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Review

Dual Immune-Regulatory Role of DAMPs in Glioblastoma Radiotherapy

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Abstract

Glioblastoma (GBM) remains among the most treatment-refractory human malignancies. It is characterized by profound radioresistance and a highly immunosuppressive tumor microenvironment, limiting the durable efficacy of radiotherapy. Beyond direct cytotoxicity, ionizing radiation can induce immunogenic cell death and the release of damage-associated molecular patterns (DAMPs), including surface-exposed calreticulin, HMGB1, extracellular ATP/adenosine, and tumor-derived DNA. These signals engage pattern-recognition receptors and cGAS–STING–type I interferon pathways, transiently promoting antigen presentation and immune activation. In GBM, however, DAMP signaling frequently evolves toward chronic inflammation and immune suppression, characterized by myeloid cell recruitment, adenosine accumulation, and immune checkpoint upregulation, thereby contributing to tumor regrowth and radioresistance. This dual immune-regulatory role of DAMPs highlights the importance of temporal and contextual interpretation of radiation-induced immune responses. In this review, we summarize current mechanistic and translational evidence on DAMP-mediated immunomodulation in GBM radiotherapy; discuss modality-dependent considerations across photon, proton, and high-LET irradiation; and evaluate the emerging potential of DAMPs as dynamic biomarkers of treatment response. We further outline how integration of DAMP profiling with liquid biopsy, imaging, and nanotheranostic platforms may support biologically informed and adaptive radiotherapy strategies for glioblastoma.

Keywords: glioblastoma; radiotherapy; proton therapy; damage-associated molecular patterns (DAMPs); immunogenic cell death; cGAS–STING pathway; tumor microenvironment; radioresistance; immune modulation; personalized radiotherapy

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1. Introduction

Glioblastoma (GBM) remains one of the most aggressive and treatment-refractory human malignancies, with median survival rarely exceeding 15 months despite maximal safe resection, concurrent chemoradiotherapy, and adjuvant temozolomide administration [1]. Standard radiotherapy regimens, including photons, provide modest improvements in local control, but tumor recurrence within the irradiation field is almost universal and strongly associated with intrinsic and acquired resistance mechanisms, including glioma stem cell survival, hypoxia, and potent immunosuppression in the tumor microenvironment (TME) [1].

Recent advances in radiation oncology have expanded interest beyond purely physical dose delivery to include biological modulation of treatment response. In particular,

there is increasing recognition that ionizing radiation can act as a stressor that not only induces DNA lesions but also activates complex immunological pathways through the release of damage-associated molecular patterns (DAMPs). DAMPs are endogenous danger signals—including high-mobility group box 1 (HMGB1), ATP, heat-shock proteins, and mitochondrial DNA—that are released during radiation-induced cell damage and immunogenic cell death (ICD), as established by Hernandez et al. [1], and can engage pattern recognition receptors (PRRs) in innate and adaptive immune cells, as demonstrated in experimental ICD models by Fucikova et al. and Krysko et al. [2,3].

This paradigm reflects an important evolution in radiobiology: Whereas early models focused primarily on direct DNA damage and clonogenic cell death, modern frameworks encompass immune reprogramming, inflammation, and systemic host responses as critical determinants of therapeutic outcome. ICD and associated DAMP signaling have been shown to promote antitumor immunity through dendritic cell maturation and T-cell priming while also contributing to chronic inflammation and tumor tolerance under certain conditions, as described by Hernández et al. [1].

In GBM, an inherently "cold" tumor with profound immunosuppressive networks, the interplay between DAMPs, ICD, and the TME is of particular relevance. Strategies that harness DAMP-mediated immune activation may enhance the efficacy of radiotherapy and immunotherapy combinations, overcome resistance, and enable personalized approaches that integrate real-time biological response monitoring [2,4]. Moreover, differential radiation modalities—including proton beam therapy with its distinct ionization patterns—may variably modulate DAMP release and immune outcomes, offering opportunities for theranostic integration [1–3].

Glioblastoma is characterized by a profoundly immunosuppressive tumor microenvironment dominated by myeloid cells, including tumor-associated macrophages and microglia, with limited infiltration and functional impairment of cytotoxic T lymphocytes. Antigen presentation is inefficient, immune checkpoint pathways are constitutively active, and metabolic constraints such as hypoxia and adenosine accumulation further restrict effective antitumor immunity. In this context, immune signaling is highly plastic and readily shifts from transient activation toward tolerance and suppression. This creates a setting in which DAMP signaling has been shown to exert divergent and context-dependent effects following radiotherapy, depending on microenvironmental conditions and signaling dynamics, as consistently reported in studies on tumor-associated macrophages and myeloid-derived suppressor cells by Li et al. and Ostrand-Rosenberg and Fenselau [5–7]. Clinical observations, including those reported by Clasen et al., demonstrate that circulating HMGB1 levels increase during radiochemotherapy and correlate with treatment response [8].

In this review, we examine the emerging role of DAMPs as key mediators linking radiotherapy-induced tumor damage with immune regulation in glioblastoma. We summarize the molecular mechanisms underlying radiation-induced immunogenic cell death, discuss how DAMP signaling can drive either antitumor immunity or immunosuppression within the GBM microenvironment, and evaluate whether different radiation modalities may differentially influence these processes. Finally, we highlight translational perspectives, including the use of DAMPs as dynamic biomarkers and their integration with advanced imaging and nanotheranostic strategies, with the aim of informing biologically guided and personalized radiotherapy approaches for glioblastoma.

2. Radiotherapy-Induced DAMP Signaling and Immunogenic Cell Death

2.1. Canonical DAMPs Released After Irradiation

Among the best-characterized DAMPs induced by radiation are calreticulin (CRT) exposure on the cell surface, extracellular ATP, nuclear proteins such as HMGB1, mitochondrial and nuclear DNA fragments, and heat-shock proteins [2]. Each has been shown to play a temporally coordinated role in immunogenic cell death, as described by Krysko et al. and Galluzzi et al. [9]. CRT [3] exposure provides an early "eat-me" signal, promoting phagocytosis of dying tumor cells by dendritic cells, which was demonstrated in studies of immunogenic cell death by Krysko et al. ATP released into the extracellular space activates purinergic receptors (e.g., P2RX7), favoring inflammasome assembly and dendritic cell maturation, as shown in the experimental models summarized by Fucikova et al. [2]. HMGB1 subsequently binds pattern-recognition receptors, including TLR4 and RAGE, supporting efficient cross-presentation of tumor antigens [4]. Meanwhile, cytosolic leakage of nuclear or mitochondrial DNA has been shown to activate the cGAS–STING pathway, resulting in type I interferon production, as demonstrated by Deng et al. and further refined by Vanpouille-Box et al. [4–6].

