

# Gamma-H2AX as a biomarker to assess individual radiosensitivity for radiopharmaceutical therapy in cancer patients: a systematic review

T. Kisananto<sup>1,2</sup>, D. Ramadhani<sup>2</sup>, E.H Purwaningsih<sup>3</sup>, R.W Hakim<sup>3</sup>, I.K.H Basri<sup>2</sup>, T.S Humani<sup>2</sup>, H.N.E Surniyantoro<sup>2</sup>, M. Syaifudin<sup>2</sup>, S. Purnami<sup>4</sup> and D.A Suryandari<sup>5,\*</sup>

<sup>1</sup> Doctoral Program in Biomedical Sciences, Faculty of Medicine, Universitas Indonesia, Jakarta 10430, Indonesia.

<sup>2</sup> Research Center for Radioisotope, Radiopharmaceutical, and Biodosimetry Technology, Research Organization for Nuclear Energy National Research and Innovation Agency, Tangerang, Indonesia.

<sup>3</sup> Department of Medical Pharmacy, Faculty of Medicine, Universitas Indonesia, Jakarta 10430, Indonesia.

<sup>4</sup> Research Center for Safety, Metrology, and Nuclear Quality Technology, Research Organization for Nuclear Energy National Research and Innovation Agency, Tangerang, Indonesia.

<sup>5</sup> Department of Medical Biology, Faculty of Medicine, Universitas Indonesia, Jakarta 10430, Indonesia.

Received: 19 July 2024 / Accepted: 31 January 2025

**Abstract** – Radiopharmaceutical therapy offers targeted and potent cancer treatment, but individual responses vary, necessitating personalized approaches for optimal outcomes. Gamma-H2AX, a phosphorylated form of histone, has become a sensitive indicator of DNA double-strand breaks caused by ionizing radiation. Gamma-H2AX assays in radiopharmaceutical therapy offer a non-invasive way to assess individual radiosensitivity, improving treatment optimization and patient classification. This systematic review examines the use of gamma-H2AX as a biomarker for determining individual radiosensitivity in cancer patients receiving radiopharmaceutical therapy. An in-depth search for supporting publications utilized three popular electronic databases: PubMed Central (PMC), ScienceDirect, and Scopus. Specific keywords determine article limits. Every existing article was examined and appraised independently using the PICO method (participants, intervention/exposure, comparison, and outcome). Eighty-one articles were identified, 49 were screened based on title and abstract, and 32 were excluded. Six articles were worthy, but only two were considered in this research. Based on these two articles, it is possible to conclude that the gamma-H2AX foci marker in peripheral blood cells of patients receiving radiopharmaceutical therapy was promising for predicting treatment response and individual radiosensitivity. However, this research must be validated with more patients for a higher prospective value in future investigations.

**Keywords:** DNA damage response / gamma-H2AX / radiosensitivity / cancer / radiopharmaceutical therapy

## 1 Introduction

Radiopharmaceutical therapy technology uses radioactive substances to treat a variety of medical conditions, particularly cancer. The radioactive substance is given orally or intravenously, allowing it to specifically target cancer cells or diseased tissue while minimizing damage to surrounding healthy tissue (Derlin *et al.*, 2021; Sgouros *et al.*, 2020). Radiation emitted from radioactive substances can kill cancer cells or inhibit their growth. These compounds are designed to be absorbed and targeted at specific types of cancer cells (Cai *et al.*, 2008; Vinnikov and Belyakov, 2022).

Radiopharmaceuticals commonly used in therapy include iodine-131 for thyroid cancer, strontium-89 and samarium-153 for bone metastases, and lutetium-177 and yttrium-90 for prostate cancer and neuroendocrine tumors (Dewaraja *et al.*, 2022; Vinnikov and Belyakov, 2022). Nuclear medicine specialists often deliver radiopharmaceutical therapy, which requires close monitoring to ensure safety and efficacy. It can be used as the primary treatment or in combination with other therapies such as surgery, chemotherapy, or external beam radiation therapy (Gong and Miller, 2019; Raavi *et al.*, 2021).

Individual radiosensitivity refers to the variation in how different individuals respond to radiation exposure. This sensitivity can influence radiopharmaceutical therapy's effectiveness and potential side effects (Chen *et al.*, 2024;

\*Corresponding author: [anitabio@yahoo.co.uk](mailto:anitabio@yahoo.co.uk)

**Table 1.** Literature article search approach.

Database	Keywords	Results
PMC	((individual radiosensitivity) AND gamma-H2AX) AND radiopharmaceuticals) AND cancer patients	43
ScienceDirect	individual radiosensitivity, gamma-H2AX, radiopharmaceuticals, cancer patients	11
Scopus	individual AND radiosensitivity AND gamma-h2ax AND radiopharmaceuticals AND cancer AND patients AND PUBYEAR > 2008 AND PUBYEAR < 2025 AND ( LIMIT-TO ( DOCTYPE, “ar”) OR LIMIT-TO ( DOCTYPE, “re”))	37

