Title: *PIK3CA* and *TP53* mutations predict overall survival of stage II/III colorectal cancer patients

Previous title: *PIK3CA* and *TP53* Mutations Cooperatively Correlate with Poor Outcome in Stage II/III Colorectal Cancer Patients Treated with 5-Fluorouracil-based Chemotherapy

Dear Editor,

Thank you very much for the consideration of our manuscript for submission in the World Journal of Gastroenterology. The comments are very valuable. We have considered the comments carefully and revise the manuscript according to the comments. Revised portion have been marked in the manuscript. The followings are the point-by-point responses to the reviewer's comments.

Yours sincerely,

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Response to the reviewer's comments

1. Introduction: Introduction caption is missing.

Response: Introduction caption has been added to the manuscript.

2. In vitro and in vivo term in italics.

Response: In vitro and in vivo were revised in italics.

3.Use CRC abbreviations across the whole manuscript.

Response: CRC abbreviations were used across the manuscript.

4. The sentence miss a verb: Tumor staging strictly abided by the TNM classification of the American Joint Committee on Cancer (AJCC).

Response: The verb has been added.

5. The QIAamp DNA FFPE Tissue Kit is specially designed for purifying DNA from formalin-fixed, paraffin-embedded tissue sections. Why QIAamp DNA Mini Kit was used for isolation of genomic DNA from FFPE?

Response：Sorry for the mistake and thank you for your detailed checking. Indeed, we extracted DNA from FFPE samples using QIAamp DNA FFPE

Tissue kit (Qiagen).

6. Which version of Stata software was used for statistical analysis?

Response: The versions 14.2 of Stata software are applied in the manuscript.

7. Please characterize in detail the *PIK3CA-TP53* mutations observed in your study. Is any mutation observed predominantly? Is OS associated with any particular mutation as well?

Response： The data of *PIK3CA* and *TP53* mutations has been uploaded in the supplementary Table 1 and Table 2. For individual sites of *PIK3CA* and *TP53* mutations, the most frequent mutation in CRC was Glu545Lys (5.1%) and Arg175His (5.1%), respectively. The amount of samples carrying both mutations was very small and not sufficient for OS analysis.

8. PIK3CA mutation were observed predominantly in rectum. Do you have hypothesis why it is so?

Response： In my manuscript, *PIK3CA*-mutated tumors were significantly correlated with proximal location (*P* = 0.036), I mistakenly wrote as 'with rectum location'. I am sorry for this mistake. In our study, the frequency of *PIK3CA* mutations in the proximal, distal and rectum location was 50% (39/78), 38.7% (29/75) and 32.7% (53/162), respectively. The present results

were consistent with those of two previous studies which were mentioned in the revised manuscript, showing that the proximal colon showed a higher frequency of *PIK3CA* mutations than any other sites.

9. The word against in sentence is controversial? Do you mean in CRC treatment? - The effectiveness of 5-FU-based chemotherapy is very limited against colorectal cancer.

Response：5-FU-based chemotherapy is regarded as a first-line chemotherapy in both adjuvant and palliative settings for advanced CRC. However, a large number of patients actually will not benefit from 5-FU-based chemotherapy but suffer from side effects. But now I realize there is ambiguity or discrepancy in this sentence. I have deleted this sentence in the manuscript.