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Liquid biopsy in patients with pancreatic cancer: Circulating tumor cells and cell-free nucleic acids

Taisuke Imamura, Shuhei Komatsu, Daisuke Ichikawa, Tsutomu Kawaguchi, Mahito Miyamae, Wataru Okajima, Takuma Ohashi, Tomohiro Arita, Hirotaka Konishi, Atsushi Shiozaki, Ryo Moriumura, Hisashi Ikoma, Kazuma Okamoto, and Eigo Otsuji

Name of Journal: World Journal of Gastroenterology

ESPS Manuscript NO: 26019

Dear Ya-Juan Ma
Science Editor Editorial office "**World Journal of Gastroenterology**"

Thank you for your kind letter concerning invited manuscript entitled "**Liquid biopsy in patients with pancreatic cancer: Circulating tumor cells and cell-free nucleic acids (ESPS Manuscript NO: 26019)**" by Imamura, Komatsu et al. We have revised the manuscript according to editor's and reviewer's comments using a red color font (highlighted revise version) and presented the outlining responses to your comments below.

We thank you for the valuable suggestions and comments for the manuscript. We have carefully revised it accordingly. Explanations have been provided point by point. We believe that our revised manuscript has been improved by these revisions, and satisfy your concerns. We cordially appreciate your work regarding our manuscript. We hope that the revised manuscript is now acceptable for publication in the "**World Journal of Gastroenterology**".

Sincerely yours,

Shuhei Komatsu, M.D. (corresponding author)
Division of Digestive Surgery, Department of Surgery,
Kyoto Prefectural University of Medicine,
465 Kajii-cho, Kawaramachihirokoji, Kamigyo-ku, Kyoto 602-8566, Japan
Phone: +81-75-251-5527
Fax: +81-75-251-5522 E-mail: skomatsu@koto.kpu-m.ac.jp
Reviewer code: 00057877

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Telephone: +1-925-223-8242 Fax: +1-925-223-8243
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Date reviewed: 2016-04-11 02:33
Classification: Grade C (Good)
Language evaluation: Grade B: Minor language polishing
Conclusion: High priority publishing
Comments to Authors:
The review is complete. it focuses on an interesting and challenging perspective in diagnostic work up of cancer, and especially of pancreatic cancer. However, the possible role of "liquid biopsy" in this particular type of tumor should be better clarified: Infact, pancreatic cancer is often diagnosed in advanced stages when ascites is present where free tumor cells can be easily found with conventional citology, which is a less expensive method with the same effectiveness (cfr. Sibio S et al. World J Gastrointest Surg. 2015 Sep 27;7(9):178-84). The authors should discuss this aspect and cite this work.

Response to reviewer's comments :
Thank you for your comment. Pancreatic cancer is often diagnosed in advanced stages with ascites and it is easy to obtain tumor cells from malignant ascites by conventional manner. However, the diagnosis by the ascites is too late to improve the prognostic outcomes of pancreatic cancer patients because there is no effective treatment for advanced pancreatic cancer. Thus, we consider that liquid biopsy is useful especially for the patients with PCa in early stage, such as PCa without malignant ascites or patients after surgery to improve their management.
As the study (Sibio S et al. World J Gastrointest Surg. 2015 Sep 27;7(9):178-84), which reviewer proposed, intra-operative peritoneal lavage fluid is an useful material to predict post-operative outcomes by examining tumor cells or cell-free nucleic acids and we found some previous reports about this. Therefore, we revised the **Current issues and future perspective** and added a description about the usefuless of intra-operative peritoneal lavage fluid as follows.
We cordially appreciate the above comments. If you have further queries, we are willing to reply them. Thank you so much for your helpful comments.

Revised
Discussion, paragraph 1, L11

Meanwhile, recent technical advances allow us to detect a slight amount of circulating cell and nucleic acids even in the body fluid other than blood, and several recent studies have already reported the possible utility of CTC and cfNAs in body fluid other than blood [118-122]. These reports have suggested the possibility of even less invasive and even more effective biomarkers in near future.