**Table 2.** Rules for screening articles.

Inclusion criteria	Exclusion criteria
<ul style="list-style-type: none"> <li>– Participants were cancer patients undergoing radiopharmaceutical therapy</li> <li>– DDR was assessed by the increase in H2AX foci</li> <li>– Gamma-H2AX predicts the individual radiosensitivity in cancer patients.</li> <li>– Articles were published during or before April 2024</li> <li>– Articles were written in English</li> </ul>	<ul style="list-style-type: none"> <li>– Gamma-H2AX did not identify individual radiosensitivity in cancer patients.</li> <li>– The entire article could not be obtained in PDF format.</li> </ul>

Purnami *et al.*, 2023; Widjaja *et al.*, 2022). Some individuals may have a higher sensitivity to radiation, meaning they may experience more severe side effects from radiopharmaceutical therapy compared to others. Factors contributing to individual radiosensitivity include genetic predisposition, age, overall health, and previous radiation exposure (Vinnikov and Belyakov, 2022).

Radiopharmaceutical therapy aims to deliver a therapeutic radiation dose to target cancer cells while minimizing damage to healthy tissues. However, individuals with higher radiosensitivity may experience increased side effects such as radiation dermatitis, nausea, fatigue, and bone marrow suppression (Fu *et al.*, 2023; Khazaei Monfared *et al.*, 2023). Healthcare providers take individual radiosensitivity into account when planning and administering radiopharmaceutical therapy. They may adjust the dosage or treatment schedule based on the patient’s sensitivity and closely monitor for adverse reactions (Dewaraja *et al.*, 2022).

Gamma-H2AX is a marker that detects DNA damage produced by ionizing radiation, such as that utilized in radiopharmaceutical therapy (Djuzenova *et al.*, 2015, 2013). When cells are exposed to radiation, double-strand breaks develop in the DNA, causing the histone variant H2AX phosphorylation. These phosphorylated H2AX molecules generate foci around DNA damage sites, which can be visualized and quantified with immunofluorescence microscopy (Derlin *et al.*, 2021; Djuzenova *et al.*, 2013; Srivastava *et al.*, 2009)

The level of gamma-H2AX foci formation can measure the extent of DNA damage induced by radiation exposure. It is often used in research to study individual radiosensitivity, as differences in the response of cells to radiation can be observed among individuals. Some people may have a higher baseline level of gamma-H2AX foci formation, indicating increased DNA damage sensitivity (Kawashima *et al.*, 2020).

Understanding individual radiosensitivity through markers like gamma-H2AX can be valuable for personalized treatment planning in radiopharmaceutical therapy. By assessing a patient’s baseline level of DNA damage sensitivity, healthcare providers can tailor the dosage and treatment regimen to minimize adverse effects while maximizing therapeutic efficacy (Djuzenova *et al.*, 2015; Srivastava *et al.*, 2009).

Additionally, research into individual radiosensitivity and gamma-H2AX may help identify biomarkers that predict patient response to radiopharmaceutical therapy, allowing for more precise patient selection and treatment optimization. Gamma-H2AX and individual radiosensitivity are essential in developing and administering radiopharmaceutical therapy, contributing to personalized treatment strategies and improved patient outcomes (Cai *et al.*, 2008; Kawashima *et al.*, 2020).

A systematic review on gamma-H2AX, individual radiosensitivity, and radiopharmaceutical therapy would aim to evaluate the current body of research on these topics comprehensively. By systematically synthesizing existing evidence, such a review can contribute to understanding the role of gamma-H2AX and individual radiosensitivity in guiding and optimizing radiopharmaceutical therapy for cancer patients.

## 2 Literature review

### 2.1 Literature search strategy

A comprehensive data search was undertaken using three electronic databases: PubMed Central (PMC), ScienceDirect, and Scopus. The literature analysis included phrases such as “individual radiosensitivity,” “gamma-H2AX,” “radiopharmaceuticals,” and “cancer patients,” as shown in Table 1. Suitable publications were discovered by reviewing the references and included if they matched the inclusion criteria (Tab. 2). The