2.2. ICD as the Immunological Outcome of Radiation-Induced Damage

ICD represents a form of regulated cell death that is recognized as pro-immunogenic, as defined by Krysko et al. and Galluzzi et al. [3,9]. Rather than being defined solely by morphology, ICD is characterized by the coordinated presence of DAMP hallmarks (CRT, ATP, and HMGB1) and robust type I interferon signaling. Radiotherapy has been shown to induce ICD, converting otherwise "silent" cell death into an immunogenic process capable of eliciting T-cell responses [2]. Importantly, the immunogenicity of ICD is context-dependent—influenced by dose, fractionation, tumor genotype, hypoxia, and the immunological state of the microenvironment [3,4].

In glioblastoma, where T-cell infiltration is limited and suppressive myeloid populations dominate, partial ICD induction may contribute to immunosuppressive outcomes under certain conditions, as suggested in studies on chronic DAMP signaling carried out by Hernández et al. Thus, understanding how radiation shapes the quality and kinetics of DAMP release is crucial for rationally combining radiotherapy with immunotherapies [3,5,7].

2.3. Receptor Pathways Linking DAMPs to Immune Activation

Multiple receptor systems translate DAMPs into immune signals. TLR2/4, RAGE, and NLRP3 coordinate early innate responses; cGAS–STING couples cytosolic DNA sensing to interferon-driven cross-priming; purinergic signaling regulates inflammasome activation and antigen presentation [2]. These pathways converge on dendritic cells, macrophages, and endothelial cells, ultimately influencing T-cell trafficking and effector function. Intriguingly, glioma cells sense DAMPs, potentially contributing to feed-forward inflammatory loops associated with radioresistance [2].

2.4. Proton Versus Photon Irradiation: Qualitative Differences in DAMP Dynamics

This section focuses on experimental and mechanistic evidence describing how different radiation modalities influence DAMP induction at the cellular and tissue level. Beyond differences in physical dose distribution, accumulating preclinical data suggest that radiation quality may influence immunological consequences of irradiation. Proton irradiation, characterized by distinct linear energy transfer profiles, has been reported in preclinical studies to modulate DAMP-related processes—including surface-exposed CRT, HMGB1 release, extracellular ATP signaling, and radiation-induced DNA stress—through mecha-

nisms that may differ quantitatively from photon irradiation, with proposed contributions from mitochondrial perturbation and cGAS–STING activation. Preliminary studies further indicate modality-dependent differences in extracellular vesicle composition, bystander signaling, and myeloid cell responses following proton exposure. While these findings support the hypothesis that radiation quality can influence DAMP-associated immune signaling, current evidence remains largely preclinical and requires rigorous validation in glioblastoma-specific models. The translational implications of these observations are discussed in Section 4. Figure 1 schematically illustrates how sustained DAMP signaling following radiotherapy can promote immunosuppression in glioblastoma through activation of TLR4, RAGE, and cGAS–STING pathways, leading to myeloid recruitment, adenosine accumulation, immune checkpoint upregulation, and ultimately tumor regrowth and radioresistance.

