

February 23, 2017

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 31615-Answering Reviewers.docx).

Title: Abnormal DNA methylation as a cell-free circulating DNA biomarker for colorectal cancer detection: a review of literature

Author: Michail Galanopoulos*, Nikolaos Tsoukalas*, Ioannis S Papanikolaou, Maria Tolia, Maria Gazouli, Gerassimos J Mantzaris

Name of Journal: *World Journal of Gastrointestinal Oncology*

ESPS Manuscript NO: 31615

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) Answering comments of reviewer with code: 00070758

Comment: In abstract section, the author mentioned “In this brief review, we epitomize the current knowledge on the research in circDNA biomarker – primarily focusing on DNA methylation – as a potential blood-based test for early detection of colorectal cancer.”. However, the author also emphasis on the other content such as molecular pathways, cell-free circulating DNA-based markers. So, the abstract needs to revise

Change: In this brief review, we epitomize the current knowledge on the research in circDNA biomarkers – mainly focusing on DNA methylation – as potential blood-based tests for early detection of colorectal cancer.

Comment: The title suggests that the content is furthermore focused in the application of circulating cell-free DNA in colorectal cancer early detection, but these expectations are not properly met. Thus “early detection” is not proper.

Change: New title: Abnormal DNA methylation as a Cell-free circulating DNA biomarker for colorectal cancer detection: a review of literature

Running title: Methylation-related circDNA biomarker for CRC detection

Comment: In “EXISTING SCREENING MODALITIES TO AVOID CRC” section, “Screening modalities” is little related to the theme of this article, so it needs to short the content.

Change: The specific section has been shortened

Comment: In “Methods to detect circDNA-related markers in blood”, the author only introduces the methylation-related circDNA detect methods. So, the subtitle needs changed.

Change: Methods to detect methylation-related circDNAMarkers in blood

(2) Answering comments of reviewer with code: 00070758

Comment: In the introduction the authors write that currently available screening tests for early detection have proven ineffective due to costs and a rather low participation rate. I am not agreeing with this statement completely. No doubt, the currently available tools/methods could be better, likewise the participation rate, but stating that there are ineffective is not correct. Screening (FOBT and colonoscopy if positive) were introduced nationwide early 2014 in Denmark, and data from the national databases already clearly shows a decline in percentage of patients that are diagnosed with stage IV disease. So currently available strategies do work – but yes there is clearly room for improvements.

Change: The appropriate change has been applied by the sentence: Nevertheless, the currently available screening tests for the early detection of CRC need improvement enough in order to increase their cost-effective status, **instead of** : Nevertheless, the currently available screening tests for the early detection of CRC have been proven ineffective due to high cost and low rate of willingness to participate

Comment: Introduction, three lines down from the above mentioned statement: New biomarkers, with higher sensitivity and specificity – how are they to reduce the expected incidence of CRC? Better markers may help identifying patients with CRC at an earlier stage, but they hardly reduce the overall incidence – unless they become so specific that the implementation leads to identification and removal of a larger number of polyps – that potentially could lead to cancer over time.

Change: The appropriate change has been applied by the sentence : Thus, it is conceivable that, there is a significant interest in using noninvasive blood biomarkers

which could be of low cost and high sensitivity and specificity to help reduce the predicted surge in the incidence of CRC by identification and removal of a larger number of polyps that potentially could lead to CRC over time, **instead of** : Thus, it is conceivable that, there is a significant interest in using noninvasive blood biomarkers which could be of low cost and high sensitivity and specificity to help reduce the predicted surge in the incidence of CRC with no risk for patients^[3]

(3) Answering comments of reviewer with code: 00070758

Comment: The paper is interesting and targets a large number of specialists in the field. Minor language polishing is required. More information about other circulating cell free DNA markers is needed. Otherwise I would recommend the title to be changed.

Change: New title has been applied: Abnormal DNA methylation as a Cell-free circulating DNA biomarker for colorectal cancer detection: a review of literature

3. References and typesetting were corrected

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

Sincerely yours,

Nikolaos Tsoukalas, MD, MSc, PhD,

Medical Oncologist, MSc in Bioinformatics,

Department of Medical Oncology, Veterans Hospital (417 NIMTS),

Gennimata N.10-12 Ampelokipi 11524 Athens, Greece.

Email: tsoukn@yahoo.gr, **telephone:** +30-697-7366056, **Fax:** +30-210-7237578