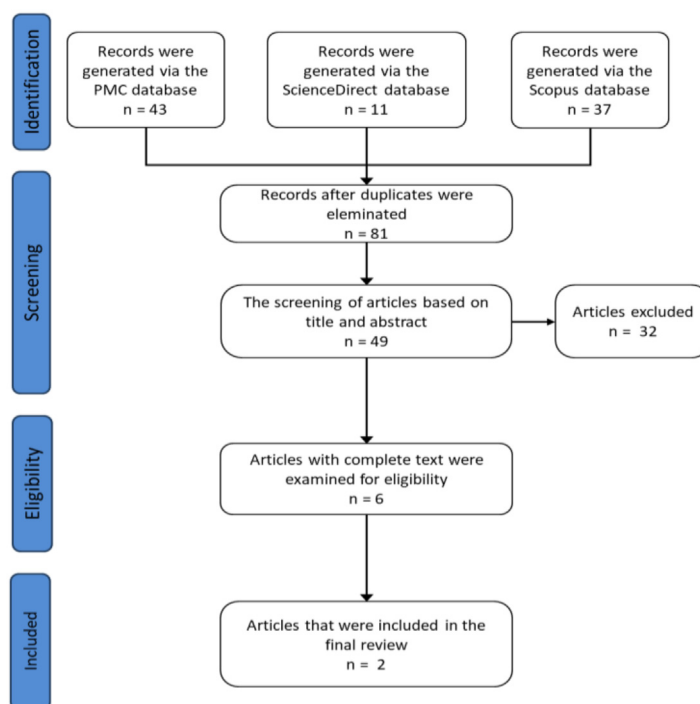


Fig. 1. PRISMA visualization for the current systematic review.

publications were screened using the PICO method (participants/patient, intervention, comparison, and outcome). PICO is a structured approach to formulating a clinical or research question. Experiments were conducted after taking into consideration the DNA damage response (DDR) evidenced by the rise in gamma-H2AX foci (C) in cancer patients (P) receiving radiopharmaceutical treatment (I) to measure individual radiosensitivity (O).

## 2.2 Eligibility and data analysis

This review implemented the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) disclosure items flowchart, as shown in Figure 1. According to searches utilizing specified keywords, 43, 11, and 27 articles were found in each database (PMC, ScienceDirect, and Scopus). The Mendeley reference manager was then used to search for duplicate articles; when the duplicates were deleted, 81 articles existed. The title and abstract were then used to pick 49 appropriate articles, while 32 were rejected. Articles containing full text were examined for eligibility, resulting in six articles that could be continued in the final assessment. According to the inclusion criteria (Tab. 2), only two articles were worthy of processing and are described in Table 3.

## 2.3 AI tools usage statement

The author uses Assistive AI tools (Grammarly) to correct grammatical errors in this manuscript. In addition, Generative AI tools (QuillBot Premium) are employed to assist in paraphrasing and plagiarism detection.

## 3 Result and discussion

The PRISMA approach identified two suitable articles that matched the inclusion requirements. The research articles were Widjaja *et al.* (2022), and Derlin *et al.* (2021). These articles will be thoroughly explained utilizing the PICO method, outlined in Table 3.

Widjaja *et al.* (2022) investigated 20 men, aged  $72.1 \pm 8.6$ , with metastatic castration-resistant prostate cancer (mCRPC) treated with  $^{177}\text{Lu}$ -prostate-specific membrane antigen ( $^{177}\text{Lu}$ -PSMA) radiopharmaceutical therapy. A DDR investigation was carried out on the patient's peripheral blood lymphocytes using immunocytofluorescence analysis. Blood samples were collected before, an hour after, and 24 hours after  $^{177}\text{Lu}$ -PSMA therapy. Before the first cycle of  $^{177}\text{Lu}$ -PSMA therapy, all patients had positron emission tomography (PET/CT scans) for measuring tumor PSMA expression (assessing the maximum standardized uptake value (SUV<sub>max</sub>) of all tumor lesions). Following the second cycle, DDR markers (gamma-H2AX and 53BP1) were tested for prediction performance against clinical and PET-based indicators for prostate-specific antigen-progressing disease (PSA-PD). The predictive value for progression-free survival (PSA-PFS, expressed as median and 95% confidence interval [CI]) was also investigated. The amount of DDR markers determined individual radiosensitivity in lymphocyte cells before and after the injection of  $^{177}\text{Lu}$ -PSMA therapy (Widjaja *et al.*, 2022).

Furthermore, Derlin *et al.* (2021) studied 21 patients with advanced gastroenteropancreatic neuroendocrine tumors (ten males and eleven females), with an average age of  $64.3 \pm 11.6$  and a range of 41.0–84.9 years. The patients underwent PRRT with 7.4 GBq  $^{177}\text{Lu}$ -DOTA-TATE per cycle every 8–16