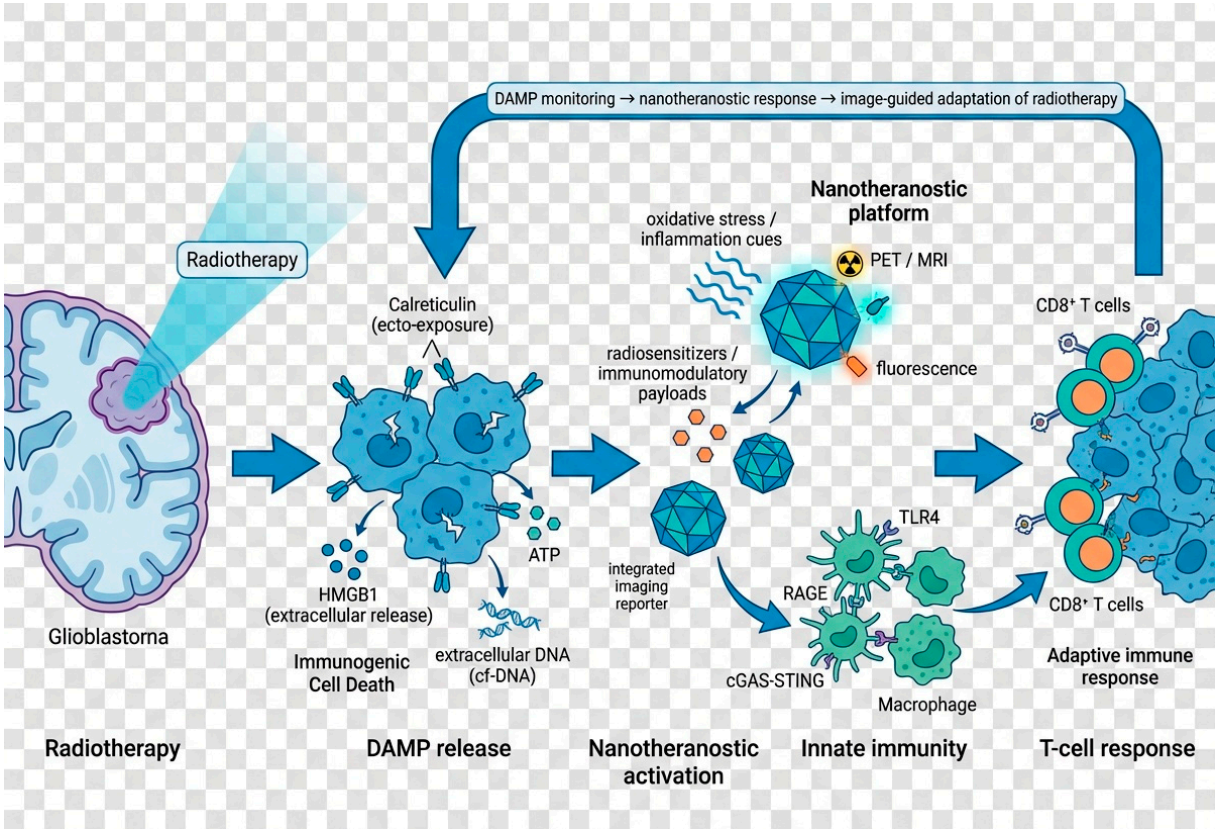


Figure 1. Chronic DAMP signaling promotes an immunosuppressive tumor microenvironment. Radiotherapy causes glioblastoma cells to release DAMPs (CRT, HMGB1, ATP/adenosine, and extracellular DNA). When signaling becomes persistent, activation of TLR4, RAGE, and cGAS–STING drives recruitment of MDSCs, polarization of M2-like macrophages, accumulation of adenosine (CD39/CD73), and upregulation of PD-L1. These processes suppress cytotoxic T-cell activity, promote angiogenesis and survival signaling, and ultimately contribute to tumor regrowth and radioresistance.

2.5. How Radiotherapy-Induced DAMPs Shape Immunity and Therapeutic Sensitivity

DAMPs released after irradiation have been shown to contribute to therapeutic sensitivity by converting radiation-induced tumor damage into ICD, thereby coupling local cytotoxicity with systemic immune engagement. Mechanistically, early exposure of ecto-CRT provides an "eat-me" signal recognized via CD91, enhancing phagocytosis of dying tumor cells and antigen transfer to dendritic cells. The ATP released into the extracellular space activates purinergic receptors, e.g., P2RX7, promoting inflammasome signaling (e.g., NLRP3) and dendritic-cell maturation. Subsequent HMGB1 release signals through TLR4

and RAGE to support antigen processing and cross-presentation. In parallel, radiation-induced nuclear and mitochondrial DNA leakage activates cytosolic DNA sensing (cGAS–STING), inducing type I interferon programs that promote dendritic-cell licensing and recruitment/priming of $CD8^+$ T cells. These pathways have been shown to increase tumor immunogenicity and enhance immune recognition.

At the same time, DAMP signaling is context- and time-dependent and may reduce therapeutic sensitivity when signaling becomes sustained. In glioblastoma, repeated tissue injury and necrosis can drive chronic PRR activation and sterile inflammation that preferentially recruits and programs suppressive myeloid populations (microglia/TAMs/MDSCs), increases ectonucleotidase activity (CD39/CD73) with adenosine accumulation, and reinforces immune checkpoint and exhaustion programs. Thus, radiotherapy-induced DAMPs have been described as a context-dependent determinant of therapeutic outcome: transient, well-orchestrated DAMP pulses favor ICD and immune activation, whereas persistent DAMP exposure favors immunosuppression, immune escape, and radioresistance.

2.6. Radiotherapy-Induced DAMPs Versus DAMPs from Other Therapies

The major DAMP species released after radiotherapy largely overlap with those induced by other cytotoxic therapies that can trigger immunogenic cell death, including ecto-CRT, extracellular ATP, HMGB1, heat-shock proteins, and nuclear/mitochondrial DNA. The principal differences are therefore not the identity of DAMPs but rather their kinetics, amplitude, and cellular routing, which are strongly influenced by the dominant stress program induced by each therapy. Radiotherapy is characterized by the coupling of membrane/ER stress, mitochondrial perturbation, and DNA damage, frequently associated with the generation of cytosolic DNA fragments that engage cGAS–STING and type I interferon signaling. In addition, fractionated radiotherapy can produce repeated "pulses" of DAMP release and inflammatory remodeling across weeks, which has been shown to either reinforce adaptive immunity or, particularly in glioblastoma, progressively favor suppressive myeloid programs. In contrast, systemic chemotherapies often generate broader systemic inflammation and immunotoxicity, while targeted therapies may induce less overt ICD; they can alter antigenicity and immune checkpoints. Therefore, radiotherapy should be viewed as a DAMP-inducing modality distinguished primarily by spatiotemporal patterning and DNA-sensing engagement rather than by a unique DAMP repertoire.

3. Immune Modulation in Glioblastoma After Radiotherapy: DAMP Signaling in a Highly Suppressive Tumor Microenvironment

Radiotherapy has been shown to induce changes within the glioblastoma microenvironment that extend beyond direct cytotoxicity [10]. Experimental and clinical studies consistently show that ionizing radiation leads to the exposure and release of canonical DAMPs, including CRT, extracellular ATP, HMGB1, and fragments of nuclear or mitochondrial DNA [11]. These signals have been shown to engage pattern-recognition receptors, such as TLR4, RAGE, and the cGAS–STING pathway, thereby linking cellular damage to antigen cross-presentation, dendritic cell maturation, and type I interferon production [12]. In glioblastoma, this sequence of events has been described as contributing to increased antigen visibility that transiently promotes conditions permissive for T-cell priming and trafficking [6,13,14].

At the same time, glioblastoma is characterized by a strongly immunosuppressive tumor microenvironment dominated by tumor-associated macrophages and microglia, regulatory T cells, and limited infiltration of cytotoxic lymphocytes. Multiple analyses demonstrate that radiation-induced inflammation in this setting is rapidly counterbalanced

by compensatory mechanisms, including upregulation of immune checkpoints, accumulation of myeloid-derived suppressor populations, and metabolic features such as hypoxia and adenosine production [15]. As a result, although DAMP signaling initiates immunogenic processes, subsequent remodeling frequently favors restoration of tolerance rather than sustained antitumor immunity [14]. This pattern reflects a reproducible biological property of glioblastoma rather than a failure of any single pathway.

