**Dear Editor and Reviewers,**

Thank you very much for your time spent reviewing and providing feedback on our manuscript. We have addressed the comments provided by the reviewers, point by point, and revised the manuscript accordingly.

Best regards,
Bing Lu, M.D.

**Reviewer(s)' Comments to Author:**

**Reviewer code: 05290162**

A unique case indeed, and well described. A few points need elaboration. 1- The staging of rectal cancer, I have doubts that a tumor at 4-5 cms from anal verge can undergo resection and joining unless preoperative radiations are given. She had stage III disease so, did she receive and post adjuvant treatment. 2- The staging of the oesophageal cancer is not mentioned properly. 3- What was done for gastric GIST? 4- The case is written in ward round history, please rewrite in medical manuscript fashion.

**Answer:** Thank you for your suggestions. We are grateful to your comments. The detailed answers are shown below.

1- The staging of rectal cancer was stage IIIB (T3N1cM0). She didn't receive any preoperative radiations or post adjuvant treatment at the patient's request.

2- The staging of the oesophageal cancer was stage III (pT4N0M0). We have revised the stage in the manuscript on line 142~143, page 5.

3- There was no postoperative treatment for leiomyoma or gastric GIST.

4- We have edited for proper English language, grammar, punctuation, spelling, and overall style. We hope that you are satisfied with our revised manuscript. Thank you very much!

**Reviewer's code: 01221666**

The author performed a next-generation whole exome sequencing for tissue specimens of rectal cancer (2016), esophageal cancer, GIST, and WBC (2019) in a case of multiple primary cancers. They reported that esophageal cancer and GIST were not genetically evolved from rectal cancer and this patient did not have a trunk driven clone. Comments 1. The authors concluded that NGS is a useful tool to study cloncal evolution of tumours and distinguish between MPMNs and intrapulmonary metastasis. This conclusion is rooted on the facts when tissue specimens are available. Moreover, what extra applications of NGS when you can reach pathological diagnoses anyway? 2. The author should demonstrate how NGS in this case aid or add clinical significance.

**Answer:** Thank you for your comments and suggestions. It will make our study more competitive. The detailed answers are shown below.

1. MPMNs are sometimes confused with multiple metastases from the same cancer. As in Reference NO. 21, In an NGS analysis of 60 patients with multifocal tumours, researchers at Memorial Sloan-Kettering Cancer Center found that prospective histological predictions were inconsistent with the sequencing results in 17 (22%) cases, particularly intrapulmonary metastases (IPMs) (44%). In comparison with NGS, standard histopathologic approach is adequate in most cases, but has notable limitations in the recognition of IPMs. In Reference NO. 22, 9 of 33 identical histological tumour pairs were misclassified, 7 were inconclusive and 2 were metastases by histological evaluation, while categorization was accurate by genetic testing.

2. In addition to studying the origin and evolution of tumours, the results of WES could also guide the medication of the patient. The patient in the case with KRAS.p. G13D should not be administrated with cetuximab. The KIT p.V559D mutation indicated that the patients might be treated with KIT inhibitor such as imatinib. We have revised in the manuscript on line 261~264, page 9.

**Reviewer's code: 06040507**

Ouyang et al. reported a case of 66-year-old female with multiple primary cancers, and investigated the genetic characteristics using the whole exome sequencing. The report described the methods detailly, presented the results clearly, and addressed the scientific question properly. This is a valuable study described a case of multiple primary cancers, of which, the newly diagnosed tumors in 2019 were not genetically evolved from rectal adenocarcinoma in 2016. However, some details need further improved. 1. Did authors perform Sanger sequence or RNA sequence to verify the results? 2. The sequencing technique is truly a practical tool for oncology research, and clinical diagnosis and treatment. Please clearly indicate the clinical value of WES for this certain case (e.g., How does WES assist in the diagnosis of patients? What is the guiding significance for clinical treatment? How does WES improve the patient's prognosis?). 3. Misdiagnosis is a common problem in multiple primary cancers cases. Is it necessary for all patients with multiple cancers to receive a WES test to clarify the diagnosis? Please highlight the enlighten of this report to the following clinical works. 4. As authors stated, the cause of MPMNs in this case remains unclear. Please highlight the value of WES in multiple primary cancers' mechanism research. 5. Please improve the sharpness of the red arrows in Figure 2 to make them clearly. 6. Please mention the patient's consent in the article. 7. Please ensure that all authors meet the criteria of ICMJE. 8. Please ensure that the format of the informed consent form meets the journal's requirements.