**Table 3.** Description of studies using the PICO method

Articles	Patients	Intervention	Comparison	Outcome
Widjaja <i>et al.</i> (2022), Germany	Twenty men with mCRPC undergoing 177Lu-PSMA therapy	A median dose of 7.32 GBq (interquartile range 7.28–7.37) of 177Lu-PSMA was given every cycle. Treatment followed established procedural parameters, with cycles repeated every 6–8 weeks (median 42 days). A routine laboratory panel was evaluated before and throughout each cycle, including blood cell counts, liver enzymes, and PSA levels.	Blood samples for DDR-marker analysis were taken before, an hour after, and 24 hours after the 177Lu-PSMA injection. DDR markers associated with intact lymphocyte cells were added and divided by the total number of cells to determine the number of foci per cell. PSA levels were measured before and 6–8 weeks after two cycles of therapy, and the percentage change from baseline was analyzed.	Low baseline DDR marker (gamma-H2AX foci) levels before therapy may represent low individual radiosensitivity and be associated with earlier PSA-PD and shorter PSA-PFS.
Derlin <i>et al.</i> (2021), Germany	Twenty-one patients with advanced gastroenteropancreatic neuroendocrine tumors (ten males and eleven females)	Eleven patients started peptide receptor radionuclide therapy (PRRT) with 7.4 GBq lutetium-177(177Lu) labeled 1,4,7,10 tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid-d-Phe(1)-Tyr(3) octreotate (177Lu-DOTA-TATE), and 10 patients had previously received at least one PRRT cycle before (6±1). All patients suffered from grade 1 (G1) or grade 2 (G2) metastatic nonfunctioning gastroenteropancreatic neuroendocrine tumors. In addition, general laboratory examinations were performed, including hematology, liver enzymes, and serum CgA levels.	The reliability of the analysis was confirmed in lymphoblastoid cells irradiated with a dose of 2 Gy from healthy donors and ataxia-telangiectasia syndrome (A–T) patients, the latter of whom showed a radiosensitivity phenotype. Blood samples of patients undergoing PRRT with 177Lu-DOTA-TATE were irradiated with a dose of 2 Gy as positive controls	The study found significant heterogeneity in gamma-H2AX foci induction and kinetics in PRRT patients' PBLs, suggesting potential for stratification and treatment response prediction. However, the data was preliminary and called for larger prospective studies.

weeks. Gamma-H2AX and 53BP1 foci growth in peripheral blood lymphocytes were assessed at baseline, one hour, and 24 h after PRRT 177Lu-DOTA-TATE delivery. 68Ga-DOTA-TATE PET/CT was used to evaluate therapy response before enrollment and after two cycles of PRRT. Individual radiosensitivity was assessed by the correlation between subclinical hematotoxicity and the onset of H2AX and 53 BP1 foci (Derlin *et al.*, 2021).

### 3.1 DNA damage and formation of gamma-H2AX foci

“DNA damage” refers to many lesions or damage to the DNA molecule. External sources, including radiation exposure, toxic chemicals, viral infections, and internal ones like DNA replication mistakes or undesired metabolic reactions, may damage DNA. DNA damage may result in genetic alterations, mutations, or cell death (Liu *et al.*, 2022).

One of the reactions to DNA damage is the development of gamma-H2AX foci. Gamma-H2AX is a post-translational alteration of the histone H2AX after DNA damage (Willers *et al.*, 2015). When DNA damage occurs, such as double-strand breaks or other DNA lesions, the protein kinase ATM

(ataxia-telangiectasia mutated) is activated. This activation of ATM is the initial step in the signalling pathway responding to DNA damage. When active, ATM phosphorylates serine residues on the C-terminal tail of histone H2AX, producing gamma-H2AX. This creates scars that specifically mark sites of DNA damage in the genome. It is part of the cellular reaction to DNA damage, to repair the damage or, if the damage is too severe, directing the cell to apoptosis, also known as “programmed cell death” (Song *et al.*, 2023; Srivastava *et al.*, 2009). DNA DSB signalling mechanism involves histone H2AX phosphorylation.

The formation of gamma-H2AX foci is critical for DNA damage detection and repair, as well as genomic stability. It is also employed in scientific studies as a marker to detect and quantify the extent of DNA damage in cells.

### 3.2 Gamma-H2AX as a biomarker of ionizing radiation exposure

The gamma-H2AX assay has gained popularity as a marker of radiation exposure due to its speed, sensitivity, ability to detect

a wide range of doses, and ability to be performed in various tissues such as lymphocytes, splenocytes, buccal cells, bone marrow cells, skin, and plucked hairs (Raavi *et al.*, 2021).

As a result, gamma-H2AX assay may serve as a sensitive and specific biomarker of ionizing radiation exposure. This approach can identify low levels of radiation exposure that other measuring methods cannot. Furthermore, because gamma-H2AX foci can remain for hours after radiation exposure, this approach can detect radiation exposure even after DNA damage has occurred (Chen *et al.*, 2024; Ferlazzo *et al.*, 2017).

The use of gamma-H2AX as a biomarker of ionizing radiation exposure has been extensively researched, both for medical applications (such as monitoring radiation dose in patients undergoing radiation therapy) and for assessing radiation dose in workers exposed to radiation in their workplace (Djuzenova *et al.*, 2015; Liu *et al.*, 2022; Sgouros *et al.*, 2020). This is a significant example of how DNA damage and cellular response mechanisms have been used in the creation of helpful technologies in health and safety (Khazaei Monfared *et al.*, 2023).

### 3.3 Gamma-H2AX as an indicator of DNA damage in external radiation therapy

External radiation therapy causes DNA damage in bodily cells, such as blood cells and hematopoietic cells in the bone marrow (Richardson, 2011). The gamma-H2AX biomarker can directly indicate the extent of DNA damage directly related to the patient's hematotoxicity. Higher radiation doses will cause more DNA damage and gamma-H2AX foci, increasing the risk of hematotoxicity. This is related to bone marrow damage caused by radiation exposure (Derlin *et al.*, 2021). As a result, it is critical to monitor the hematological status of patients receiving radiation therapy constantly.