DAMP signaling therefore functions as an organizing node that integrates radiation-induced cell injury with immune reprogramming [16]. Early exposure of CRT and ATP release has been shown to facilitate phagocytosis and inflammasome activation, while HMGB1 and DNA sensing promotes interferon-driven recruitment of effector cells. Parallel studies indicate that persistent activation of these same pathways correlates with myeloid polarization toward tissue-repair phenotypes and functional exhaustion of infiltrating lymphocytes [15,17]. Importantly, these observations have been reported across multiple preclinical glioma models and validated in analyses of patient material, establishing DAMP-driven immune dynamics as a reproducible feature of radiotherapy response in this disease [18].

These immune consequences are closely linked to the structural and metabolic organization of glioblastoma, including hypoxic niches, disrupted vasculature, and high intracellular antioxidant capacity [19]. Such characteristics influence both the magnitude of DAMP release and the downstream composition of infiltrating immune cells. In parallel, radiation-conditioned extracellular vesicles containing proteins, nucleic acids, and DAMP-related molecules have been documented as additional mediators of communication between tumor cells and the surrounding microenvironment [20]. The findings show a biologically consistent framework in which radiotherapy induces a defined cascade of immune-relevant signals that are subsequently shaped by local microenvironmental constraints [19,20].

Because DAMP levels and associated immune signatures reflect the biological effectiveness of radiotherapy, they are being increasingly investigated as measurable indicators of treatment response and as candidates for integration into precision-oriented treatment strategies [11]. This evidence-based perspective provides a logical entry point for translational research aimed at linking molecular monitoring with therapeutic decision-making, particularly in the context of biologically informed radiotherapy approaches [16].

4. DAMP-Driven Biomarkers and Pathways Relevant to Precision Radiotherapy

Importantly, the evaluation of DAMPs extends beyond individual molecules. Composite signatures integrating HMGB1, surface-exposed CRT, and interferon-related responses have been shown to align with hallmarks of immunogenic cell death across several tumor types, including glioma, as described by Galluzzi et al. and Krysko et al. [3,9]. In clinical contexts, such integrated signatures have been associated with immune activation and, in selected settings, with improved outcomes when radiotherapy is combined with immunomodulatory approaches, underscoring their relevance for biomarker development rather than isolated experimental observations, as reported in clinical and translational analyses by Vaes et al. [19].

A further translational application of DAMP biology lies in treatment monitoring. Serial sampling studies demonstrate that radiation-induced DAMP release is dynamic over the course of therapy and can be detected using liquid biopsy approaches, including plasma and cerebrospinal fluid analyses, as demonstrated in clinical observations by Clasen et al. [10]. In parallel, imaging techniques capable of capturing inflammation, perfusion changes, or microenvironmental remodeling provide complementary information

on downstream immune effects [12,15]. The integration of molecular DAMP biomarkers with functional imaging, therefore, offers a realistic framework for biologically guided adaptation of radiotherapy, in which treatment effectiveness is assessed through biological response rather than anatomical change alone [12,15,21].

Nanotechnology further supports this paradigm by enabling spatially resolved delivery and monitoring within the irradiated tumor microenvironment [18]. In preclinical glioma models, nanotheranostic platforms incorporating radiosensitizers or immunomodulators have been shown to enhance local radiation effects while allowing in vivo visualization of distribution and response, as shown in preclinical glioma models by Wu et al. [22]. When combined with DAMP-related biomarkers, such systems provide complementary insights, with DAMPs reflecting biological impact and nanotheranostic probes informing localization and treatment kinetics [23,24].

Finally, radiation modality represents an additional translational dimension. Comparative studies suggest that different radiation qualities may influence the magnitude and kinetics of DAMP release and associated immune signaling, as demonstrated in comparative radiobiological studies by Gameiro et al., Yoshimoto et al. and Huang et al. [16,17,25]. While these observations do not establish clinical superiority of any modality, they indicate that radiation type contributes to the biological signature generated by therapy. Aligning dosimetric parameters with molecular and imaging biomarkers may therefore support biologically informed radiotherapy planning and personalization [22].

In Table 1, we summarized published evidence comparing photon, proton, carbon-ion, and BNCT irradiation with respect to the induction of canonical DAMPs [15]. Across modalities, the same core DAMPs (ecto-CRT, HMGB1, ATP, and extracellular DNA) are consistently observed, with differences mainly reflecting quantitative and kinetic variation rather than modality-specific DAMP species, which is consistent with findings reported by Gameiro et al. and Huang et al. [24].

DAMP-related signatures and ICD hallmarks provide quantifiable indicators of radiation-induced immune engagement; imaging-enabled nanoplatforms allow localized therapy and simultaneous monitoring; modality-specific biological effects create opportunities to match treatment delivery with tumor biology [19]. The cumulative evidence indicates that integrating these components is feasible within prospective clinical research, offering a structured pathway toward precision radiotherapy that is grounded in measurable biological parameters rather than theoretical assumptions.

4.1. Do Different Radiation Modalities Induce Different DAMPs?

Across preclinical and early translational studies, photon, proton, and high-LET radiations (carbon ions and BNCT) consistently induce the same canonical DAMPs associated with ICD: ecto-CRT, extracellular ATP, HMGB1 release, and the presence of nuclear or mitochondrial DNA in the extracellular space. These DAMPs signal through shared receptor systems—CD91 for CRT, P2RX7/P2RY2 for ATP, TLR4 and RAGE for HMGB1, and the cGAS–STING pathway for cytosolic DNA—driving the established ICD cascade. Comprehensive reviews by Vaes et al. and Zhu et al. emphasize that these molecules and pathways are common across different radiation regimens rather than being specific to a particular modality [19,23].

For photons vs. protons, the most direct mechanistic comparison comes from Gameiro et al. In multiple human tumor cell lines, including radioresistant subsets, they showed that proton and photon irradiation at iso-effective doses induced a “common immunogenic modulation signature”, resulting in the upregulation of MHC class I, ICAM-1, tumor antigens (CEA and MUC1), and CRT exposure on the cell surface, which in turn increased susceptibility to antigen-specific T-cell killing [16]. The authors explicitly concluded that

tumor cells surviving proton or photon radiation share the same immunogenic phenotype, with no evidence of modality-specific DAMPs [16,20–29].

Table 1. Overview of radiation modalities and their reported DAMP responses.

