

Response to Reviewer Comments

We thank the Senior Editor and the Reviewer (ID: 03003422) for their thoughtful and insightful comments. We have modified our manuscript to address their concerns as below.

1. In “INTRODUCTION”, we added one conclusion sentence:

In this review, we have updated a review published in 2014^[6]. We examine molecular (genetic, epigenetic, protein) biomarkers associated with CRC and discuss their role in cancer screening, early detecting of disease recurrence and as prognostic and predictive factors. (Page 6, paragraph 1)

2. According the reviewer’s suggestion, we added the title “*Blood and stool genetic and epigenetic markers*” in Page 6 and provided related content:

These investigations have concentrated on the detection of mutated KRAS, TP53, APC and markers for microsatellite instability (MSI)^[7,8,9]. A faecal DNA test targeted at molecular biomarkers has been commercially available for twelve years, with reported sensitivity for cancer ranging from 25% up to 92% for the latest tests based on BEAMing technology, and 94-98% specificity^[10,11,12]. Apart from genetic alterations, the DNA promoter hypermethylation silencing the tumour suppressor genes has been widely investigated. Epigenetic changes, depending on the markers or their combinations evaluated, have been detected in CRC patients with 70%-96% sensitivity and 72%-96% specificity^[9,13,14]. Many combinations of genetic and epigenetic markers have been studied, but until now, the results have not endorsed their use in clinical practice. Using blood instead of stool as a screening material could offer some obvious advantages. Several studies have evaluated potential plasma DNA genetic and epigenetic biomarkers in CRC detection. The overall sensitivity ranges from 30% to 87%, with specificity of up to 96%. The use of RNA biomarkers in stool has not been investigated as

extensively as was the case for DNA biomarkers, mainly because stool environment is responsible for mRNA degradation, although improving laboratory retrieval methods seems to solve this problem. Koga et al analysed mRNA expression of MMP7, PTGS2, TP53, MYBL2 in colonocytes isolated from stool by quantitative real-time RT-PCR, to find out that these markers can identify CRC patients with 58% sensitivity and 88% specificity. Sensitivity was found to depend on tumour size and tumour location, but not cancer stage^[15]. Most recently, the so called transcriptomic studies have investigated the expression of microRNAs – short, non-coding 18-22 nucleotide RNA molecules in stools of CRC patients. The most extensively studied miR21, miR106a, miR135, miR17-92 were found to be overexpressed in CRC patients compared with healthy individuals^[16,17]. As was the case with RNA markers in stool, many studies have been evaluating mRNA of different tumour genes in whole blood, plasma or circulating tumour cells to identify new CRC screening markers. Most of them investigated mRNA molecules of CK19, CK20, or CEA. The overall sensitivity of these markers was up to 72%, specifically when combinations of these markers were used^[18,19]. The specificity was very high with healthy control samples or much lower when compared to other cancer or inflammatory bowel diseases samples^[20]. Recent studies have indicated that circulating microRNAs may be involved in the process of oncogenesis. The use of miRNA as a biomarker is now being evaluated. A large number of miRNA molecules have been assessed, with a focus on miR145, miR143, miR135, miR17-92. More specifically, Huang et al. has found that plasma miR29a and miR92a demonstrated a significant diagnostic value for advanced neoplasia with 83% and 84% sensitivity and specificity, respectively, in discriminating CRC patients^[21]. These studies need to be validated in randomised trials to define their value in CRC screening.

3. According to the reviewer's suggestion, we cited "**Grady WM, Pritchard CC. Molecular alterations and biomarkers in colorectal cancer. *Toxicol Pathol* 2014; 42: 124-139 [PMID: 24178577 DOI: 10.1177/0192623313505155]**" as reference **87**, and has been described in the manuscript.

4. The Table was provided and modified the format.

5. All formats advised by editor have been updated.

Thanks again!

Sincerely yours,

A handwritten signature in dark ink, appearing to read 'Gustaw Lech', with a stylized, flowing script.

Gustaw Lech MD, PhD

Department of General, Gastroenterological and Oncological Surgery

Medical University of Warsaw

Banacha 1a, 02091 Warsaw

Poland

gustaw.lech@wum.edu.pl