A comparison of gamma-H2AX foci levels before and after radiation therapy reveals the quantity of DNA damage and may also indicate the severity of hematotoxicity in patients. The gamma-H2AX assay could be an effective technique for personalizing radiation therapy and identifying patients at high risk of hematotoxicity (Lassmann *et al.*, 2010). Further research relating this biomarker to hematological results could aid in developing safer and more effective therapeutic techniques and lower the risk of radiation-induced side effects.

In nuclear medicine,  $\gamma$ -H2AX is used to evaluate DNA damage at the cellular level during external radiation therapy and isotope radiation therapy evaluations. One of its key applications is determining an individual's radiation sensitivity. Clinical investigations have indicated that this biomarker can be used to assess DNA damage in radiation therapy patients and help decide the best radiation dose for each individual.

Several clinical trials have investigated using  $\gamma$ -H2AX in external beam radiation therapy. Sak *et al.* (2007) found an increase in  $\gamma$ -H2AX foci in patients' peripheral blood cells after radiation therapy, with a clear link between the number of foci and radiation dose received. These findings support using  $\gamma$ -H2AX as a sensitive, non-invasive indicator of DNA damage that can provide a direct picture of the biological

response to radiation therapy. Additionally,  $\gamma$ -H2AX can potentially monitor the effectiveness of radiation treatment in nuclear medicine.

In a study of head and neck cancer patients, Li *et al.* (2013) found that higher levels of  $\gamma$ -H2AX foci before therapy were associated with more DNA damage and better outcomes.  $\gamma$ -H2AX can assess patients' radiation sensitivity, allowing clinicians to regulate dosages more precisely, reduce side effects, and improve therapeutic success. In addition,  $\gamma$ -H2AX can be combined with other medicines, such as chemotherapy or radiation therapy and DNA repair inhibitors.

The use of  $\gamma$ -H2AX in nuclear medicine has the potential to be a helpful tool in personalizing radiation therapy. This biomarker enables real-time monitoring of DNA damage in patients and more exact therapy adjustments.  $\gamma$ -H2AX identifies individuals' radiation sensitivity, leading to more effective and focused therapy with fewer adverse effects.

### 3.4 Gamma-H2AX and radiopharmaceutical therapy

Radiopharmaceutical therapy uses beta and alpha particles to eliminate cancer cells while minimizing damage to healthy tissue surrounding the tumor. As the energy passes through the tissues, it accumulates inside the cells, causing DNA SSB and DSBs. To ensure optimal destruction of the targeted cells while minimizing ionization interactions with healthy cells, it is critical to consider multiple factors such as the particle energy, emission range, and linear energy transfer (LET), in addition to the physical or biochemical characteristics (phenotype), dimensions or physical extent (size), and location of the target cells within the tumors (Khazaei Monfared *et al.*, 2023).

Peptide receptor radionucleotide therapy (PRRT) is a generally safe treatment however modest hematologic damage is expected. Continuous PRRT treatment raises the danger of accumulating unrepaired DNA damage in normal tissues, possibly leading to genetic instability and carcinogenesis. Aside from acute myelotoxicity, four out of 504 individuals in one study developed myelodysplastic syndrome (MDS) (Denoyer *et al.*, 2015; Vinnikov and Belyakov, 2022). To reduce the toxicity of PRRT, organ dosimetry using radioisotope biodistribution and kinetics is recommended. Image-based dosimetry can not accurately predict blood problems in particular people. There is no agreement on the best radiobiologic model for short-range, low-dose-rate radiopeptides. Studying DNA damage at the single-cell level may provide light on PRRT-induced toxicity pathways and aid in predicting the biological effects of radiation dosage *in vivo* (Denoyer *et al.*, 2015).

Gamma-H2AX has been used as a "biodosimeter" to assess radiation exposure in peripheral blood lymphocytes (PBLs) after external body irradiation because of its immediate reaction and sensitivity (Raavi *et al.*, 2021; Ramadhani *et al.*, 2020; Vinnikov and Belyakov, 2022). Recent studies suggest that the gamma-H2AX assay may identify radiosensitive patients with early chronic radiation-induced normal-tissue damage and guide individualized treatment (Djuzenova *et al.*, 2013; Lobachevsky *et al.*, 2016).

Radiopharmaceutical-induced DNA damage is more complex than that caused by external ionizing radiation. This is due to the nature of radionuclide emissions, the

biodistribution of the agent within cells and organs, and the time course of radiation delivery determined by the radionuclide's physical decay and tissue clearance kinetics (Denoyer *et al.*, 2015; Khazaei Monfared *et al.*, 2023; Purnami *et al.*, 2023). The use of gamma-H2AX as a biomarker of toxicity and biodosimeter following radiopharmaceutical administration is mainly based on research on <sup>131</sup>I treatment in thyroid cancer patients and <sup>18</sup>F-FDG PET/CT imaging (Vinnikov and Belyakov, 2022). The studies identified gamma-H2AX as a biomarker for DNA damage following internal irradiation. Validating the use of gamma-H2AX for specific radiopharmaceuticals is necessary due to the correlation between the chemical form of the radiopharmaceutical and the energy of the beta particles, which affects the proportion of DNA single-strand breaks (DSBs) (Mahmoud *et al.*, 2022; Willers *et al.*, 2015).

