Professor Lian-Sheng Ma,
President and Company Editor-in-Chief,
World Journal of Gastroenterology

Dear Professor Lian-Sheng Ma,

Manuscript: 81663

"Novel biomarkers for early detection of gastric cancer", by Tasuku Matsuoka and Masakazu Yashiro

As per invitation by the Editor, please find attached a revised version of the original version of our manuscript previously entitled "Novel biomarkers for early detection of gastric cancer", for which the resubmission fee has been waived. We are most grateful to you and the reviewers for the helpful comments and have addressed each concern with either new data or modification of the manuscript text. A brief overview of changes made has been included below, and we hope that the explanations and revision of our work is satisfactory.

In writing this paper, English grammar was carefully checked again by a colleague whose native language is English.

In addition to a number of editorial corrections, the following significant changes were made to the manuscript.

Thank you for your kind attention.

Best regards.

Sincerely yours

Tasuku Matsuoka, M.D., Ph. D.

Reviewers' comments to the authors

Reviewer #1:

This study provides a comprehensive overview of relevant detection techniques for gastric cancer, and the content is relatively rich. However, there are also the following problems:

1. There are too many references cited, and the IF of the references is not high, please delete them appropriately;

Response: We appreciate the reviewer's comments. According to the reviewer's suggestion, we delete the references of which IF is not high as possible along the reviewer's recommendation.

2. There are too many tables in the manuscript, please delete them appropriately.

Response: According to the reviewer's suggestion, we combined Table 1-3 and deleted Table 5, 6.

3. The author does not list Please make appropriate additions to the application status of MRD technology in gastric cancer;

Response: We appreciate the reviewer's comments. The prospective cohort study showed that ctDNA for detection of MRD can be an emerging clinical biomarker for disease monitoring in GC. However, applications of ctDNA need to be examined more comprehensively in prospective trials. We add these comments into the Discussion Section on page 22, line 22- page 23, line 2.

4. Please add more relevant mechanism diagrams instead of listing tables.

Response: According to the reviewer's suggestion, we made more relevant figure which

summarized the non-coding RNAs as biomarker for early detection of gastric cancer.

Reviewer #2:

1. Develop detailed inclusion and exclusion criteria.

Response: We appreciate the reviewer's comments. As the reviewer indicated, we added inclusion and exclusion criteria into Methods section.

2.The content of the paper is simplified and descriptive statements are reduced.

Response: We carefully checked our manuscript and revise sentences more simple and luculent. We also have shortened the article and delete tables to the possible extent.

Reviewer #3:

Dear editors and reviewers, Thank you for the invitation to submit the manuscript entitled "Novel biomarkers for early detection of gastric cancer". It is well known that the prognosis of progressive gastric cancer is poor. Therefore, early diagnosis and systematic and comprehensive treatment would be beneficial to improve the prognosis of this group of patients. In some parts of China, the majority of patients diagnosed with gastric cancer are already in the progressive stage. The most commonly used diagnostic tests for gastric cancer include endoscopic histopathological examination and imaging, but this also requires the active cooperation of the patient and the experience and skill of the endoscopist to make a definitive diagnosis, but this has limitations for early gastric cancer. The advent of tumor markers may open up new avenues for the diagnosis of early gastric cancer. The authors provide a detailed description of the current state of research and methods for CTCs, cfDNA, noncoding RNAs, exosomes,

DNA methylation, and STOMACH SPECIFIC BIOMARKER. Thus, the detection of new biomarkers for the early diagnosis of GC will be a potential therapeutic strategy to improve the survival prognosis of patients. This latest biomarker information will contribute to further research and development of GC biomarkers and clinical applications.

Response: We appreciate the reviewer's kind comments. I would like to use these in my future research.

