Peer Review File

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Review Comments

This study evaluated the value of γ-H2AX in the peripheral blood as a biomarker for treatment response in locally advanced rectal cancer patients undergoing preoperative concurrent. This is very interesting issue and peripheral γ-H2AX would be expected to be useful if the value of peripheral γ-H2AX could be proven. However, there is several limitations in study to be published. Please, consider the following issues and provide appropriate supplementation.

Comment 1: This study evaluated the performance of peripheral γ-H2AX to predict the response only based on the MRI finding after the neoadjuvant CCRT for locally advanced rectal cancer. Although the mrTRG shows significant association with pathologic TRG (pTRG) in many studies, the ultimate endpoint needs to be the performance of peripheral γ-H2AX to predict the pTRG which is the more ultimate outcome than mrTRG. So, please, provide the outcome regarding the pTRG.

Reply 1: We agree that the pathologic Tumor Regression Grade (pTRG) is the ultimate outcome for evaluating treatment response. In our study, There were only 13 patients had pTRG grading performed by a pathologist. Due to the limited number of patients with pTRG data, a detailed statistical analysis of the correlation between peripheral γ-H2AX and pTRG may be underpowered.

Changes in the text: We added the **Table 3** showed pathological outcomes of patients underwent oncologic surgery (see Page 24, line 492)

Comment 2. Similarly, the correlation between peripheral γ-H2AX and pathologic outcomes such as downstaging and resection margin status comparing with mrCRM, etc is also important outcome. Please, provide the results of diverse analyses regarding the association between peripheral γ-H2AX and various pathologic data.

Reply 2: Thanks to your valuable suggestion, we have included the results of the association between peripheral γ-H2AX and pathologic outcomes, including both pCR and non-pCR patients. Our findings reveal a distinction in γ-H2AX levels between pCR and non-pCR patients, aligning with our results based on mrTRG. Your insightful recommendation has greatly enhanced our analysis and underscored the importance of understanding the relationship between γ-H2AX activation and pathologic outcomes.

Changes in the text: We have added **Table 3, Table 4**, and **Figure 4** (see Page 24, line 492), a paragraph discussing pathological outcomes (see Page 12, line 236), and expanded on these findings in the abstract (see Page 3, line 58) and discussion section (see Page 15, line 318).

Comment 3. Is there any association between peripheral γ-H2AX and survival outcomes such as disease-free survival, distant metastasis-free survival, intrapelvic recurrence-free survival, or overall survival? What is the median follow-up duration? Please, provide this information.

Reply 3: Our study protocol involved assessing patients using MRI after completing CCRT, the protocol did not carried patients to proceed the oncologic surgery. The surgery depended on surgeon and patient decision. Nevertheless, we continued to follow up with patients, resulting in a median follow-up duration of 3.06 years from diagnosis. We have conducted Cox proportional hazards analyses to explore the association between peripheral γ-H2AX and survival outcomes, including overall survival and distant metastasis-free survival. However, no significant correlation or trend was observed between γ-H2AX levels and these survival outcomes.
Changes in the text: We have added the survival outcomes (see Page 13, line 255), with detailed data provided in Supplementary Appendix Table A1 and Table A2 (see Page 28, line 530), along with an expanded discussion on these findings (see Page 16, line 338).

Comment 4. Peripheral γ-H2AX may reflect the DNA double-strand damage of not only the cancer cells but also normal cells such as peripheral blood cells. Is there any association between peripheral γ-H2AX and hematological toxicities such as lymphopenia, anemia, neutropenia, or thrombocytopenia? Please, show the results regarding these issues.
Reply 4: We have collected data on white blood cell (WBC) count and lymphocyte count. Following your suggestion, we performed Pearson correlation analysis to investigate the relationship between γ-H2AX levels and these cell counts across five different time points. However, our analysis did not reveal any significant correlation between γ-H2AX levels and either WBC count or lymphocyte count.
Changes in the text: We have added Table A3 and Table A4 to the Supplementary Appendix section (see Page 28, line 535).

Comment 5. Although the authors mentioned some of the results about acute toxicity in the Discussion section, the results regarding toxicity should be written in Results section, not in Discussion section. Despite the low rates of the toxicities, the rates according to the toxicity profiles and relevant representative activated γ-H2AX ratio look necessary to be shown in Results section.
Reply 5: We have updated the Results section to include specific data on acute toxicity experienced during CCRT treatment, along with the rates according to the toxicity profiles. We appreciate the suggestion to include these details in the Results section, as it provides a clearer presentation of the findings.
Changes in the text: We have added the text including the toxicity in result section (see Page 13, line 260).

Comment 6. Please, provide the p-values for each number of PBMC collection in addition to adjusted difference between the groups using proper statistics such as post-hoc analysis or t-test. These may provide the information regarding the optimal timing for evaluation of peripheral γ-H2AX.
Reply 6: According to your suggestion, we have added the p-values for each PBMC collection, calculated using a t-test in **Table 2** and additional **Table 4**.

Changes in the text: In **Table 2**, we have added the p-values for each time point using a t-test to identify differences (see Page 23, line 490). Additionally, we have included a new **Table 4** based on your suggestion (see Page 24, line 495).

Comment 7. Please, provide the information about the expertized experience period of the radiologist who evaluated the mrTRG and mrCRT

Reply 7: We have included details about the qualifications and experience of our gastrointestinal (GI) radiologist in the MRI assessment in the Methods section.

Changes in the text: We have updated the text to provide a detailed description of the radiologist's specialization in this study (see Page 10, line 189).