Reference
118 Zavesky L, Jandakova E, Turyna R, Langmeierova L, Weinberger V, Zaveska Drabkova L, Hulkova M,

Horinek A, Duskova D, Feyereisl J, Minar L, Kohoutova M. Evaluation of Cell-Free Urine microRNAs Expression for the Use in Diagnosis of Ovarian and Endometrial Cancers. A Pilot Study. *Pathology oncology research : POR* 2015; **21**(4): 1027-1035 [PMID: 25827090 DOI: 10.1007/s12253-015-9914-y]

119 Erbes T, Hirschfeld M, Rucker G, Jaeger M, Boas J, Iborra S, Mayer S, Gitsch G, Stickeler E. Feasibility of urinary microRNA detection in breast cancer patients and its potential as an innovative non-invasive biomarker. *BMC cancer* 2015; **15**: 193 [PMID: 25886191 PMCID: PMC4383066 DOI: 10.1186/s12885-015-1190-4]

120 Duz MB, Karatas OF, Guzel E, Turgut NF, Yilmaz M, Creighton CJ, Ozen M. Identification of miR-139-5p as a saliva biomarker for tongue squamous cell carcinoma: a pilot study. *Cellular oncology (Dordrecht)* 2016; **39**(2): 187-193 [PMID: 26650483 DOI: 10.1007/s13402-015-0259-z]

121 Tokuhisa M, Ichikawa Y, Kosaka N, Ochiya T, Yashiro M, Hirakawa K, Kosaka T, Makino H, Akiyama H, Kunisaki C, Endo I. Exosomal miRNAs from Peritoneum Lavage Fluid as Potential Prognostic Biomarkers of Peritoneal Metastasis in Gastric Cancer. *PloS one* 2015; **10**(7): e0130472 [PMID: 26208314 PMCID: PMC4514651 DOI: 10.1371/journal.pone.0130472]

122 Sibio S, Fiorani C, Stolfi C, Divizia A, Pezzuto R, Montagnese F, Bagaglini G, Sammartino P, Sica GS. Detection methods and clinical significance of free peritoneal tumor cells found during colorectal cancer surgery. *World journal of gastrointestinal surgery* 2015; **7**(9): 178-184 [PMID: 26425265 PMCID: PMC4582234 DOI: 10.4240/wjgs.v7.i9.178]

Reviewer code: 00043819
Date reviewed: 2016-04-14 17:29
Classification: Grade B (Very good)
Language evaluation: Grade B: Minor language polishing
Conclusion: Minor revision
Comments to Authors:
This is a well-written review on circulating tumor cells and cell-free nucleic acids from liquid biopsy in patients with pancreatic cancer. I think that this technique may have useful application in the diagnostic work-up of pancreatic cystic neoplasms, particularly IPMNs; the Authors should discuss this point.
Response to reviewer's comments:
Thank you for your valuable comment. As we mentioned in **Current issues and future perspective** section, mucinous cystic neoplasms, intraductal papillary neoplasms, pancreatic intraepithelial neoplasia, and intraductal tubular papillary neoplasms were identified as premalignant lesions of PCa [1] that develop to invasive PCa through stepwise progression with the accumulation of several genetic aberrations. If these important genetic aberrations could be captured by liquid biopsy, screening and monitoring tests for high-risk lesions or early detection could be realized. Recently, we demonstrated that plasma miR-223 could predict malignant potential of IPMN [2]. we revised the **Current issues and future perspective** and added a description about the perspectives in pancreatic cystic neoplasms, particularly IPMNs as follows.

We cordially appreciate the above comments. If you have further queries, we are willing to reply them. Thank you so much for your helpful comments.

Revised

Current issues and future perspective, paragraph 2, L11

Furthermore, mucinous cystic neoplasms, intraductal papillary neoplasms, pancreatic intraepithelial neoplasia (IPMN), and intraductal tubular papillary neoplasms were identified as premalignant lesions of PCa [124] that develop to invasive PCa through stepwise progression with the accumulation of several genetic aberrations. If these important genetic aberrations could be captured by liquid biopsy, screening and monitoring tests for high-risk lesions or early detection could be realized. To date, there are few reports about the usefulness of liquid biopsy in these premalignant lesions of PCa [125]. Recently our study successfully demonstrated that plasma miR-223 could predict malignant potential of IPMN[126]. We believe that further studies of liquid biopsy in premalignant lesions of PCa could contribute to improve the prognostic outcomes of PCa patients and the biomarker for premalignant lesion is nearing the clinical application.

