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Dr Jing Yu
Science Editor
World Journal of Gastroenterology

Please find enclosed the revised manuscript (file name: 26758-Revised_MS.docx)

Title: Circulating tumor DNA as a liquid biopsy target for detection of pancreatic cancer

Authors: Erina Takai, Shinichi Yachida

Name of Journal: *World Journal of Gastroenterology*

ESPS manuscript NO: 26758

We thank the reviewers for their insightful comments and have incorporated them into our manuscript as recommended. Our detailed comments are listed below.

REVIEWER 1 (03478343)

Comment 1

I have attached a word doc with all the minor typos and comments of where to add references. Since you are writing a review for a wide audience it is important to give as much literary support as possible whenever making a claim

Thank you so much for checking the manuscript carefully. According to the reviewer's comment, we have corrected the typos and grammatical mistakes, and also added the references (ref 10-12, 14, 15, 17, 19, 20, 22, 41, 44, 45).

Comment 2

Was Figure 1 drawn from scratch or based on someone else's artwork?

The illustration in Figure 1 is our original.

REVIEWER 2 (03478298)

Comment 1

The content that ctDNA as a liquid biopsy target for detection of pancreatic cancer needed further discussing.

Based on the reviewer's comment, we have added the following sentences:

"As mentioned above, currently available tumor biomarkers, such as CA19-9, insufficient to detect PDAC due to low sensitivity and low specificity. Somatic mutations, on the other hand, are highly specific to DNA derived from cancer or precancerous cells. Especially, *KRAS* is the most frequently mutated gene in PDAC and the mutations occur at the very early stage of carcinogenesis. As technology advances, ctDNA discriminated by *KRAS* mutation may have great potential as a blood-based biomarker for PDAC." (Page 10)

Comment 2

This review not expressing their original insights.

According to the reviewer's comment, we have added the following sentences:

"At present, as NGS assays are still costly and the sensitivities of standard sequencing technologies are limited, targeted deep sequencing of cfDNA may not be practical in clinical settings for all patients. Since *KRAS* mutation is a good cancer biomarker in pancreatic cancer patients, our two-step approach combining dPCR and NGS could be cost-effective and applicable in the clinic. It may be possible to apply such ctDNA assays to broader range of patients by using a larger volume of plasma because the sensitivities of these assays should depend on the amount of input cfDNA. In addition, the use of novel techniques, including molecular barcoding, and error reduction methods by bioinformatics approaches could improve the sensitivities of sequencing analysis [41,42]." (Page 12)

REVIEWER 3 (03017300)

Comment 1

In my opinion, a section and a synoptic table regarding new agents targeting circulating tumor DNA as a liquid biopsy target for detection of pancreatic cancer is suggested.

As suggested, we have added the following sentences regarding new agents and products targeting ctDNA as a liquid biopsy target for detection of pancreatic cancer:

"Recently, there are an increasing number of new products for cfDNA processing including blood collection tubes (e.g., Cell-Free DNA BCT® (Streck) and Cell-Free DNA Collection Tube (Roche)) and cfDNA extraction kits (e.g., Quick-cfDNA™ Serum & Plasma Kit (Zymo Research), Maxwell® RSC ccfDNA Plasma Kit (Promega), and MagMAX™ Cell-Free DNA Isolation Kit (Thermo Fisher Scientific)). For sequencing of cfDNA, new library preparation kits optimized for small amounts of fragmented DNA, such as Accel-NGS® DNA Library Kits (Swift Biosciences) and ThruPLEX® Plasma-seq Kit (Rubicon Genomics), have also been available. It is worth evaluating the new products to establish standardized methods of ctDNA analysis."

Comment 2

It is better to introduce the detailed value of the sensitivity and specificity for detection of PDAC for *KRAS* detection with or without the combined detection of the serum CA19-9 level, especially for the prognostic significances.

According to the reviewer's suggestion, we have added the following sentences:

"Maire *et al.* reported that the sensitivity and specificity of serum *KRAS* mutations for the diagnosis of pancreatic cancer were 47 and 87%, respectively, whereas the combination of serum *KRAS* mutations and CA19-9 had a sensitivity and specificity of 98 and 77%, respectively [32]. Analysis by Dąbritz *et al*. also suggested that detectable *KRAS* mutations in the plasma were associated with progressive disease (75%), whereas the association was more evident when combining plasma *KRAS* mutations and elevated CA19-9 (92%) [33]." (Page 9)

"Multivariate analysis also showed that *KRAS* mutations in plasma DNA were stronger prognostic factor for survival (hazard ratio 7.39, $P<0.001$) than elevated CA19-9 (hazard ratio 2.49, $P=0.087$) [34]." (Page 9-10)

Yours sincerely,

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