**Answer:** Thank you very much for your time spent reviewing and providing feedback on our manuscript. The detailed answers are shown below.

1. We didn't perform Sanger sequence or RNA sequence to verify the results. Because compared with Sanger sequencing or normal PCR, WES sequencing can cover a comprehensive range of genes and loci, which is more conducive to the study of tumour evolution and origin, and its detection results could provide suffcient reference information for medication.

2. We have described these in detail in the article on line 233~260, page 8~9 and line 265~269, page 9.

3. Due to limited data, this research only provides a reference for the identification of multiple primary tumours and metastatic tumours from the genetic level. For large-scale clinical application, more prospective research results with large sample size are needed to guide clinical decision-making, and the cost of detection and the economic cost of patients are also important factoring that must be considered.

4. WES sequencing can cover a comprehensive range of genes and loci, which is more conducive to the study of tumour evolution and origin.

5. We have modified the way we label tumors in Figure 2 in this manuscript. The red circles indicate tumour lesions.

6. We have mentioned the patient's consent in the article on line 164~165, page 6.

7. We ensure that all authors meet the criteria of ICMJE.

8. We ensure that the format of the informed consent form meets the journal's requirements.

**Reviewer's code: 00070191**

This study presents a case where next-generation whole-exome sequencing confirmed that ESCC and GIST did not genetically evolve from a previous rectal adenocarcinoma. The study is remarkable in terms of emphasizing the importance of whole-exome sequencing in such cases. The authors stated that esophageal SCC and GIST were not found together. However, there are cases in the literature with GIST, SCC in the esophagus, and other tumors 1. Therefore, this statement should be changed. Information on the occurrence of GIST and other tumors in the same and different organs should be emphasized more comprehensively. 1. Zhou Y, Wu X, Shi Q, Jia L. Coexistence of gastrointestinal stromal tumor, esophageal and gastric cardia carcinomas. World J Gastroenterol 2013; 19: 2005–8 [ PMID: 23569349. DOI: 10.3748/wjg.v19.i12.2005 ]

**Answer:** Thank you for your suggestions. We really appreciate your advice which will make our paper more complete. As you suggested, we have carefully reviewed the statement and changed on line 80~91, page 3. And we added references NO. 10~12 in the manuscript. Thank you very much!

10 Zhou Y, Wu XD, Shi Q, Jia J. Coexistence of gastrointestinal stromal tumor, esophageal and gastric cardia carcinomas. *World J Gastroenterol* 2013; 19: 2005-2008 [PMID:23569349 DOI: 10.3748/wjg.v19.i12.2005]

11 Suzuki T, Suwa K, Hanyu K, Okamoto T, Fujita T, Yanaga K. Large gastrointestinal stromal tumor and advanced adenocarcinoma in the rectum coexistent with an incidental prostate carcinoma: A case report. *Int J Surg Case Rep* 2014; 5: 640-642 [PMID: 25052916 DOI: 10.1016/j.ijscr.2014.06.012]

12 Fan H, Lu P, Xu L, Qin Y, Li J. Synchronous occurrence of hereditary gastric adenocarcinoma, gastrointestinal stromal tumor, and esophageal small cell and squamous carcinoma in situ: An extremely rare case report. *BMC Cancer* 2017; 17: 720 [PMID: 29115925 DOI: 10.1186/s12885-017-3736-0]