Author’s response to reviews

Title: Impact of Primary Colorectal Cancer Location on the KRAS Status and its Prognostic Value

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Reviewer reports:

Desmond Yip (Reviewer 1):

Some language and grammatical corrections required
Response: We are highly grateful for your invaluable comments. We have revised the WHOLE manuscript carefully and tried to avoid any grammar or syntax error. If you think additional editing needs to be done in order to further improve the language of manuscript, please feel free to contact us.

Suggest that a CONSORT diagram be included to indicate how many papers the search found and how these fitted into each of the analyses the number of excluded studies and reasons for exclusion. Figure 1 and 3, 4 need labelling of the x axis especially what each side of the graph represents.
Response: Thank you for your valuable comment. We added a low chart (Figure 1) of the article selection process in the revised manuscript. We described the meaning of forest plots in the corresponding figure legends. Letters marked in red in the figure legend of revised manuscript.

Combine survival analysis of the patients in the paper was done with inclusion of 5 papers that had survival data. Need some details on the patient population ie early stage cancers or included metastatic cases. Did patients in these series have access to anti EGFR therapies in the palliative setting?
Response: Thank you so much for your thoughtful advice. There were four studies that provided the data of OS, we corrected this mistake in the revised manuscript. However, the data of patients in each study was incomplete; we could not list the details, such as tumor stage, in the Table 3. In addition,
although these studies reported that the patients receiving chemotherapy and/or radiotherapy, no studies presented the data of anti-EGFR therapies, so we could not make a stratify analysis base on the different stage of treatment.

Charlton paper listed in table 3 does not have mutational status. Was this a subset of the Chalton dataset used in the analysis in Figure 4
Response: Thank you so much for the comment. The data of Chalton dataset was not used in the analysis in Figure 4 (Figure 5 in the revised manuscript).

Matthew Burge (Reviewer 2): This paper analyses the prognostic impact of KRAS status by tumour location.
Unfortunately, I find that there are flaws in this paper which need to be addressed.
Specifically:
- Introduction: Authors should detail what is defined by a right and left sided colon cancer. How were transverse colon cancers classified? It's not clear why they also divided the left colon and rectum and compared these 2 entities
Response: Thank you for your valuable comment. We stated the definition of right and left sided colon cancer in first paragraph of Method part of the revised manuscript (Letter marked in red). The transverse colon cancers were classified based on the site of tumor, and the splenic flexure of colon was the site divided colon into right and left sided colon. The rectum was not belonged to the left colon, so we need to divide the left colon and rectum for further analysis as previous study did.

A discussion/reference to RAS mutations outside codons 12 and 13 should take place. Why were these patients excluded? What might their impact on the results be?
Response: Thank you for your critical comment. We discussed the other RAS mutations in the revised manuscript. Because the patients with other RAS mutations, such as NRS and BRAF mutation, was much fewer compared with the RAS mutations of codons 12 and 13, we therefore did not included these patients. However, this exclusion might lead to selected bias in the analysis. We discussed this limitation in Third point of the fourth paragraph of the revised manuscript.

Methods: A more detailed description of this study is needed. Was it prospective? Why was there no survival data?
Response: Thank you so much for your comment. We made a more detailed description of this study in the revised manuscript. Letter marked in red in the first paragraph of Method section. This study was retrospective design. Because a large part of the patients were admitted to hospital after 2017, some patients remain receiving treatment; thus little survival data was available for these patients, so we could not extract the survival data.

More detail is needed regarding the types of studies included in the meta-analysis. Were they prospective or retrospective? randomised? An important paper on this topic comes from the QUASAR study:11)
Response: Thank you so much for thoughtful advice. We added the details of the included in the revised manuscript. See the revised Table 3. The Hutchins et al study was such a good paper, thank you give us a chance to learn it, we modified our manuscript according to the Hutchins et al study.

The statistical section is inadequate. Was a multivariate analysis performed to take into account confounding prognostic factors? How many patients were included in the meta-analysis of the
The study population includes all stages of colorectal cancer hence is very heterogeneous and I can't see how any definitive conclusions can be drawn based on the data presented. The effect size of KRAS appears modest. What were the endpoints? "Survival time' is inadequate. Overall survival; disease free survival?

Response: Thank you so much for important suggestions. The multivariate analysis performed in the included study was adjusted by the TNM stage, age, gender, but not all the prognostic factors. The number of patients included in the meta-analysis of the prognostic impact of KRAS was four studies with 6697 patients; we added this number in the revised manuscript.

Yes, this study includes all stages of colorectal cancer, which will undermine the robustness of conclusion; we discussed this limitation in the revised manuscript.

Yes, the effect size of KRAS appears modest.

The endpoint of the included study was overall survival.

There needs to be justification for the inclusion/exclusion criteria. Why were patients receiving pre-operative chemotherapy/radiotherapy excluded? The target population for this study is not well.

Response: Thank you so much for critical comment. We described the inclusion/exclusion criteria in the revised manuscript. Letter marked in red in the first paragraph of Method section. Actually, the patients were receiving pre-operative chemotherapy/radiotherapy after resection of tumor. The detection of KRAS was before the pre-operative chemotherapy/radiotherapy.

Results: The figures are somewhat confusing and need better explanation. In addition figures 3 and 4 appear to be the wrong way around.

Response: Thank you so much for the advice. We corrected this mistake in the revised figures, and modified the figure legend to better describe the figures.

Discussion: I do not agree with the concluding statement that this studies data indicate the crucial importance of categorising CRC by primary side and KRAS status. This is important in metastatic colorectal cancer, but this is already well established. The utility of these same factors in early stage CRC is less.

Response: Thank you so much for thoughtful comment. We agreed with your opinion. We removed this sentence in the conclusion to turn down the importance of our study in the revised manuscript.