ANSWERING REVIEWERS

September 9, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 13065-review.doc).

Title: An improved method increases sensitivity for circulating hepatocellular carcinoma cells

Author: Hui-Ying Liu, Hai-Hua Qian, Xiao-Feng Zhang, Jun Li, Xia Yang, Bin Sun, Jun-Yong Ma, Lei Chen, Zheng-Feng Yin

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 13065

The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

(1)Reviewer No. 111771:

Comments To Authors：The paper is well written, clear and concise. The topic is interesting. The methods are sound. Conclusions are consistent with the results. References are generally adequate: I would add some more comprehensive overview on the biological meaning and detection methods of circulating tumor cells such as "Circulating tumor cells: the 'leukemic phase' of solid cancers. Trends Mol Med 2006 Mar;12(3):130-9".
Classification：Grade A (Excellent)
Language evaluation：Grade A: priority publishing
Conclusion：Accept

Response：
Thanks for your comments. The spread of circulating tumor cells (CTCs) in the blood plays a major role in the initiation of metastases and tumor recurrence after surgery. Basically, the questions raised by the reviewer have been overviewed in our manuscript.

(2)Reviewer No. 503125:

Comments To Authors: In this manuscript a double antibody screening technique was described to better detect circulating tumor cells in patients with hepatocellular carcinoma. In particular use of a single antibody often fails to detect certain circulating tumor cells since tumor cells do not uniformly express certain common tumor antigens. The results demonstrate an improved sensitivity for identifying circulating tumor cells upon use of an antibody cocktail for both asialoglycoprotein and carbamoyl phosphate synthetase 1

Classification：Grade B (Very good)

Language evaluation：Grade B: minor language polishing

Conclusion：High priority for publication

Response：
Thank you very much for your comments.

(3)Reviewer No. 503857:

Comments To Authors: In this manuscript, the authors described a new method of detecting circulating HCC tumor cells by using CD45 depletion of leucocytes+ antibody cocktail selection with ASGPR and CPS1. The idea of using this strategy is novel. However, I have a minor suggestion to this article. 1. In Table 2, six HCC patients were in stage I (T1N0M0, solitary tumor without vascular invasion). However, CTCs were detected in 3 out of 6 HCC patients in stage I. Please address more about this point. In my opinion, early detection of CTCs using this new strategy is promising.

Classification：Grade B (Very good)

Language evaluation：Grade A: priority publishing

Conclusion：Minor revision

Response：
Thank you for your kind suggestion. We examined peripheral blood samples from 32 patients with HCC at various stages with this system. The HCC patients were classified according to the widely accepted sixth edition of UICC TNM staging system. Results showed that CTCs could be detected in most HCC patients (>90%), even in patients at early stage or in patients with tumor size of less than 5 cm. Importantly, both the positivity rate and the number of detected CTCs were positively correlated with the disease extent as classified by the TNM classification. With tumor progression, the number of detected CTCs had also been increased. We have created a better method to detect CTCs in patients with hepatocellular carcinoma even it can't detect all.

(4)Reviewer No. 51398:

Comments To Authors: refer to comments to editor

Classification：Grade C (Good)

Language evaluation：Grade C: a great deal of language polishing

Conclusion：Major revision

Response：
Thank you for your meticulous review. We have corrected several grammatical/spelling errors. And revision has been made according to the suggestions.

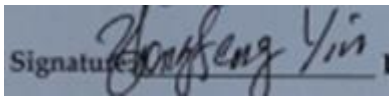
3 References and typesetting were corrected

4 We have sent my paper to a professional English language editing company-AmEditor and will get a

language certificate.

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,



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