### 3.5 Gamma-H2AX for predicting the radiosensitivity

Irradiation leads to three distinct cell death pathways: mitotic death, apoptosis, and senescence, all contributing to the global inactivation of clonogenic potential. Mitotic death causes the development of permanently damaged chromosomal fragments (micronuclei), which are expelled from the nucleus. It is the most common kind of radiation-induced death in proliferative cells. The quantity of remaining micronuclei has been associated with radiation-induced clonogenic inactivation (Ferlazzo *et al.*, 2017; Lobachevsky *et al.*, 2016).

Senescence leads to a persistent and irreversible stop in the G1 phase. The most common cause of mortality for inactive cells due to radiation-induced damage is seen, particularly for doses over 4 Gy. CDKN1A/p21 expression is a dependable indicator of senescence. CDKN1A/p21 expression was shown to be dramatically decreased in radiosensitive breast cancer patients after irradiation (Ferlazzo *et al.*, 2017; Huang and Zhou, 2020). Meanwhile, apoptosis is one of the most well-documented pathways of cell death, although it is one of the rarest. Apoptosis mainly occurs in lymphocytes and is quite rare in fibroblasts (Derlin *et al.*, 2021; Ferlazzo *et al.*, 2017; Fu *et al.*, 2023).

Unrepaired DSB appears to be the most significant endpoint for radiosensitivity among all DNA damage, given that unrepaired DSB is the primary cause of micronuclei and unrepaired chromosome breaks, and diseases associated with DSB repair defects are systematically linked to radiosensitivity (Khazaei Monfared *et al.*, 2023; Srivastava *et al.*, 2009). It was demonstrated that the Ataxia Telangiectasia Mutated (ATM-dependent phosphorylation) of the variant histone H2AX phosphorylated on serine 139 (gamma-H2AX) was among the earliest radiation-induced events by its immunofluorescence quantification of discrete nuclear foci (Srivastava *et al.*, 2009).

Nonhomologous end-joining (NHEJ), the primary DSB repair and signalling route in mammals, recognizes DSBs *via* developing gamma-H2AX foci. The gamma-H2AX assay determines each radiation-induced DSB using a one-to-one connection between radiation-induced DSB and gamma-H2AX foci. In human fibroblasts, the number of unrepaired DSBs measured by the gamma-H2AX assay typically varies from 0–12 after 2 Gy and 24 hours for repair (Huang and Zhou, 2020; Purnami *et al.*, 2023).

The generated gamma-H2AX foci may indicate the degree of DNA damage caused by radiation exposure. It is often employed in research to investigate individual radiosensitivity, since changes in cell response to radiation may be found between people (Berthel *et al.*, 2019; Lobachevsky *et al.*, 2016; Widjaja *et al.*, 2022). Some persons may have a more incredible baseline amount of gamma-H2AX foci formation, suggesting heightened DNA damage sensitivity. Radiosensitivity is linked to DSB repair defects, but not all are. The challenge lies in predicting intermediate radiation responses (Huang and Zhou, 2020).

## 4 Conclusion

This review gives further information on the efficacy of the gamma-H2AX biomarker in determining individual radiosensitivity in cancer patients receiving radiopharmaceutical treatment. Based on the two articles analyzed, it can be concluded that the gamma-H2AX foci marker in peripheral blood cells of radiopharmaceutical therapy patients is promising for predicting treatment response and individual radiosensitivity but requires validation with more patients. In addition, it is necessary to consider the optimal time for sampling. Sampling timing significantly affects biodosimetry data accuracy, especially for high and low radiation doses. High doses may allow DNA repair early, while low doses require later sampling to observe significant changes in  $\gamma$ H2AX foci. Analysis at this time provides more accurate information on cellular DNA damage and individual biological responses.

### Acknowledgments

The authors would like to thank the Grant of Ministry of Education, Culture, Research, and Technology 2024.

### Funding

This research did not receive any specific funding.

### Conflicts of interest

The authors declare that they have no conflict of interest.

### Data availability statement

The research data associated with this article are included within the article.

### Author contribution statement

**T. Kisananto:** Conceptualization, methodology, investigation, and writing the original draft. **D. Ramadhani:** Conceptualization, methodology, and reviewing. **E.H Purwaningsih:** Conceptualization, methodology, and investigation. **R.W Hakim:** Methodology and investigation. **I.K.H Basri:** Methodology and investigation. **T.S Humani:** Investigation and reviewing. **H.N.E Surniyantoro:** Investigation and reviewing. **M. Syaifudin:** Reviewing. **S. Purnami:**

Reviewing. **D.A. Suryandari**: Conceptualization, methodology, investigation, reviewing.