Radiation Modality	Main DAMP(s) Reported	Timing of Detection	Model/Clinical Setting	Key Findings	Evidence for Modality-Specific Differences in DAMPs
Photons (clinical IMRT + CRT)	HMGB1 (plasma)	Weekly during RT; post-treatment follow-up	HNSCC patients receiving definitive radiochemotherapy	Serial plasma sampling showed dynamic rise of HMGB1 over the course of RT, with patterns associated with outcome and toxicity.	No qualitative difference in DAMP species vs. other modalities; clinical data only for photons. Quantitative behaviour reflects tumor burden, inflammation and toxicity, not unique photon biology [1]
Photons (fractionated, preclinical)	HMGB1, HSP70 and other ICD-associated DAMPs	~24–72 h after irradiation; repeated pulses with fractionation	Glioma and other tumor cell lines; small-animal models	Fractionated photon RT induces pulse-like release of HMGB1/HSP70 after each fraction, consistent with ICD kinetics.	Same DAMP panel as for other RT types; no evidence of photon-specific DAMPs. Differences are dose-, time-, and cell-line-dependent [2,3]
γ-rays vs. BNCT (high-LET neutrons)	HMGB1 (cell supernatant, plasma)	In vitro: ~24 h; in vivo: peak ~day 3	Xenograft model of BNCT vs. γ-irradiation	BNCT induced early extracellular HMGB1 release in vitro and a marked plasma HMGB1 peak around day 3 in mice; γ-rays also induced HMGB1 but in a less pronounced fashion.	Same DAMP (HMGB1) for γ and BNCT. Some quantitative enhancement with BNCT, but no new DAMP species; LET mainly affects magnitude and kinetics [4,11]
Photons vs. protons (in vitro)	ecto-CRT	12, 24, 48 h (max at ~48 h)	Multiple human tumor cell lines, including U251 glioma	Photon, proton, and carbon-ion beams all increased ecto-CRT in a time- and dose-dependent manner. At iso-doses, photons and protons showed very similar CRT exposure; carbon ions required lower doses to reach comparable or higher CRT.	No qualitative difference in DAMP (CRT) between photons and protons. High-LET carbon ions show stronger CRT at lower doses, but the DAMP itself is the same [5,18]
Photons vs. protons (in vitro)	Ecto-CRT, MHC I, ICAM-1, tumor antigens (immunogenic modulation)	48–72 h after RT	Multiple tumor cell lines	Proton and photon irradiation produced a shared immunogenic modulation signature, including increased CRT and antigen presentation, rendering cells more sensitive to T cell killing.	Authors explicitly conclude that tumor cells surviving proton or photon RT share a common immunogenic profile; no DAMP unique to one modality. Differences are not consistent enough to claim modality-specific DAMP biology [18]
Carbon ions (high LET) vs. X-rays	HMGB1	24–72 h after RT	Various human cancer cell lines	Carbon-ion beams induce HMGB1 release at levels comparable to X-rays; subsequent work shows HMGB1 release increases with LET, but remains the same molecule.	High-LET RT increases amount and persistence of HMGB1, but does not introduce new DAMPs; pattern is quantitative, not qualitative [7,8]

Huang et al. extended this observation by directly comparing ecto-CRT induction after photon, proton, and carbon-ion irradiation in several tumor cell lines, including U251 glioma. All three modalities increased ecto-CRT in a time- and dose-dependent manner. At iso-doses, proton and photon beams were essentially equivalent in inducing ecto-CRT, whereas carbon ions produced stronger CRT exposure at 2 and 4 Gy but not at higher doses. This confirms that the DAMP species are the same for low-LET photons and protons; high-LET heavy ions change the intensity, not the identity, of DAMP signaling [27–29].

The same pattern holds for HMGB1, as demonstrated in radiobiological studies by Yoshimoto et al. and Onishi et al. Yoshimoto et al. examined HMGB1 release from multiple human cancer cell lines after X-ray versus carbon-ion irradiation and found that both modalities induced HMGB1 release, and when doses were normalised to iso-survival levels, X-rays and carbon ions were equally effective at inducing HMGB1 [17]. Immamichi et al. later showed that increasing LET within carbon-ion beams further enhances HMGB1 release, reinforcing that high-LET radiation can quantitatively boost HMGB1 while still utilising the same molecular mediator [25].

For BNCT, Imamichi et al. demonstrated that both BNCT and γ-irradiation trigger extracellular HMGB1 release in vitro, but BNCT produces a more pronounced and sustained HMGB1 elevation in mouse plasma, peaking around day 3 after treatment [15]. Again, the DAMP is HMGB1 in both conditions; BNCT modifies the kinetics and amplitude, not the DAMP repertoire [15]. The data support a conclusion: Photons, protons, carbon ions, and BNCT all induce the same canonical DAMPs associated with ICD. High-LET modalities can enhance the magnitude and timing of DAMP release, but no modality-specific DAMP species have been consistently identified. Differences are primarily quantitative and kinetic, not qualitative [13,15].

4.2. How Can DAMPs Influence Radiotherapy Outcomes and How Does RT Choice Interact with This Biology?

DAMPs influence radiotherapy outcomes by shaping the balance between effective antitumor immunity and tolerogenic or chronically inflamed microenvironments.

Mechanistically, the sequence is well defined. Early after irradiation, CRT translocates to the cell surface, acting as an "eat-me" signal that facilitates phagocytosis of dying tumor cells by dendritic cells via CD91. Extracellular ATP is released and engages P2RX7, promoting NLRP3 inflammasome activation and IL-1β secretion, which supports dendritic cell maturation [13]. Subsequently, HMGB1 is released and binds TLR4 and RAGE on antigen-presenting cells, enhancing antigen processing and cross-presentation. In parallel, leakage of nuclear and mitochondrial DNA into the cytosol or extracellular space activates cGAS–STING, leading to type I interferon production and a transcriptional programme that recruits and primes $CD8^+$ T cells [9].

Vaes et al. demonstrated the clinical relevance of ICD-associated DAMPs and systematically reviewed clinical data in which radiotherapy-induced ICD markers were assessed in patients. Vaes et al. found that elevated HMGB1 and ecto-CRT and the activation of IFN-I-related signatures correlate with features of immune activation and, in selected settings, with improved outcomes in combination with immunotherapies [9]. Zhu et al. summarized similar relationships across radiotherapy trials where ICD hallmarks were linked to enhanced infiltration of effector T cells and better responses to immune checkpoint blockade, particularly in tumors historically considered "cold" [23].