Reference

125 Permuth-Wey J, Chen DT, Fulp WJ, Yoder SJ, Zhang Y, Georgeades C, Husain K, Centeno BA, Magliocco AM, Coppola D, Malafa M. Plasma MicroRNAs as Novel Biomarkers for Patients with Intraductal Papillary Mucinous Neoplasms of the Pancreas. *Cancer prevention research (Philadelphia, Pa)* 2015; **8**(9): 826-834 [PMID: 26314797 PMCID: PMC4560649 DOI: 10.1158/1940-6207.capr-15-0094]

Reviewer code: 03473803
Date reviewed: 2016-04-30 11:15

Classification: Grade C (Good)
Language evaluation: Grade B: Minor language poloshing
Conclusion: Minor revision
Comments to Authors:
This is a review article on circulation tumor cells and nucleic acid markers specially in pancreatic cancer. The authors performed an extensive review on the current literature - Introduction: could you please provide more references (e.g., on line 5 and 7) - how could these markers be used in practice? There is no specific population to target the screening and in advance stages It is too late to use.
Response to reviewer's comments :

Thank you for your comment. In introduction section, we added some references as follows. As reviewer indicated, how could these markers be used in practice is most important point. As mentioned in **Current issues and future perspective,** several problems remain to be solved before application in a clinical setting. We consider that these markers are useful especially for the patients with high risk, such as the patietns with precursor lesions of PCa at this time. Therefore we revised the **Current issues and future perspective** and added a description about the biomarker for the patients with premalignant lesions of PCa as target of the screening as follows.
We cordially appreciate the above comments. If you have further queries, we are willing to reply them. Thank you so much for your helpful comments.

Revised

Introduction, paragraph 1, L3-6

In recent years, as a result of advances in surgical techniques and perioperative management, the perioperative mortality rate has decreased and perioperative chemotherapy and radiotherapy have greatly improved; however, prognostic outcomes for PCa remain poor[2][3]. Even now, the median survival time of patients with PCa is 5–8 months and their 5-year survival rate is less than 10%[2][3].

Reference

2 Stathis A, Moore MJ. Advanced pancreatic carcinoma: current treatment and future challenges. *Nature reviews Clinical oncology* 2010; **7**(3): 163-172 [PMID: 20101258 DOI: 10.1038/nrclinonc.2009.236]
3 Wolfgang CL, Herman JM, Laheru DA, Klein AP, Erdek MA, Fishman EK, Hruban RH. Recent progress in pancreatic cancer. *CA: a cancer journal for clinicians* 2013; **63**(5): 318-348 [PMID: 23856911 PMCID: PMC3769458 DOI: 10.3322/caac.21190]

Current issues and future perspective, paragraph 2, L11

Furthermore, mucinous cystic neoplasms, intraductal papillary neoplasms, pancreatic intraepithelial neoplasia (IPMN), and intraductal tubular papillary neoplasms were identified as premalignant lesions of PCa [124] that develop to invasive PCa through stepwise progression with the accumulation of several genetic aberrations. If these important genetic aberrations could be captured by liquid biopsy, screening and monitoring tests for high-risk lesions or early detection could be realized. To date, there are few reports about the usefulness of liquid biopsy in these premalignant lesions of PCa [125]. Recently our study successfully demonstrated that plasma miR-223 could predict malignant potential of IPMN[126]. We believe that further studies of liquid biopsy in premalignant lesions of PCa could contribute to improve the prognostic outcomes of PCa

patients and the biomarker for premalignant lesion is nearing the clinical application.

Reference

125 Permuth-Wey J, Chen DT, Fulp WJ, Yoder SJ, Zhang Y, Georgeades C, Husain K, Centeno BA, Magliocco AM, Coppola D, Malafa M. Plasma MicroRNAs as Novel Biomarkers for Patients with Intraductal Papillary Mucinous Neoplasms of the Pancreas. *Cancer prevention research (Philadelphia, Pa)* 2015; **8**(9): 826-834 [PMID: 26314797 PMCID: PMC4560649 DOI: 10.1158/1940-6207.capr-15-0094]

We cordially appreciate the above comments. If you have further queries, we are willing to reply to them.