**MINISTRY OF HEALTH, RUSSIA**

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Prof. Hiten RH Patel
Editor-in-Chief
World J Clin Oncol
Prof. Lian-Sheng Ma
Science Editor, Company Editor-in-Chief, Editorial Office
Baishideng Publishing Group Inc

Dear Prof. Patel,
Dear Prof. Ma,

We appreciate greatly your positive consideration of our manuscript #67430 entitled "Tumor irradiation may facilitate the detection of tumor-specific mutations in plasma" by Kuligina et al.

We have managed to address the issues raised during the evaluation process:

**Reviewer #1:**

*Comment 1:* Please add the detailed captions of each figure.

*Response:* We have added the captions:

***Figure 1.*** *The flowchart describing the recruitment of the patients for the study. Consecutive patients with locally advanced rectal cancer were subjected to the KRAS/NRAS/BRAF mutation testing. Seventeen tumors did not contain mutations in the mentioned genes, 2 tumors carried rare substitutions unsuitable for the available ddPCR assays, and 2 patients failed to receive neoadjuvant treatment. The remaining 9 patient underwent preoperative RT and provided serial blood sample for the study.*

***Figure 2****. Schedule for serial blood-takes and irradiation fractions. Serial blood samples were obtained at 11 different time points. Irradiation was delivered every day with 24 hours intervals.*

***Figure 3.*** *Changes in ctDNA content occurring within first 96 hours after the start of radiotherapy. Patients #ArAS and #GaZM had no detectable ctDNA at baseline, but showed the emergence of mutated gene copies in the bloodstream upon RT. At least some serial plasma samples had increased concentration of ctDNA as compared to the base-line in patients #DaKS, #ArTP and #MaNK. RT did not result in the elevation of ctDNA level in patient #MaLI.*

*Comment 2:* In the discussion part, please add the detailed advantages and reasons for locally advanced RAS/RAF-mutated rectal cancer as the research object.

*Response:* We have added the comment:

*Patients with locally advanced rectal cancer provide a good opportunity for the analysis of RT-induced changes in the ctDNA level, as these malignancies frequently contain RAS/RAF mutations and the tumor irradiation is a part of routine clinical management of this disease*[21].

*Comment 3:* In the discussion part, the authors mentioned that "If this intervention was to increase the rate of EGFR T790M allele detection in the plasma, the proposed approach would have significant potential for clinical use". I hope that the author will pay attention to the side effects of local radiotherapy and put forward some strategies.

*Response:* We have incorporated the comments regarding the risk of this intervention:

*It is essential to minimize the risks of this procedure by considering the anatomic location of targeted tumor foci (particularly, the vicinity of large blood vessels), ensuring a highly precise topical delivery of the irradiation dose and accounting for potentially significant comorbidities. If this intervention, was to increase the rate of EGFR T790M allele detection in the plasma while being sufficiently safe, the proposed approach would have significant potential for clinical use.*

*Comment 4:* Please add the limitations of this article in the discussion section.

*Response:* We have added to the *Discussion* the comments regarding the limitations of the study:

*There are several limitations of this investigation. Human studies involving multiple serial blood takes are logistically complicated and need to be well balanced with ethical issues, therefore it is understandable that our study and similar reports[14-16] are of limited size. Furthermore, the range of "natural" variations of ctDNA measurements occurring due to imperfect reproducibility of laboratory protocols or physiological fluctuations of ctDNA content is largely unknown. Therefore, although our study demonstrated a trend towards the RT-induced increase of ctDNA concentration in some rectal cancer patients, it is not clear how these observations need to be adjusted for the described above confounding factors. This limitation is also applicable to other published data sets[14-16].*

**Reviewer #2:**

*Comment Major 1:* It is widely known fact that radiation and chemotherapy can increase amount of ctDNA in blood and tumor specific DNA detection. While authors focused on rectal cancer which was not selected for similar study design, scientific novelty of this study was unclear. Authors should emphasize this point.

*Response:* We have provided additional clarification of this issue in the first paragraph of the *Discussion*:

*Patients with locally advanced rectal cancer provide a good opportunity for the analysis of RT-induced changes in the ctDNA level, as these malignancies frequently contain RAS/RAF mutations and the tumor irradiation is a part of routine clinical management of this disease*[21]. *The data obtained within this study are consistent with prior*

*investigations, which were performed on lung cancer patients and demonstrated that radiotherapeutic or chemoradiotherapeutic intervention may result in a transient increase of the level of ctDNA in some cases[14-16]. As compared to published reports[14-16], our study considered multiple evenly distributed time points within the first day after tumor irradiation. We anticipated, that this additional effort may help us to identify a time interval characterized by maximal RT-induced ctDNA release. However, there was a significant interpatient variability with regard to the timing of ctDNA concentration peaks (Table 2, Figure 3).*

*Comment Minor 1:* Please clearly describe information of concurrent chemotherapy. It might affect somehow amount of ctDNA.

*Response:* We have inserted appropriate in formation in the Figure 3, Table 1 and Table 2.

We hope that the manuscript is now acceptable for publication and look forward to the decision of the Editorial Board.

With best regards,

Evgeny Imyanitov