresectable. Reference 3 and 4 were cited to support the sentence, "CTCs measurement as a non-invasive detection method which can monitor therapeutic responses of cancer patients dynamically." However, reference 3 addressed the issue of prostate cancer. Reference 4 addressed the issue of taking metastasis by CTCs. In my opinion, the first 4 references (the first three at least) seemed inadequately cited. I suggest the authors find other references closely related to esophageal cancer instead of current reference 3.

The mistake of citation errors would make readers extremely confusing.

(4-b) The authors cited several very old references for CTC and failed to cite some recent references addressing new insights into clinical values in patients with esophageal cancer. For instance, Reference R1 and R2 were recently published evidence and review of the role of CTC in esophageal cancer. I was wondering the reasons why the author avoid these references in this manuscript?

(4-c) The field of CTC investigation and application evolves very quickly, hundreds of new reference have been published in the literature. Among all the 22 references the authors used, only one reference was published after the year 2017. That implies the citation was too old for readers to catch up with the updated information considering liquid biopsy has been a very hot topic in the most recent decade. I strongly recommend that the authors cite/replace some newer pieces of evidence to support the current work.

(4-d) Reference 13 mentioned about circulating "endothelial" cells, not circulating tumor cells, which was epithelial by definition.

Minor concerns:
5. The background of the CTC was too short/brief. I recommend that the authors cite more about the subtypes (another epithelial, stemness or platelet-associated markers) of CTC in the literature.

6. Some English problems exist, such as "4-cycles chemotherapy", "2-cycles chemotherapy"; "After then these patients....", "positive correlated." ,,etc. Please check the wording carefully.

7. Figure 2. The P value was missing.

8. Table 1 and 2 were crude information. I suggest the authors present them more readable in a statistic way and might consider to move the detailed tables to the appendix.

Reference R1 and R2:

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

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