

Dr. Jing Yu
Science Editor
World Journal of Gastroenterology

September 26, 2016

Dear Dr Yu,

We would like to thank the reviewers for their useful comments. We have revised our manuscript accordingly, as outlined below.

Reviewer 1 (Code: 02456089)

- Comments; 4

1. OBP-401 is a new method to label CTCs. But, the introduction about it is not enough. So, more attention on relation of CTCs and prognosis, the detail and advantage of OBP-401 are necessary.

- We have added sentences about OBP-401 on page 8, line 13–15.

2. Authors should consider if OBP-401 accurately label CTCs or add the number of patients in this study.

- GFP-positive cells were detected in blood samples from healthy volunteers in this study. This result indicates that the OBP-401 assay detects cells with high telomerase activity cells other than CTCs. To improve the accuracy of OBP-401 assay, we determined a cut-off based on GFP fluorescence intensity and cell size.

We recognize that the sample size of this study was small, and we carefully analysed the data by using adequate statistical methods. We agree that there are some limitations in this study, and these have been described on page 18, lines 1–16.

This manuscript is a final report of a pilot study with a small sample size including more than 50 patients and 10 volunteers. We have already started the next, larger study.

3. The language should be more simplify.

- We initially submitted this article after English language editing by Cactus Communications Inc. The revised article was submitted after a second round of English language editing.

4. The picture of the cell lines infected OBP-401 should be included.

- We added a picture of the cell lines in Figure 1 (Fig. 1A), description on page 14, line 2–3 and figure legend on page 34, line 2–3.

Reviewer 2 (Code: 02520549)

- Comments; 2

1. good work, could define better the treatment that the patients received after your evaluation and if more CTCS means metastases in sanctuary sites?

- Detailed analysis of this study indicates a positive relationship between the number of CTCs and recurrence. Thus, we believe that evaluation of CTCs using OBP-401 is useful for treatment selection, including neoadjuvant and adjuvant therapy in patients.

2. Pls reduce references.

- We removed three references.

Other revision

1. Since Katsuhiko Murakami changed places with Shun I in this study, we deleted Shun I and added Katsuhiko M as a co-author.

2. CD45 staining was confirmed in the early samples in this series, and there were samples without CD45 stain. Then, we deleted the last sentence "Since CD45 is the leukocyte common antigen, CD45-positive cells were not classified as CTCs, and were excluded from the analysis." of "Cell counting and analysis" section on page 12.

We believe that our prospective translational research will be of great interest to oncologists, molecular biologists and gastroenterologists. As a journal covering international issues relevant to gastroenterological specialists, and one of the leading journals worldwide for original research with a focus on gastroenterology, *World Journal of Gastroenterology* represents the perfect platform for us to share these results with the international research community.

We confirm that this manuscript has not been published elsewhere and is not under consideration by another journal. All authors have approved the manuscript and agree with submission to *World Journal of Gastroenterology*. The authors have no conflicts of interest to declare.

Please address all correspondence to:

Hiroaki Ito, MD
Department of Surgery,
Digestive Disease Center,
Showa University Koto Toyosu Hospital,
5-1-38 Toyosu
Koto-ku
Tokyo 135-8577, Japan
Tel: +81- 3-6204-6000; Fax: +81- 3-6204-6396; E-mail: h.ito@med.showa-u.ac.jp

We look forward to hearing from you at your earliest convenience.

Yours sincerely,

Hiroaki Ito, MD

Dr. Jing Yu
Science Editor
World Journal of Gastroenterology

October 18, 2016

Dear Dr. Yu,

We would like to thank Professor Andrzej S. Tarnawski and the reviewers for their useful comments. We have revised our manuscript accordingly, as outlined below.

Professor Andrzej S Tarnawski, Editor-in-Chief
- Comments; 2

Comments.

1) The authors used relatively old references. It is somewhat surprising since there are numerous recent publications regarding this topic I see below - including those in WJG. The authors should cite these references and state why their method of identifying circulating cancer cells is superior to previously used methods.

Shimazu K, Fukuda K, Yoshida T, Inoue M, Shibata H. High circulating tumor cell concentrations in a specific subtype of gastric cancer with diffuse bone metastasis at diagnosis. World J Gastroenterol. 2016 Jul 14;22(26):6083-8. doi: 10.3748/wjg.v22.i26.6083.

- We referenced this article as reference number 16 on page 7, line 14.