Another clinical example is provided by Clasen et al., who performed weekly plasma sampling in head-and-neck squamous cell carcinoma patients receiving definitive radiochemotherapy with photons [10]. Plasma HMGB1, measured by ELISA, increased dynamically over the course of treatment in most patients. Importantly, specific HMGB1 trajectories correlated with oncologic outcomes: Patients with a sustained increase in HMGB1 during radiochemotherapy had more favorable local control, whereas decreasing or flat profiles were associated with higher progression risk. HMGB1 dynamics also correlated with tumor volume, CRP, and treatment-related toxicity [27]. This study shows that DAMP levels are not merely a byproduct of damage but carry prognostic information relevant to treatment success [14].

In preclinical models, high-LET modalities (carbon ions and BNCT) amplify these same DAMP-mediated mechanisms. Yoshimoto and Han demonstrated that carbon ions

induce HMGB1 release at levels comparable or higher than X-rays at iso-survival doses and can more strongly enhance downstream immune responses in combination with immunotherapy [17,25]. The authors showed that BNCT produces an early and sustained HMGB1 plasma peak, which they propose as a biomarker of BNCT effectiveness. High-LET radiations magnify the same DAMP-driven immunological cascade and can, under controlled conditions, translate into superior antitumor immunity but the underlying DAMP biology remains shared [16].

For proton versus photon radiotherapy, the key message from Gameiro and Huang is that at the tumor-cell level, DAMP-related immunogenic modulation is very similar: Both modalities induce ecto-CRT, upregulate antigen presentation machinery, and enhance susceptibility to CTL killing. The clinically relevant difference arises more at the systemic immune level than at the level of DAMP species per se. Multiple clinical and dosimetric studies (in glioblastoma and other tumors) have demonstrated that proton plans substantially reduce low-dose exposure of circulating blood and lymphoid organs compared to photons, resulting in lower rates of severe radiation-induced lymphopenia [10]. Because DAMP-driven ICD requires functional dendritic cells and T-cell populations to translate into effective immunity, preservation of the host immune compartment is likely to enhance the clinical impact of a given DAMP/ICD signal.

Thus, from a translational standpoint,

- Different RT types do not fundamentally change which DAMPs are generated;
- High-LET beams (C-ions and BNCT) can increase how much and how fast they are released;
- Protons may improve the conditions in which DAMP-induced immunity operates by sparing blood and immune organs, even if the local DAMP pattern is similar to photons.

This implies that the choice of RT modality can influence outcomes indirectly via DAMP biology: by modulating the intensity of ICD (high-LET) or preserving the immune system's ability to respond (protons), rather than by switching to entirely different DAMP pathways.

4.3. Can DAMPs Be Markers of Radiotherapy Success?

The accumulated evidence strongly supports the idea that DAMPs and ICD signatures are promising biomarkers of radiotherapy effectiveness, although they are not yet fully validated predictive or surrogate markers in glioblastoma.

In the clinical setting, Wang-Bishop et al. [30] summarised multiple studies where ICD markers were measured during or after radiotherapy in different tumor types. HMGB1 increases after CRT for oesophageal, rectal, and head-and-neck cancers; ecto-CRT in tumor biopsies correlates with dendritic cell infiltration and favourable prognosis in cervical cancer; type I interferon-related gene signatures have been associated with better response to RT plus immunotherapy in several solid tumors. Clasen's weekly HMGB1 study is a particularly clear demonstration that serial DAMP measurements during RT carry prognostic information and can be used to stratify patients by risk, fulfilling key criteria for biomarkers of treatment success [28].

Preclinical data, presented by Liao et al. and Alhasan et al. [14,15], provide mechanistic support: Blocking HMGB1, CRT, or ATP signaling attenuates the immune contribution to tumor control after RT, while enhancing these pathways (e.g., via STING agonists) augments T-cell responses and abscopal effects. These interventions modulate radiotherapy outcome in a manner tightly coupled to DAMP biology, strengthening the rationale to use DAMP signatures as biomarkers and potential therapeutic targets.

In glioblastoma specifically, systematic liquid-biopsy studies have shown that circulating DNA, exosomes, and immune-related proteins can be monitored over time and

correlate with disease burden and treatment response, even though DAMP-specific panels (HMGB1, ecto-CRT, and mtDNA) are only beginning to be explored [24]. The analytical tools—ELISA, qPCR/digital PCR, EV proteomics, and, increasingly, DAMP-sensitive imaging probes—already exist and have been proven feasible in other tumor types [24].

DAMP-related markers fulfil the criteria of "promising biomarkers" of radiotherapy success: They can be repeatedly measured, show dynamic changes over the course of treatment, and correlate with immune activation and clinical outcome in several tumor entities. However, they have not yet reached the status of fully validated predictive or surrogate endpoints in glioblastoma, and their integration into clinical decision-making will require prospective, biomarker-driven trials.

In this framework, our own experience, that proton radiotherapy appears to produce a qualitatively different immune context than photon RT, supports the available data: the DAMP biology at the tumor level is shared; the systemic environment in which these signals act is improved by proton plans that better preserve the immune system [24,26,28].

Although no late-phase clinical trials have been designed to directly manipulate DAMP signaling as a therapeutic target, several early-phase and observational clinical studies provide proof of principle that DAMPs can be monitored in patients receiving radiotherapy and may reflect treatment-induced immunogenic cell death. For example, clinical protocols such as NCT03783364 incorporate biological endpoints related to radiotherapy-induced immune activation, including features consistent with ICD and DAMP release, to explore links between radiation delivery and immune response. In parallel, prospective and retrospective clinical studies—most prominently in head and neck and other solid tumors—have demonstrated that circulating DAMPs such as HMGB1 can be measured serially in plasma during radiochemotherapy and that their temporal dynamics correlate with treatment response, toxicity, and immune activation markers. Additional feasibility studies have extended this concept by evaluating extracellular vesicles and related danger-associated cargo as potential radiation response biomarkers, further supporting the technical feasibility of longitudinal DAMP monitoring in clinical settings. Importantly, these investigations remain predominantly correlative, vary in sampling strategy and analytical endpoints, and do not yet establish causality or standardized thresholds for clinical decision-making. However, it has been demonstrated that DAMP dynamics can be captured in vivo during radiotherapy and provide a clinical foundation for future prospective trials specifically designed to validate DAMPs as predictive and stratification biomarkers, particularly in immunologically complex tumors such as glioblastoma.

