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Athens, 1/8/2016

**To:**

Editor, World Journal of Gastrointestinal Oncology

Re: Revised manuscript entitled " **The role of circulating free DNA in colorectal cancer** " ESPS Manuscript NO: **27814**

Dear Editor,

We would like to thank you for considering our manuscript for possible publication in your journal. The manuscript has been revised on a point-by-point basis addressing all the comments raised by the reviewers. Additions have been marked with the yellow highlight tool in the revised manuscript. Our responses to the reviewers' comments and the corresponding changes are presented below.

**Reviewer #1**

*Comment 1. Abstract is not informative and serves no purpose, it contains no data, no conclusions. Needs rewriting.*

**Response:** In accordance with the reviewer's comment, the abstract was partially rewritten with several phrases deleted and added. It now follows a more logical course, first laying the current framework, then summarizing data on cfDNA and finally concluding that cfDNA may soon be a part of everyday practice.

*Comment 2: Rationale for prognostic and predictive biomarkers is not given. Authors try to impress with percentages, but all mentioned incidences are irrelevant. What is important is that we don't know which patients harbour occult metastases (those are ones that will 'relaps'). So it is important that we know which patients already have*

*occult metastases at the time of initial diagnosis, and those patients need to receive maximal treatment (e.g. adjuvant therapy). The practice today is that we determine risks of relaps based on Dukes' classification (i.e. positivity of lymph nodes and some other hystological findings such as vascular invasion etc.), but that is not sufficient. See comments 6 and 8.*

**Response:** The reviewer raises a valid point. Consequently, we now clearly verbalize this suggestion (page 4 first paragraph, page 7 first paragraph)

*Comment 3. Liquid biopsy is not explained. How is it different from normal biopsy ? How it "...addresses these pressing requirements" ?*

**Response:** Several additions were made (page 4, second paragraph) and now the term liquid biopsy and its advantages are briefly presented. Also, the term “pressing requirements” was rewritten.

*Comment 4. What is "Asymptomatic screening" ? Can we talk about screening in patients with symptoms ? Screening is always asymptomatic, if patients have symptoms, we talk about diagnostic workup.*

**Response:** The reviewer is correct to note that “asymptomatic screening” is a redundancy and the phrasing was corrected.

*Comment 5. "Colonoscopy is regarded as the preferred technique, since it clearly improves disease specific survival, it doesn't require additional therapeutic interventions..." Colonoscopy is gold standard, and how is it advantageous that it "doesn't require additional therapeutic interventions" ? What does it mean ? I would delete that.*

**Response:** This phrase was meant to underline the fact that while most other screening modalities need a confirmatory endoscopic biopsy, colonoscopy obviously doesn't. To avoid confusion, we have omitted the phrase (which should have stated “additional diagnostic interventions”).

*Comment 6. "In a high risk population with positive fecal occult blood test that subsequently underwent colonoscopy, Perrone et al demonstrated that the quantification of cfDNA by qPCR was predictive for CRC but not premalignant lesions" this sentence is pivotal for your work, I would give it more focus.*

**Response:** Following the reviewer's suggestion, we now underscore the importance of the findings of this study (page 6, second paragraph).

*Comment 7. "otherwise clinically insignificant malignancies" ? What would that be ? Malignant = capable of metastasizing. How can it be insignificant ? Maybe you meant neoplasia ?*

**Response:** To avoid confusion, we have rephrased to "overdiagnosis" (page 6 second paragraph)

*Comment 8. "Following curative surgery for localized CRC, approximately 50% of stage III patients according to the American Joint Committee on Cancer (node-positive disease) and 20% of stage II patients (T3N0 and T4N0) are expected to experience disease relapse without adjuvant chemotherapy." This is another pivotal sentence I would focus on.*

**Response:** Same as comment 2, the importance of this fact is now clearly verbalized in page 4 first paragraph, page 7 first paragraph, following the reviewer's suggestion.

*Comment 9 "Also, several ethical issues will need to be considered, such as the management of healthy subjects with detectable cfDNA at presymptomatic screening and the possibility of barriers regarding the access to certain agents despite their regulatory approval (as an example of a possible scenario, the use of anti-EGFR treatment is not reimbursed for a patient with a RAS WT tumor and KRAS mutant cfDNA)." Why would there be such an ethical issue ? You have cfDNA on screening, you undergo further diagnostic workup or enhanced monitoring (consider e.g. prophylactic mastectomy for MCIS or Brca+). As for reimbursements, cfDNA testing might actually turn the tables and force insurances to reimburse as it might eventually be cheaper than treatment of advanced disease. I would not go into that line of thinking.*

**Response:** Per the reviewer's request, the last phrase of the discussion has been omitted from the revised manuscript.

**Reviewer #2**

*It is a very interesting review of the work concerning the clinical value of circulating free DNA in colorectal cancer. The m/s is suitable for publication.*

**Response:** We thank the reviewer for his comments

We are grateful to the reviewers for their critical contribution. We believe that our manuscript has been significantly improved and we hope that you will find it suitable for publication in the World Journal of Gastrointestinal Oncology.

Sincerely yours,

Alexios Matikas