Peer Review File

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Reviewer A

In the manuscript by Zhang S, et al, the authors analyzed 258 plasma samples from 198 patients with gastrointestinal (GI) cancers to detect circulating tumor DNA (ctDNA) using a tumor-informed multiplex PCR-NGS assay, and abstracted their clinicopathological data via chart review. ctDNA-positivity was significantly associated with advanced stage and the presence or extent of metastases. Serial time-point analysis showed that 22% patients cleared ctDNA following treatment, and ctDNA was detected in all patients who recurred. The findings presented in this study would be of interest for the journal's readers. However, there are several concerns to be addressed.

We would like to thank the reviewers very much for their time and insightful comments. We believe the manuscript is enhanced after addressing the reviewers suggestions.

Comments,
1. In the Methods section, more details regarding multiplex PCR-NGS assay (Signatera) should be described.

Response: Further detailed description added (page 5, lines 2-9).

2. In this study, the authors sought to focus on the positivity rate of ctDNA according to ethnicity. However, sample size was too small to show statistically significant difference across the ethnicity. The authors should minimize the description about the ethnicity-dependency throughout the manuscript.

Response: We agree the sample sizes are too small and adjusted the text accordingly. We removed all sentences that referred to a potential causal association between ethnicity and ctDNA positivity.
Page 7 (bottom of last paragraph); Results: We included 'trends' to highlight the fact that no statistical statements are being made. We also removed last sentence which had indicated positivity rates might be related to ethnicity.
Page 10, lines 2-10; Discussion: We removed two sentences which highlighted ctDNA positivity association with ethnicity.

3. In the Discussion section (page 11, lines 13-16), the authors proposed mechanisms underlying the higher rates of positive ctDNA in patients receiving active systemic treatment. They argued that ctDNA positivity rates are higher during active disease when patients are receiving treatment due to higher tumor burden, compared to patients who are not receiving therapy. How about other hypothesis such as temporary increase in ctDNA from tumor cells damaged by chemotherapy?

Response: Thank you for this suggestion. We agree and added a sentence to the discussion including the proposed mechanism by the reviewer as a potential explanation of the observed ctDNA levels. Page 12, lines 5-6.

Reviewer B

The authors investigated the utility of ctDNA in GI malignancies.
1) Regarding surgery for stage IV disease, please clarify whether the radical surgery or palliative surgery was performed.

Response: No palliative surgeries were performed. We clarified this in Methods, page 5, line 3-5.

2) Figure 1 was not described in the main text. I guess that figure 1A was the fourth case and figure 1B was the second case. Furthermore, please describe the exact time of ctDNA positive and recurrence after surgery in each case.

Response: Results page 8: Figure included in the case description. Please note: Fig 1A and 1B are switched to reflect the correct order of case presentation.
We added the time from surgery to recurrence in months to the case descriptions (page 8).

3) ref.16 is an article for MRD in not GI malignancies but multiple myeloma. Please change or delete the reference #16.

Response: Thank you for noticing the oversight. The appropriate reference was included and the original reference #16 was removed.

4) Even if the follow-up time is short, KM curve for DFS or RFS is necessary for this kind of research.

Response: We very much agree with the reviewer that survival endpoints are important and fully intend to follow up the patients longer (and over time also with more patients included) in order to be able to describe differences in RFS, PFS, and OS based on ctDNA dynamics. The main purpose of survival curves would be to compare ctDNA positive with negative patients, and their recurrence pattern. We reviewed the database and at this time, due to short follow-up, only 3 patients with original Stage I-III have recurred. Thus, at this time it would not be feasible to generate survival curves with only 3 events.

5) Ethnic difference should not be referred because it might be due to social problem including healthcare access.

Response: Please also refer to the response #2 to Reviewer A: Several passages were removed.

Also, we described some of the barriers to care in Discussion, page 10, line 2-4.