

Peer Review File

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Reviewer A

The paper describes a useful overview of clinical trials in colon cancer that use liquid biopsies. However, the manuscript still needs some work before it can be considered for publication.

General comments:

Comment 1: *The paper discusses "colorectal cancer" trials, but only "colon cancer" trials are searched for on ClinicalTrials.gov. What about "rectal cancer"? ClinicalTrials.gov even has a category "Colorectal Cancer".*

Reply 1: We repeated our search for “colorectal cancer” and added two additional clinical trials, specifically to the bottom of Table 3 and Table 4.

Changes in the text: changes in the text are in Table 3 and Table 4

Comment 2: *Do you expect different findings for other types of cancer?*

Reply 2: This review is focused on current, active trials for colorectal cancer only. We did not review other types of cancers, though we would expect there may be differences for other types of cancers.

Changes in the text: N/A

Comment3: *"Colon Cancer was used and cross reference with Liquid Biopsies or ctDNA." --> Please include the exact search terms so that the query can be reproduced. Did you use the "Advanced Search"?*

Reply 3: “Colorectal cancer” was used as the search term under “Condition or disease” “liquid biopsies” and “ctDNA” were used as the search terms for “Other terms”.

Changes in the text: We added these sentences to the Methods, second sentence.

Comment4: *- In the introduction and discussion sections, I would expect that certain statements are accompanied by a reference. For example, the complete page 3 does not contain a single reference. Nine references is very little for a narrative review paper. Furthermore, the references are not in the right*

order (1,5, etc.) and references 2 and 3 seem to be missing in the text. Please use round brackets everywhere, as requested by the journal.

Reply 4: We apologize for this oversight.

Changes in the text: Additional relevant references have been added throughout the manuscript, increasing the references by nearly three-fold. Round brackets have been used.

Comment 5: *Grammar/spelling errors:*

Reply 5: *We apologize for these errors and thank the reviewer for identifying them. These have been corrected as requested.*

Changes in the text; - *Line 67: cell-free DNA (ctDNA) --> Cell-free DNA is cfDNA. Circulating tumor DNA is ctDNA.*

- *Line 78: I believe the term that's widely used is "tumor-uninformed testing"*

- *Line 161: "North American" --> "North America"*

- *Table 1, etc.: "CRC" --> explain abbreviation (e.g. at line 88)*

As I have added some info, the line is not 88, but I corrected it.

- *Line 195: "CEA"--> explain abbreviation (e.g. at line 154)*

- *Line 207: "ACT" --> explain abbreviation*

Reviewer B

Comment 1: *The authors use liquid biopsy interchangeably with ctDNA throughout the paper; lump cancer detection studies with cancer monitoring/surveillance studies together. There is lack of description and discussion on the techniques used in each study as well as in local institute.*

Reply 1: We thank the review for this comment.

Changes in the text: We have added a significant amount of additional detail to the listed trials in each of the tables.

Comment 2: *Authors are recommended to review the following papers/presentation: 1) M.R. Speicher and K. Pantel - "Tumor signatures in the blood" Nat Biotechnol, 32 (5) (2014), pp. 441-443; 2)Computational and Structural Biotechnology Journal Volume 16, 2018, Pages 190-195, ctDNA and CTCs in Liquid Biopsy – Current Status and Where We Need to Progress, by Martin et al.; 3) Circulating tumor DNA analysis for assessment of recurrence risk, benefit of adjuvant therapy, and early relapse*

detection after treatment in colorectal cancer patients by Henriksen et al. DOI: 10.1200/JCO.2021.39.3_suppl.11 Journal of Clinical Oncology 39, no. 3_suppl (January 20, 2021) 11-11.

Reply 2: Thank you for these recommendations.

Changes in the text: We have reviewed and added each of these references to our manuscript.

Reviewer C

Comment 1: *This is a fairly written manuscript with several apparent weaknesses:*

1. Table 2: AMPLIFY-201 is a ctDNA guided study and hence not a non-interventional study.

Reply 1: Thank you for identifying this.

Changes in the text: We have moved this study to Table 2 (Non-interventional studies).

Comment 2: *Line 160: ‘Though there is currently much discussion of the possible uses of LB in cancer care, there are limited publications on the actual clinical use’- not an accurate statement. The authors should modify this statement and state that liquid biopsy-based technologies are far from reaching their full potential.*

Reply 2: We agree and have changed this sentence as per the reviewer’s request.

Changes in the text: We have changed this sentence as per the reviewer’s request.

Comment 3: *The Mayo Clinic experience (line 159-197): Where does it come from? Did Mayo Clinic publish an internal institutional guideline? What is the source of this information? The authors should provide data to support these statements as “The Mayo Clinic experience.” Does this experience come from a survey or an organized study?*

Reply 3: We apologize for this confusion. Our medical and surgical oncologists practice based on published (not ongoing, active studies), and not from our own survey or study.

Changes in the text: We have changed section heading to “The Current Mayo Clinic Practice” to provide clarification that these are not guideline-based recommendations, but rather practice based on existing studies.

Comment4: *Several sections are nebulous: for example (line 169-173), 'In the non-interventional setting, LB is performed for patients presenting with stage IV disease. This has three potential uses: to establish baseline ctDNA characteristics, to compare to primary tumor and correlate LB levels with primary tumor characteristics, and if primary tumor biopsy tissue is insufficient for sequencing.' The meaning and the purpose of this paragraph are unclear.*

Reply 4: We have re-written this paragraph to be hopefully be clearer about our use of LB in practice for stage IV colorectal cancer.

Changes in the text: We have re-written this paragraph to be hopefully be clearer about our use of LB in practice for stage IV colorectal cancer.

Comment 5: *A section should be added to include the literature on the utility of ctDNA in treatment response assessment, particularly while patients are receiving immunotherapy (Bratman. Nature cancer 2020).*

Reply 5: Thank you for this suggestion.

Changes in the text: We have cited this reference and added to the related section in the Introduction.

Comment 6: *The MRD section is weak. This is one of the most important aspects of ctDNA technology.*

Reply 6: Thank you for this recommendation.

Changes in the text: We have added more regarding MRD to the last paragraph of the Introduction.