4.4. When DAMPs Promote Tolerance Rather than Immunity

DAMP signaling is fundamentally dualistic [23]. Although acute, well-orchestrated release of CRT, ATP, HMGB1, and extracellular DNA promotes ICD, persistent or dysregulated DAMP release generates a biological milieu that paradoxically sustains tumor survival and treatment resistance [11,25]. In the context of chronic tissue injury, repeated fractions of radiotherapy and persistent tumor necrosis, DAMP-driven activation of pattern-recognition receptors (PRRs)—including TLR4, RAGE, and cGAS–STING—can shift from initiating protective immunity toward reinforcing chronic, tumor-supportive inflammation [1–3].

This tolerogenic reprogramming proceeds through several partly overlapping mechanisms. First, chronic HMGB1 exposure enhances NF-κB signaling, angiogenesis, DNA repair capacity, and autophagy, ultimately conferring radioresistance at the tumor-cell level [4–7]. Experimental models demonstrate that blocking extracellular HMGB1 reverses myeloid recruitment and restores radiosensitivity after irradiation, highlighting HMGB1 as a central node of therapy resistance [8–10].

Second, ATP released as an early DAMP is rapidly catabolized by CD39 and CD73, generating adenosine, a potent immunosuppressive metabolite [24]. Adenosine signaling via A2A and A2B receptors limits cytotoxic T- and NK-cell function, promotes regulatory T cells, and fosters vascular remodeling—thereby attenuating radiotherapy-induced antitumor immunity and reducing responses to immune checkpoint inhibitors. Therapeutic targeting of the CD39–CD73–A2AR axis restores sensitivity to radiotherapy in multiple preclinical models, illustrating how a DAMP that initially promotes ICD can evolve into a dominant suppressive pathway when chronically present [11–13].

Third, persistent DAMP signaling drives the accumulation and functional polarization of myeloid-derived suppressor cells (MDSCs) and tumor-associated macrophages (TAMs), which inhibit antigen presentation, suppress T-cell infiltration, and upregulate immune checkpoints such as PD-L1. This phenomenon has been extensively documented across tumor types, where DAMP-dependent sterile inflammation correlates with immune escape, metastatic dissemination, and therapeutic failure [23]. Recent genomic signatures enriched for DAMP-related genes further link these pathways with immunosuppressive tumor microenvironments and inferior clinical outcomes [17]. Figure 2 presents DAMP-guided nanotheranostics enhance radiotherapy by sensing radiation-induced stress, amplifying immunogenic cell death, activating innate and adaptive immunity, and enabling adaptive, biology-guided treatment through integrated molecular and imaging feedback.

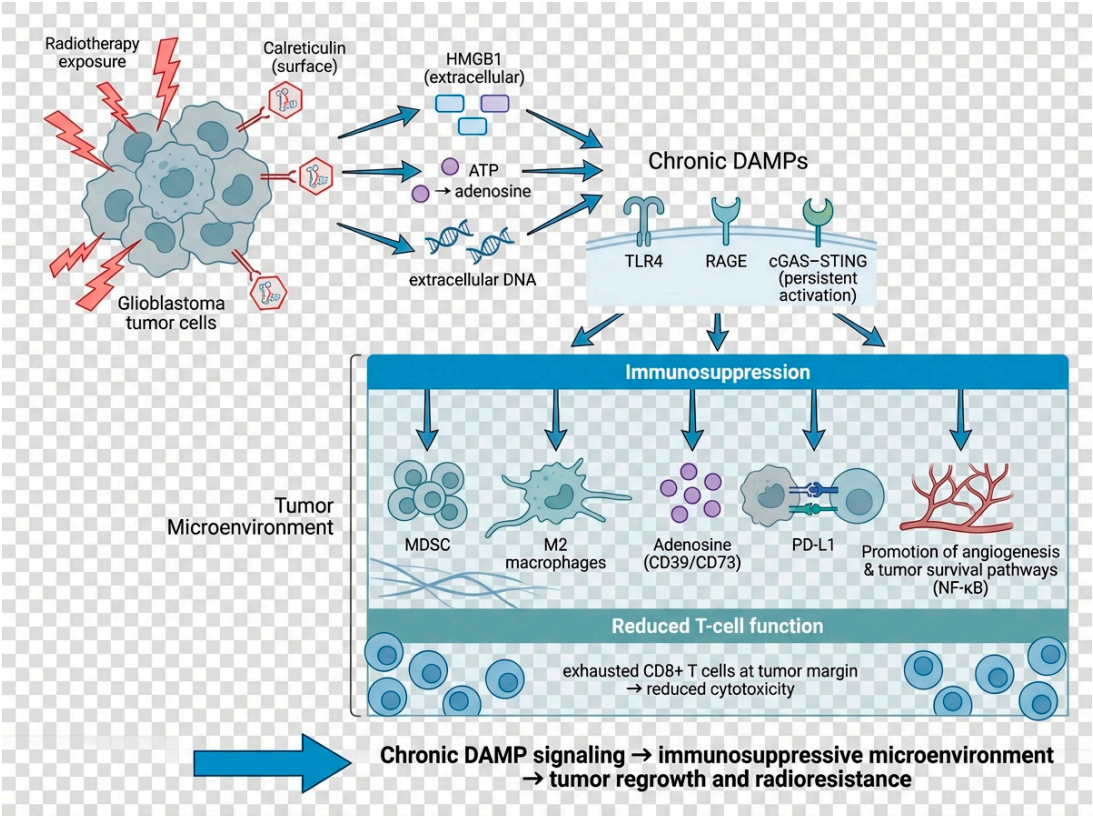


Figure 2. DAMP-guided nanotheranostics enhance radiation-induced antitumor immunity. Radiotherapy induces immunogenic cell death and release of DAMPs (CRT, HMGB1, ATP, extracellular DNA). Nanotheranostic platforms sense radiation-induced stress, deliver radiosensitizers or immunomodulatory payloads, and provide imaging feedback (PET/MRI/fluorescence). DAMP signaling activates dendritic cells and macrophages via TLR4, RAGE, and cGAS–STING, leading to T-cell priming and tumor infiltration. Integration of molecular monitoring with imaging enables adaptive, biology-guided radiotherapy.