## References

- Berthel E, Ferlazzo ML, Devic C, Bourguignon M, Foray N. 2019. What does the history of research on the repair of DNA double-strand breaks tell us?—a comprehensive review of human radiosensitivity. *Int J Mol Sci.* 20. <https://doi.org/10.3390/ijms20215339>
- Cai Z, Chen Z, Bailey KE, Scollard DA, Reilly RM, Vallis KA. 2008. Relationship between induction of phosphorylated H2AX and survival in breast cancer cells exposed to 111In-DTPA-hEGF. *J Nucl Med* 49: 1353–1361.
- Chen Z, Wakabayashi H, Kuroda R, Mori H, Hiromasa T, Kayano D, Kinuya S. 2024. Radiation exposure lymphocyte damage assessed by  $\gamma$ -H2AX level using flow cytometry. *Sci Rep.* 14. <https://doi.org/10.1038/s41598-024-54986-x>
- Denoyer D, Lobachevsky P, Jackson P, Thompson M, Martin OA, Hicks RJ. 2015. Analysis of <sup>177</sup>Lu-DOTA-octreotate therapy-induced DNA damage in peripheral blood lymphocytes of patients with neuroendocrine tumors. *J Nucl Med* 56: 505–511.
- Derlin T, Bogdanova N, Ohlendorf F, Ramachandran D, Werner RA, Ross TL, Christiansen H, Bengel FM, Henkenberens C. 2021. Assessment of  $\gamma$ -H2AX and 53BP1 foci in peripheral blood lymphocytes to predict subclinical hematotoxicity and response in somatostatin receptor-targeted radionuclide therapy for advanced gastroenteropancreatic neuroendocrine tumors. *Cancers (Basel).* 13. <https://doi.org/10.3390/cancers13071516>
- Dewaraja Y, Frey ED, Hobbs R, Sc D, Xiao, Y. 2022. Current status of radiopharmaceutical therapy. *Int J Radiat Oncol Biol Phys* 109: 891–901.
- Djuzenova CS, Elsner I, Katzer A, Worschech E, Distel LV, Flentje M, Polat B. 2013. Radiosensitivity in breast cancer assessed by the histone  $\gamma$ -H2AX and 53BP1 foci. *Radiat Oncol* 8: 1–12.
- Djuzenova CS, Zimmermann M, Katzer A, Fiedler V, Distel LV, Gasser M, Waaga-Gasser A-M., Flentje M, Polat B. 2015. A prospective study on histone  $\gamma$ -H2AX and 53BP1 foci expression in rectal carcinoma patients: correlation with radiation therapy-induced outcome. *BMC Cancer* 15: 1–10.
- Ferlazzo ML, Bourguignon M, Foray N. 2017. Functional assays for individual radiosensitivity: a critical review. *Semin Radiat Oncol* 27: 310–315.
- Fu H, Huang J, Zhao T, Wang H, Chen Y, Xu W, Pang Y, Guo W, Sun L, Wu H, Xu P, Su B, Zhang J, Chen X, Chen H. 2023. Fibroblast activation protein-targeted radioligand therapy with <sup>177</sup>Lu-EB-FAPI for metastatic radioiodine-refractory thyroid cancer: First-in-human, dose-escalation study. *Clin Cancer Res* 29: 4740–4750.
- Gong F, Miller KM. 2019. Histone methylation and the DNA damage response. *Mutat Res – Rev* 780: 37–47.
- Huang R-X., Zhou P-K. 2020. DNA damage response signaling pathways and targets for radiotherapy sensitization in cancer. *Signal Transduct Target Ther* 5. <https://doi.org/10.1038/s41392-020-0150-x>
- Kawashima S, Kawaguchi N, Taniguchi K, Tashiro K, Komura K, Tanaka T, Inomata Y, Imai Y, Tanaka R, Yamamoto M, Inoue Y, Lee SW, Kawai M, Tanaka K, Okuda J, Uchiyama K. 2020.  $\gamma$ -H2AX as a potential indicator of radiosensitivity in colorectal cancer cells. *Oncol Lett* <https://doi.org/10.3892/ol.2020.11788>
- Khazaei Monfared Y, Heidari P, Klempner SJ, Mahmood U, Parikh AR, Hong TS, Strickland MR, Esfahani SA. 2023. DNA damage by radiopharmaceuticals and mechanisms of cellular repair. *Pharmaceutics* 15: 1–26.
- Lassmann M, Hänscheid H, Gassen D, Biko J, Meineke V, Reiners C, Scherthan H. 2010. In vivo formation of  $\gamma$ -H2AX and 53BP1 DNA repair foci in blood cells after radioiodine therapy of differentiated thyroid cancer. *J Nucl Med* 51: 1318–1325.
- Li P, Du C-r, Xu W-c, Shi Z, Zhang Q, Li Z. 2013. Correlation of dynamic changes in  $\gamma$ -H2AX expression in peripheral blood lymphocytes from head and neck cancer patients with radiation-induced oral mucositis. *Radiat Oncol* 8: 155.
- Liu T-T., Li C-F., Tan K-T., Jan Y-H., Lee P-H., Huang C-H., Yu S, Tsao C, Wang J, Huang H. 2022. Characterization of aberrations in DNA damage repair pathways in gastrointestinal stromal tumors: *Cancers (Basel)* 1–22.
- Lobachevsky P, Leong T, Daly P, Smith J, Best N, Tomaszewski J, Thompson ER, Li N, Campbell IG, Martin RF, Martin OA. 2016. Compromised DNA repair as a basis for identification of cancer radiotherapy patients with extreme radiosensitivity. *Cancer Lett* 383: 212–219.
- Mahmoud AS, Hassan AME, Ali AA, Hassan NM, Yousif AA, Elbashir FE, Omer A, Abdalla OM. 2022. Detection of radiation-induced DNA damage in breast cancer using gamma H2AX biomarker: a possible correlation with their body mass index. *Genome Integr* 13.
- Purnami S, Suwifan VA, Ramadhani D, Lusiyanti Y, Darlina D, Rahajeng N, Syaifudin M, Pujianto DA, Widowati R. 2023. Phosphorylated Ataxia Telangiectasia Mutated (pATM) Enzyme-Linked Immunosorbent Assay (ELISA) for predicting radiation induces normal tissue toxicity in radiotherapy patients: a systematic review. *Indones J Cancer* 17: 235.
- Raavi V, Perumal FD, Paul S. 2021. Potential application of  $\gamma$ -H2AX as a biodosimetry tool for radiation triage. *Mutat Res – Rev* 787: 108350.
- Ramadhani D, Syaifudin M, Purnami S, Naroeni A. 2020. The use of image processing and analysis in automated biological dosimetry. *Atom Indones* 46: 127–133.
- Richardson RB. 2011. Stem cell niches and other factors that influence the sensitivity of bone marrow to radiation-induced bone cancer and leukaemia in children and adults. *Int J Rad Biol* 87: 343–359.
- Sak A, Grehl S, Erichsen P, Engelhard M, Grannaß A, Levegrün S, Stuschke M. 2007. Gamma-H2AX foci formation in peripheral blood lymphocytes of tumor patients after local radiotherapy to different sites of the body: dependence on the dose-distribution, irradiated site and time from start of treatment. *Int J Rad Biol* 83: 639–652.
- Sgouros G, Bodei L, McDevitt MR, Nedrow JR. 2020. Radiopharmaceutical therapy in cancer: clinical advances and challenges. *Nat Rev Drug Discov* 19: 589–608.
- Song H, Shen R, Liu X, Yang X, Xie K, Guo Z, Wang D. 2023. Histone post-translational modification and the DNA damage response. *Genes Dis* 10: 1429–1444.
- Srivastava N, Gochhait S, de Boer P, Bamezai RNK. 2009. Role of H2AX in DNA damage response and human cancers. *Mutat Res – Rev* 681: 180–188.
- Vinnikov V, Belyakov O. 2022. Clinical applications of biological dosimetry in patients exposed to low dose radiation due to radiological, imaging or nuclear medicine procedures. *Semin Nucl Med.* 52: 114–139.

