Peer Review File

Article information: https://dx.doi.org/10.21037/tcr-24-812

Reviewer A

Comment 1:

Title:

The title should be clearer regarding the role of Urinal 8-hydroxy-2'-deoxyguanosine level assessment during the treatment with radium-223. This marker is not frequently used and not well-known. It would be more inviting to read if the author wrote its finality.

- **Reply 1: Thank you for your valuable comment. The title was changed to "Predicting bone marrow suppression from urinal 8-hydroxy-2'-deoxyguanosine level during the treatment with radium-223 in patients with cancer bone metastasis" in the revised version (see P1L3-4).**

Comment 2:

Objective:

The objective of the study is not clear.

- **Reply 2: Following the reviewer comment, the section of abstract and introduction were corrected in the revised version (see P3L47-51).**

Comment 3:

Analysis of bone metastasis:

Bone scintigraphy is not considered the best method to follow-up bone lesions. Despite using BSI on the analysis that improves this evaluation, PSMA PET would be the gold standard.

- **Reply 3: Thank you for the comment. PSMA-PET is effective for analyzing bone metastasis caused by prostate cancer. However, this exam is not a common in Japan, where this analysis was conducted. Because it is too expensive for patients, and the supply of this chemical is also supplying unstable from the related company. Therefore,**

it is not a popular test in Japan today. In this study, we used the widely used BSI evaluation method, which has been confirmed to be safe and effective, to understand the status of bone metastases. However, we understand that PSMA-PET is a valid testing method. In the future, we plan to focus on the evaluation of bone metastases as PSMA-PET becomes more widespread. Please understand this point.

Comment 4:

Results:

The presentation of the results is confusing.

- **Reply 4: Thank you for the comment. The result section were rechecked and corrected in the revised version (see P9L201 – P10L314).**

Comment 5:

Discussion:

"We focused on RIT with Ra-223 and investigated whether blood sampling data and changes in the urinary oxidative stress marker 8-OHdG of patients with CRPC with bone metastases could predict its treatment efficacy and side effects."

Which side effects the object of the study could predict? Bone marrow suppression? Bone events such as fractures?

- **Reply 5: Thank you for the comment. It was suggested that this object of the study could predict bone marrow suppression and the associated weakened immune system. This point was added in the section of discussion (see P10L324-325).**

Comment 6:

Could the presence of bone-modifying drugs in some patients be a bias?

- **Reply 6: Thank you for the comment. In 4 cases of our study, they were administrated the bone-modifying drugs (Table 1). In general, the treatment guideline for bone metastasis in each country, such as United States and Japan, recommends administering this drug to reduce SRE (Saylor PJ et al., *JCO Oncol Pract.* 16: 389-393, 2020, Ishioka C and Baba (editors). 2022). In the comparison analysis, the authors did not consider it to be biased as in previous reports. This point was added in the revised version (see**

P10L332-P11L342, Reference 13 and 14).

Comments 7:

It does not seem that an increase in urinary 8-OHdG concentration, that indicates an increase in oxidative damage to DNA due to radiation stress, is necessarily related to the body's reserve ability to resist oxidative stress. Is it possible to distinguish between the DNA damage of the bone metastasis from healthy cells?

- **Reply 7: Thank you for the valuable comment. Although it has been previously reported that 8-OHdG is released when tumors and surrounding risk organs are exposed to high-dose radiation during cancer treatment, no such analysis has been reported in patients with bone metastases treated with radium-223. Urinary 8-OHdG can originate from both normal and tumor cells. The data of this study were derived from indirect detection of urinary 8-OHdG concentrations released from tumor or normal tissues locally exposed to alpha particles and represent a comprehensive biomarker. Currently, it is not possible to directly assess the concentration of 8-OHdG from tumor cells or normal cells oxidatively damaged by radium-223. As the next step in this research, we are currently accumulating detailed data from animal models. Thank you for your understanding about it. The limitations of this study have been added in the Discussion section (see P11L364-P12L378).**

Comment 8:

"A previous study that investigated the relationship between serum 8-OHdG levels and the prognosis and incidence of side effects during chemo/radiotherapy revealed that the lower the 8-OHdG level before treatment or the higher the 8-OHdG level after treatment, the better the prognosis and the fewer side effects caused by chemo/radiotherapy" Did the patients undergo a baseline sampling of the urinary 8-OHdG? Which side effects? If the objective was to evaluate the 8-OHdG as a predictor of bone marrow suppression, would it not be interesting and desirable to analyze the correlation between the 8-OHdG urinary level with complete blood cell?