Reviewer #4:

Improving the early diagnosis rate of gastric cancer is one of the main ways to improve the overall prognosis of patients with gastric cancer. Currently, the main screening methods are endoscopy and pathological biopsy, which is difficult to popularize. Therefore, the screening of biomarkers that are easy to detect and have high specificity and sensitivity is a hot issue in recent years. This review also reviews the studies on some novel biomarkers in gastric cancer. The following are my comments for this review:

1. There are many studies and reviews on biomarkers for gastric cancer screening. Please explain the uniqueness of this review compared with other reviews.

Response: We appreciate the reviewer's comments. As you pointed out, there are many studies and reviews on biomarkers for gastric cancer screening. However, many of these biomarkers are not specific for the early stages, being detected in advanced stages of gastric cancer, and cannot be used for early detection. Besides, comprehensive review focusing on early detection of gastric cancer is still lacking. Thus, in this paper, we would

like to claim that this review summarized the novel candidates of biomarkers focusing on early detection of gastric cancer comprehensively. We add these comments into Introduction section on page 6, line 1-6.

2. Morphological detection of diseased gastric epithelial cells has always been the standard means for the diagnosis of gastric cancer. Whether the detection based on biomarkers can completely replace the above methods?

Response: As the reviewer pointed out, upper gastrointestinal endoscopy has been well established as the standard for gastric cancer screening. In contrast, the false-positive rates in the first round are said to be 14.9% for endoscopic screening. Notably, endoscopy is easily missed during screening, even when it is performed by qualified endoscopists. Thus, it is desirable to develop a method that can detect molecular level changes associated with gastric cancer at an early stage even before the patient becomes symptomatic. In this paper, we undertook a comprehensive and systematic biomarker discovery to develop blood-based biomarkers for the early detection of gastric cancer. If high sensitivity is achieved, biomarkers for gastric cancer screening may result in unnecessary upper gastrointestinal endoscopy. We added these comments into Discussion section on page 22, line 18- Page 23, line3.

3. Serum tumor markers such as CA724 and CA199 are used for early screening and assessment of disease changes in clinical practice, but their sensitivity and specificity for gastric cancer are still limited. ctDNA, cfDNA and other indicators mentioned in the paper have been showed high diagnostic value. Do their research results have selection bias? Have any prospective clinical trial results been reported?

Response: We appreciate the reviewer's comments. Certainly, circulating biomarker

described in this article showed high sensitivity and specificity compared with conventional tumor markers. The possibility is not denied these results had selection bias. As we mentioned in this paper in Discussion section on page 29, line11-18, several clinical trials have explored the use of a liquid biopsy in an early diagnosis of setting for patients with gastric cancer. However, all of the trials were observational, case-control, and cohort trials, and no randomized control trial enlisted pertaining liquid biopsy could be found, suggesting the difficulty on the future of integrating liquid biopsies into clinical practice for gastric cancer To really substantiate that circulating biomarker, such as liquid biopsy, would reveal high sensitivity and specificity, more multicenter studies and prospective evaluations in large clinical trials are necessary to realize the integrations of such biomarkers into GC screening platforms supporting the daily clinical treatment of GC patients.

4. A large number of studies in gastric cancer have suggested that many non-coding RNAs play a crucial role in the occurrence and development of gastric cancer. However, it is very difficult to screen out several key miRNAs or lncRNAs, and the miRNAs or lncRNAs screened for diagnosis by different research teams are different. What's your opinion on this situation?

Response: We appreciate the reviewer's comments. As the reviewer mentioned, a variety of circulating biomarkers have been reported in different research team. The clinical value of all these factors is not still defined. Some discrepancies among different studies can be caused by the heterogeneity of gastric cancer. Thus, a more precise patient inclusion criteria or enlarging of the cohort size could overcome the differences in miRNA or lncRNAs expression caused by the intrinsic heterogeneity of the disease. More

multicenter, larger, longer-term studies are warranted to achieve the clinical use of liquid biopsies, in terms of early diagnosis of gastric cancer. We added these comments into Liquid biopsy section on page 16, line 14-21.