Wan QS, Zhang KH. Noninvasive detection of gastric cancer. Tumour Biol. 2016 Jul 6. [Epub ahead of print] Review. PMID: 27058412 Liu WL, Liu D, Cheng K, Liu YJ, Xing S, Chi PD, Liu XH, Xue N, Lai YZ, Guo L, Zhang G. Evaluating the diagnostic and prognostic value of circulating cathepsin S in gastric cancer. Oncotarget. 2016 May 10;7(19):28124-38. doi: 10.18632/oncotarget.8582. PMID: 27058412

- We recognize that cathepsin S is a promising biomarker. However, cathepsin S is not directly associated with CTCs. Therefore, we did not reference this article in our article.

Zhong J1, Chen Y1, Wang LJ. Emerging molecular basis of hematogenous metastasis in gastric cancer. World J Gastroenterol. 2016 Feb 28;22(8):2434-40. doi: 10.3748/wjg.v22.i8.2434

- We referenced this article as reference number 27 on page 16, line 1.

Beeharry MK, Liu WT, Yan M, Zhu ZG. New blood markers detection technology: A leap in the diagnosis of gastric cancer. World J Gastroenterol. 2016 Jan 21;22(3):1202-12. doi: 10.3748/wjg.v22.i3.1202.

- We referenced this article as reference number 24 on page 15, line 12.

Inoue M, Otsuka K, Shibata H. Circulating tumor cell count as a biomarker of a specific gastric cancer subgroup characterized by bone metastasis and/or disseminated intravascular coagulation - an early indicator of chemotherapeutic response. Oncol Lett. 2016 Feb;11(2):1294-1298. Epub 2015 Dec 24. PMID: 26893733 Free PMC Article

- We referenced this article as reference number 7 on page 5, line 6.
We also modified the sentence as 'gastric[7], and esophageal cancer[8,9]' on page 5, line 6-7.

Wang HY, Wei J, Zou ZY, Qian XP, Liu BR. Circulating tumour cells predict survival in gastric cancer patients: a meta-analysis. Contemp Oncol (Pozn). 2015;19(6):451-7. doi: 10.5114/wo.2015.56651. Epub 2016 Jan 13. PMID: 26843841 Free PMC Article

- We referenced this article as reference number 24 on page 15, line 12.

Kolostova K, Matkowski R, Gürlich R, Grabowski K, Soter K, Lischke R, Schützner J, Bobek V. Detection and cultivation of circulating tumor cells in gastric cancer. Cytotechnology. 2016 Aug;68(4):1095-102. doi: 10.1007/s10616-015-9866-9. Epub 2015 Apr 11. PMID: 25862542

- We referenced this article as reference number 31 on page 16, line 15.

Okabe H, Tsunoda S, Hosogi H, Hisamori S, Tanaka E, Tanaka S, Sakai Y. Circulating Tumor Cells as an Independent Predictor of Survival in Advanced Gastric Cancer. Ann Surg Oncol. 2015 Nov;22(12):3954-61. doi: 10.1245/s10434-015-4483-6. Epub 2015 Mar 17. PMID: 257

- We referenced this article as reference number 15 on page 7, line 14.

2)The authors stated "Since Katsuhiro Murakami changed places with Shun I in this study, we deleted Shun I and added Katsuhiro M as a co-author". WHAT DOES IT MEAN???? A person who did the work was removed/deleted????? Please explain

- During this study, Katsuhiro Murakami was replaced by Shun I as a member of the research team. Katsuhiro Murakami has become a co-author of the paper. Because Shun Ito was the early member of the research and his contribution was very limited in the research, he declined to be a co-author.

We believe that our prospective translational research will be of great interest to oncologists, molecular biologists and gastroenterologists. As a journal covering international issues relevant to gastroenterological specialists, and one of the leading journals worldwide for original research with a focus on gastroenterology, *World Journal of Gastroenterology* represents the perfect platform for us to share these results with the international research community.

We confirm that this manuscript has not been published elsewhere and is not under consideration by another journal. All authors have approved the manuscript and agree with submission to *World Journal of Gastroenterology*. The authors have no conflicts of interest to declare.

Please address all correspondence to:

Hiroaki Ito, MD
Department of Surgery,
Digestive Disease Center,
Showa University Koto Toyosu Hospital,
5-1-38 Toyosu
Koto-ku
Tokyo 135-8577, Japan
Tel: +81- 3-6204-6000; Fax: +81- 3-6204-6396; E-mail: h.ito@med.showa-u.ac.jp

We look forward to hearing from you at your earliest convenience.

Yours sincerely,

Hiroaki Ito, MD