- **Reply 8: Thank you for the important comments. The sample baseline of 8-OHdG was set to before the start of RIT (day0), and it was shown as 13.1 ng/mgCRE in Pt.1, 38.6 ng/mgCRE in Pt.2, 45.4 ng/mgCRE in Pt.3, and 25.2 ng/mgCRE in Pt.4, respectively. In**

addition, the correlation of white blood cell (WBC), red blood cell (RBC) and platelet (PLT) was compared with 8-OHdG concentlation (as new Fig. 5 and Table 3). These points were added in the revised version (see P9L209-P10L310).

Comment 9:

Conclusion:

"In conclusion, our results reveal that urinal 8-OHdG is a convenient biomarker that complements hotspot volume and existing markers of bone metastasis."

Why is 8-OHdG convenient? The casuistry is too small to determine the utility of the marker. It must raise more questions than answers and propose larger studies.

- **Reply 9: Thank you for the valuable comment. The conclusion was changed to "In conclusion, our results suggest that urinal 8-OHdG concentration has a potency of biomarker for bone marrow suppression under the administration of radium-223 in the patient with bone metastasis.**

Reviewer B

1. You mentioned "A previous study" but 2 references are cited here.

thus it may include the body's reserve ability to resist oxidative stress (18). A previous study that investigated the relationship between serum 8-OHdG levels and the prognosis and incidence of side effects during chemo/radiotherapy revealed that the lower the 8-OHdG level before treatment or the higher the 8-OHdG level after treatment, the better the prognosis and the fewer side effects caused by chemo/radiotherapy (19-20). However, because the urinary baseline in this study was very similar

   - Thank you for the point out. The sentence was corrected in the revised manuscript [P10L323-324].

2. Figures and tables

- There is no ▼, △ and ◇ in Figure 1 but their explanations in the legends. Please check and revise.
  - ➢ Thank you for the point out. The legend of Figure 1 was corrected in the revised manuscript [P15L452-P16L464].

- **All abbreviations in figures/tables and legends should be explained.** BMA in Figure 1 for example. Please check all abbreviations and provide the full names in the corresponding legends/footnote. E.g., Figure 1. xxx. Abbreviations: xxx, xxxx.
  - ➢ Thank you for the point out. All abbreviations in Figures were checked and added the full names to legends [P16L464, figure 1, P16L467, P16L469-471, P16L473, P16L476, P16L481-482].

- Please unify the expressions of NTx and NTX in the main text and the figures.

ed that BAP and NTX demonstrated no ad ditionally, serum NTx was at the same lev

  - ➢ Thank you for the point. It was unified to NTX in the revised manuscript and figures [L126, 259, 310, 312, 315].

- The following legends in the main text do not match Figure 5A. Please check if Figure 6 is the one which should be cited. Please also double check the content related to the figures to ensure the accuracy of this article.

exposed to higher dose rate of ionizing radiation *in vitro* model. In the expression of gamma-H2AX, exposure of 1 to 8 Gy showed a significant up-regulation of 1.24 ± 0.15 to 9.33 ± 1.83 folds compared to 0 Gy (Figure 5A). In addition, 8-OHdG into cell culture supernatant secreted from the cell exposed to 1 to 8 Gy also showed a significant up-regulation of 1.10 ± 0.11 to 13.00 ± 0.56 folds compared to 0 Gy (Figure 5A). A significant positive correlation was shown between gamma-H2AX and 8-OHdG (R^2=0.86).

➢ Thank you for the point out. These were typos and corrected to Figure 6A in the revised manuscript [P9L281-284].

- **All abbreviations in the Tables should be defined.** TNM, RT, RIT, CBZ, G-CSF, M in Table 1 for example. Please check all abbreviations and provide the full names in the corresponding table foot.

➢ Thank you for the point out. All abbreviations in Tables were checked and added the full names to footnotes [Table 1 to 2].

- Table 3: Please indicate how data are presented. “mean ± SD”.

R	−0.57	−0.75
Slope	−0.17±0.013	−0.11±0.047
R	−0.92	−0.66
Slope	−0.0077±0.0017	−0.018±0.010

➢ Thank you for the point out. The descrition of presence “mean ± SD” was added to footnote [Table 3].

- Table 3: Please provide a header of the second column.

	Pt. 1
R	−0.57
Slope	−0.17±0.0
R	−0.92
Slope	−0.0077±0.0

➢ Thank you for the point out. The descrition of header “Correlation parameters” was added to revised Table3.

3. Please check if it should be Table 1.

at the Mutsu General Hospital (Aomori, Japan) from October 2016 to December 2020. Table summarizes each patient’s characteristics. The Committee of Medical Ethics of the Hirosaki University Graduate School of Health Sciences, Hirosaki, Japan (no. 2016–051) approved this patient study to ensure donors’

- Thank you for the point out. This point was corrected to “Table 1” [P6L110].