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December 4th, 2019

RE: Revised manuscript No. 52253

Dear Editor,

thank you for the opportunity to further revise our manuscript entitled "The significance of postoperative follow-up of patients with metastatic colorectal cancer using circulating tumor DNA".

Thank you for reviewing and comments concerning our manuscript. We have dealt with all suggestions and have made all of the revisions using Track Changes function to the revised manuscript. We have added notes to each Editor's comment and in case of doubt we have asked a specific question.

We have resubmitted a revised version. The manuscript has been formatted according to the instructions. The order of the authors has been unified in the system and in all uploaded files as well as the order of signatures in copyright license agreement. The manuscript number has been edited in all uploaded files. The repeated references have been removed and minor revisions have been made in reference format. The supplementary tables were uploaded in a separate file.

We hope that the revised manuscript will meet the Editor's opinion and will now be accepted for publication. We look forward to hearing from you soon.

Sincerely,

Dr. Lucie Benesova
Centre for Applied Genomics of Solid Tumors, Genomac Research Institute
Drnovská 1112/60, Prague 6, 161 00, Czech Republic

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November 26th, 2019

RE: Revised manuscript No. 52253

Dear Editor and Reviewers,

thank you for your consideration of our manuscript entitled "The significance of postoperative follow-up of patients with metastatic colorectal cancer using circulating tumor DNA" for publication in *World Journal of Gastroenterology*.

We thank the Reviewers for their careful reading and valuable comments concerning our manuscript. We do accept all suggestions and include a point-by-point response to the Reviewers' comments bellow.

We have resubmitted a revised version in accordance to the Guidelines and Requirements for Manuscript Revision and the Format for Manuscript Revision. Running title, open-access statement, copyright statement and article highlights have been added to the manuscript file. Audio core tip and approved grant application form have been newly uploaded and the order of one of the co-authors has been changed. All of the revisions were highlighted in yellow.

We hope that the manuscript in its revised form will meet the Reviewers' opinion and will now be accepted for publication. We look forward to hearing from you soon.

Sincerely,

Dr. Lucie Benesova
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Reviewer's code: 00724702

SPECIFIC COMMENTS TO AUTHORS

Well written manuscript which would add value to the current established knowledge and evidence. The authors report that "In 22 cases of recurrence, ctDNA positivity was detected 22 times (22/22, 100%) compared to 16 positives (16/22, 73%) by imaging methods and 15 cases (15/22, 68%) of elevated tumor markers. Based on this they conclude that "ctDNA detection in patients with mCRC is a viable tool for early detection of disease recurrence". They should state the adequacy of the sample size to reach the stated conclusion.

RESPONSE TO REVIEWER

Thank you for your positive feedback, we are very pleased that you have found our manuscript valuable. Regarding your comment on the adequacy of the sample size, in comparison with recent publications and to our knowledge to this date, we present the largest number of curative resections whose postoperative development was monitored using ctDNA. Our study included 47 patients who met narrow entry criteria - stage IV of colorectal cancer and positive ctDNA at the time of diagnosis immediately before surgery. Out of a total of 40 curative R0 resections, 30 long-term postoperative follow-ups were performed over the subsequent months and years using standard imaging techniques, tumor markers and ctDNA testing so all three parameters could be compared. In 22 cases (73%), recurrence was detected and 8 (27%) were recurrence-free, with a corresponding positive and negative result of ctDNA, respectively. Thus, the specificity of the ctDNA test was 100%. Moreover, in four patients the disease recurrence was detected by ctDNA only (while at the same time, imaging methods and tumor markers were negative). Thank you for your very helpful remarks, indeed it is unusual that the manuscript lacked comment on the sample size. We have added a statement about the adequacy of the sample size and added the appropriate references to the

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discussion. If you still feel uncertain about our concluding statement that "ctDNA detection in patients with mCRC is a viable tool for early detection of disease recurrence", we may consider adjusting its wording.

Reviewer's code: 03017516

SPECIFIC COMMENTS TO AUTHORS

The study is very interesting, and it is very important to analyze the correlation between ctDNA, resection margin status, recurrence, metastases. The methodology is well explained. I have some minor remarks: - Introduction: may you focus more on the topic? The first part of the introduction is very generic, the paragraph starting with "other common sites." is not so useful. I think you should shorten the Introduction eliminating most of the first part. - Methods: may you mention the costs of the ctDNA extraction? - Is the test reliable, did you test several times the same samples to assess the reproducibility of the test? - Did you compare the efficacy of ctDNA versus MRI in patients with liver metastases? - Did the detection of ctDNA change patients' management?

RESPONSE TO REVIEWER

We greatly appreciate your interest in our study. Thank you for your helpful remarks. The answers to your questions are as follows:

Introduction: may you focus more on the topic? *We fully accept your concern about the length of the first part. We have reduced most of the first two paragraphs and made up only one general paragraph regarding surgical removal of metastatic colorectal cancer. We have also modified the references to reflect this revision.*

Methods: may you mention the costs of the ctDNA extraction? *ctDNA extraction from one plasma sample costs approximately 9 euros (including material, operating and personnel costs). In this context, it is also worth mentioning the total cost of ctDNA analysis (i.e. ctDNA extraction,*

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PCR with the formation of heteroduplexes and denaturing capillary electrophoresis), which is approximately 60 euros per sample.

Is the test reliable, did you test several times the same samples to assess the reproducibility of the test? *Yes, the test is reliable. Reproducibility of our ctDNA test was fully validated. The method has been used in our laboratory for over 10 years and has been successfully applied in the detection of ctDNA in various cancers such as non-small-cell lung carcinoma, pancreatic ductal adenocarcinoma, gastric cancer and others (publication in preparation). For selected targets (KRAS and EGFR) the method has been accredited according to the EN ISO 15189:2013.*

Did you compare the efficacy of ctDNA versus MRI in patients with liver metastases? *Comparison of the efficacy of ctDNA with that of MRI was not performed as the standard imaging method was CT and MRI was only indicated when the CT finding was unclear.*

Did the detection of ctDNA change patients' management?

Yes, but only in a way that in case of detection of ctDNA elevation, an additional follow-up CT scan was performed earlier than planned according to the original schedule.