Author's response to reviews

Title: BRAF V600E mutation and KRAS codon 13 mutations predict poor survival in Chinese colorectal cancer patients

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Editor of BMC Cancer

Dear Editor,

Hereby we would like to resubmit to the Editorial Office of "BMC Cancer" the manuscript titled: "BRAF V600E mutation and KRAS codon 13 mutations predict poor survival in Chinese colorectal cancer patients”.

Thank you for your careful review of our manuscript and your encouraging messages. We have revised our manuscript according to the reviewers’ comments and the editor’s request. We have explained point by point in this letter (please see below) how we have addressed each revision request or comment.

Thank you very much for your time and consideration, and we are looking forward to your reply.

Yours sincerely,

Prof. Youji He M.D. Ph.D

Answer to Editor
We thank you very much for your careful reading of our text and supportive comments on the significance of the study. We have addressed your request in detail.

Copyedit:
We recommend that you copyedit the paper to improve the style of written English.
We have carefully copyedited the manuscript. Prof. Wouter H Lamers from University of Amsterdam, who has great experience in writing scientific papers in English, helped in language editing.

Answers to reviewer 1
We thank the reviewer for his careful reading of our text and supportive comments on the significance of the study. We have addressed each of the questions in detail. The page- and line-numbers refer to the revised version.

This is a well-conducted study. I have a few comments for improvement. 
The authors should examine KRAS mutations and tumor location in more detail.

We agree with the reviewer and had indeed examined KRAS mutations and tumor location in detail. However, no difference was found for KRAS mutation comparing proximal colon, distal colon and rectum (Please find the enclosed table below). Since mutations in BRAF or PIK3CA showed significant difference between colon and rectum, we have listed only colon and rectum in Table 2A (corresponding text is at page 7 line 148-151).


<table>
<thead>
<tr>
<th>Tumor location</th>
<th>KRAS exon2 mutation</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No (%)</td>
<td>Yes (%)</td>
<td>P (chi-square)</td>
</tr>
<tr>
<td>proximal colon</td>
<td>31 (26.3)</td>
<td>33 (34.3)</td>
<td>0.057</td>
</tr>
<tr>
<td>distal colon</td>
<td>42 (35.6)</td>
<td>20 (20.8)</td>
<td></td>
</tr>
<tr>
<td>rectum</td>
<td>45 (38.1)</td>
<td>43 (44.8)</td>
<td></td>
</tr>
</tbody>
</table>

Especially, is KRAS mutation more common in cecal cancers, as previously shown by M Yamauchi, T Morikawa, et al. Gut 2012; and C Rosty et al. Mod Pathol 2013? Please discuss findings together with the data by Yamauchi and Rosty.

There were not many cecal cancers in this study cohort. Among the 436 consecutive patients diagnosed as colorectal cancer at Zhongda Hospital Affiliated to Southeast University (Nanjing, China) from 2007 to 2012, only 6 patients had cancer in the cecum. In the final 214 patients included in this study, 3 patients had cecal cancers, among them, 2 had a KRAS mutation.

We agree with the reviewer that it is important to assess the linearity and non-linearity of molecular relationships by bowel subsite. However, as mentioned in the reference of Yamauchi, subsite analyses require an adequate statistical power. Although we have detailed colorectal subsites data, we don’t think that our sample size of 214 permits meaningful subsite analyses. For your reference, please find the summary of distribution of KRAS-mutated cancers by bowel subsite in 208 of the 214 patients included in this study (we have no detailed subsite data on 6 patients).
<table>
<thead>
<tr>
<th>Tumor location</th>
<th>KRAS exon2 mutation</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No (%)</td>
<td>Yes (%)</td>
<td>P (chi-square)</td>
<td></td>
</tr>
<tr>
<td>cecum</td>
<td>1 (0.90)</td>
<td>2 (2.1)</td>
<td>0.378</td>
<td></td>
</tr>
<tr>
<td>ascending colon</td>
<td>15 (13.2)</td>
<td>18 (19.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>hepatic colon</td>
<td>7 (6.1)</td>
<td>7 (7.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>transverse colon</td>
<td>4 (3.5)</td>
<td>4 (4.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>splenic colon</td>
<td>2 (1.8)</td>
<td>2 (2.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>descending colon</td>
<td>8 (7.0)</td>
<td>5 (5.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sigmoid colon</td>
<td>32 (28.1)</td>
<td>13 (13.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rectum</td>
<td>45 (29.5)</td>
<td>43 (45.7)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Also please discuss the colorectal continuum model proposed by Yamauchi, Lochhead, et al. (Gut 2012), given continuum of gut microbiota and immunity. Recent reviews by D Colussi et al. (Int J Mol Sci 2013) and F Bishehsari et al. (World J Gastroenterol) are worth mentioning.

We agree with the reviewer that the colorectal continuum is an interesting topic. However, since we don’t have adequate statistical power to assess the linearity and non-linearity of molecular relationships by bowel subsite and we already have an extensive discussion (as commented by the second reviewer), we would like not to add this topic in the discussion of this paper. When we have enlarged our sample size by recruiting more CRC patients from other clinical centers, we hope that we will have adequate statistical power to address this topic in a next paper.

Answers to reviewer 2
We thank the reviewer for her careful reading of our text and supportive comments on the significance of the study. We have addressed each of the questions in detail. The page- and line-numbers refer to the revised version.

The study design is carefully planned and thoroughly described, the chosen methods are appropriate and results are presented comprehensively. Limitations of the work are clearly stated.

Major Compulsory Revisions:
There are no major aspects requiring revision by the authors.

Minor Essential Revisions:
• The authors state that “140 patients were excluded as they were lost during follow-up period” (p.4 ll.86-87). Were any differences observed between those 140 patients and the 214 finally included patients with regard to clinical parameters or known mutation status that might bias the obtained associations?

What is specifically meant by “lost during follow-up period” (required minimal post-operative observation period for inclusion in study?)?
140 patients were excluded, because these patients didn’t come back to Zhongda Hospital for the post-surgery surveillance. Therefore, we have no follow-up data on them.

We did the comparison of clinicopathologic characteristics of the patients included and excluded in this study, but no difference was observed. We have added this comparison as the Additional file 1 in the revision. The corresponding text has been added to the Materials and Methods, page 5 line 90.

3-year OS for KRAS c.38G>A mutants/BRAF wt is indicated as 27.9%. However, Figure 3E indicates a 3-year OS for this patient group of about 55%. Please correct and double-check calculations.

We thank the reviewer for her careful reading of our text. It was indeed a typo. We have changed it to the correct number of 55.8% (page 8 line 189).

• While the discussion targets many of the obtained results in great detail, it is extensive and should be focused more on the major findings of the study.

We have now eliminated ~20% (22 lines) of the Discussion in the revised version. Besides the major findings of the study, we believe the remained discussion points are important as well, because 1. Limited data is available for BRAF and PIK3CA in Chinese CRC patients. 2. Few studies had follow-up data. 3. Many issues haven’t been deeply discussed in Chinese CRC patients yet. Therefore, if the space of the journal allows, we would like to keep them.

Quality of written English: Needs some language corrections before being published

We have carefully copyedited the manuscript and corrected the errors. Prof. Wouter H Lamers from University of Amsterdam, who has great experience in writing scientific papers in English, helped in language editing.