Widjaja L, Werner RA, Krischke E, Christiansen H, Bengel FM, Bogdanova N, Derlin T. 2022. Individual radiosensitivity reflected by  $\gamma$ -H2AX and 53BP1 foci predicts outcome in PSMA-targeted radioligand therapy. *Eur J Nucl Med Mol Imaging* <https://doi.org/10.1007/s00259-022-05974-8>

Willers H, Gheorghiu L, Liu Q, Efstathiou JA, Wirth LJ, Krause M, von Neubeck C. 2015. DNA damage response assessments in human tumor samples provide functional biomarkers of radiosensitivity. *Semin Radiat Oncol* 25: 237–250.

**Cite this article as:** Kisananto T, Ramadhani D, Purwaningsih EH, Hakim RW, Basri IKH, Humani TS, Surniyantoro HNE, Syaifudin M, Purnami S, Suryandari DA. 2025. Gamma-H2AX as a biomarker to assess individual radiosensitivity for radiopharmaceutical therapy in cancer patients: a systematic review. *Radioprotection* 60(3): 285–292. <https://doi.org/10.1051/radiopro/2025004>



**Please help to maintain this journal in open access!**

This journal is currently published in open access under the Subscribe to Open model (S2O). We are thankful to our subscribers and supporters for making it possible to publish this journal in open access in the current year, free of charge for authors and readers.

Check with your library that it subscribes to the journal, or consider making a personal donation to the S2O programme by contacting [subscribers@edpsciences.org](mailto:subscribers@edpsciences.org).

More information, including a list of supporters and financial transparency reports, is available at <https://edpsciences.org/en/subscribe-to-open-s2o>.