Glioblastoma exemplifies this biology. Necrotic tumor cores continuously release DAMPs—including HMGB1, heat-shock proteins, and other TLR ligands—driving polarization of glioma-associated macrophages toward an M2-like phenotype, suppression of MHC expression, and induction of checkpoint molecules [18]. These effects create a TME that is highly resistant to radiotherapy and poorly responsive to immunotherapy, despite the presence of abundant inflammatory signals [18]. Thus, in GBM, DAMP signaling frequently becomes tolerogenic rather than immunogenic, converting radiation-induced injury into a chronic, self-reinforcing program of immune suppression [22].

The published data argue that ICD should not be conceptualized as a binary switch but rather as a continuum. At one end, coordinated, transient DAMP release promotes effective dendritic-cell activation and cytotoxic T-cell recruitment; at the other, sustained or excessive DAMP signaling drives myeloid suppression, checkpoint induction, vascular remodeling, and radiotherapy resistance [10,28]. Understanding this spectrum—and the conditions that shift DAMP signaling from immunity to tolerance—is essential for designing biomarker-guided strategies and rational combinations capable of converting radiation-induced damage into durable antitumor immunity [28].

5. Measuring DAMPs: From Biological Readouts to Actionable Nanotheranostics

The translational relevance of DAMP biology depends on whether these signals can be reliably quantified, monitored longitudinally, and integrated with therapeutic decision-making. Over the past decade, multiple platforms have matured to the point where DAMP assessment is technically feasible and increasingly suitable for clinical research [29]. Rather than focusing on methodological detail, the key question is how each compartment informs treatment biology and how such information could be linked to image-guided and nanotechnology-enabled interventions.

Canonical DAMPs such as HMGB1, extracellular ATP/adenosine axis components, ecto-CRT signatures, and extracellular DNA are not tumor-specific molecules; they are endogenous signals released by stressed or dying cells and therefore can originate from both tumor and normal tissues. Nevertheless, DAMPs can serve as informative liquid-biopsy biomarkers because they report on the biological intensity and temporal dynamics of tissue damage, immunogenic cell death programs, and downstream inflammation during therapy, as supported by translational analyses of radiotherapy-induced ICD markers by Vaes et al. [19]. In practice, the utility of DAMPs is strengthened by longitudinal sampling (baseline-to-on-treatment trajectories), integration with tumor-enriched readouts (e.g., circulating tumor DNA features or extracellular vesicle cargo), and interpretation within a clinical context (dose/fractionation, steroids, infection, and surgery). Importantly, DAMPs can be released and detected even in the absence of radiotherapy due to spontaneous tumor necrosis, hypoxia-driven cell stress, or other treatments; therefore, their biomarker value is primarily dynamic and contextual rather than purely diagnostic in isolation.

5.1. Blood-Based Assays

Plasma HMGB1, circulating cfDNA/mtDNA, ecto-CRT-related signatures, and interferon-stimulated gene panels can be obtained repeatedly during therapy. Clasen et al. [8], Yoshimoto et al. [17], and Han et al. [25] have shown that serial sampling studies in solid tumors demonstrate that these markers change dynamically over the course of radiotherapy and correlate with tumor burden, inflammation, and, in some settings, outcome [10,18,22]. Their minimally invasive nature makes them attractive for biologically guided treatment adaptation, particularly when combined with other systemic biomarkers.

5.2. Cerebrospinal Fluid

For brain tumors, CSF provides a compartment that is biologically closer to the tumor microenvironment than peripheral blood. DAMP-related nucleic acids and proteins detected in CSF show greater specificity for intracranial pathology and may capture microenvironmental remodeling that is not visible on structural imaging. Although still underexplored for DAMP biology specifically, CSF profiling is poised to become a high-value matrix for response monitoring in glioblastoma [29].

5.3. Extracellular Vesicles (EVs)

EVs package DAMPs, danger-associated peptides, nucleic acids, and immunomodulatory mediators released after irradiation. Their stability and ability to reflect tumor–immune crosstalk make EV-associated DAMPs promising candidates for liquid-biopsy theranostics, potentially linking biological activity with both therapeutic delivery and monitoring strategies demonstrated in glioblastoma biomarker studies by Wang-Bishop et al. [30].

5.4. Imaging Correlates

Advanced MRI and PET tracers can visualize inflammation, perfusion, hypoxia, and innate immune activation—processes closely intertwined with DAMP signaling. The development of probes targeting ICD-associated pathways (e.g., STING activation or macrophage/microglial dynamics) creates opportunities to co-register molecular DAMP activity with functional imaging, moving toward biologically informed, image-adaptive radiotherapy [31].

These approaches transform DAMPs from purely mechanistic phenomena into measurable, clinically interpretable signals [32]. Importantly, they provide the informational backbone for nanotheranostic strategies: Nanosystems can be engineered to respond to DAMP-associated cues, co-deliver radiosensitizers or immunomodulators, and simultaneously report biological effects through imaging, as demonstrated in preclinical nanotheranostic studies in glioblastoma models by Onishi et al. [33]. In this emerging paradigm, radiotherapy induces damage; DAMPs quantify the biological consequence; nanotheranostic platforms close the loop by enabling tailored intervention and monitoring. This integration offers a realistic pathway to truly personalized, DAMP-guided radiotherapy in glioblastoma and head-and-neck cancers [31,32].

5.5. Tumor-Specific DAMPs

DAMPs are endogenous danger molecules shared across cell types and released during stress or regulated cell death; thus, canonical DAMPs (HMGB1, ATP, ecto-CRT signatures, heat-shock proteins, and extracellular DNA) are not unique to tumor tissue. What can be tumor-specific or tumor-enriched in liquid biopsy is the source context and the molecular co-signature accompanying DAMP release. In glioblastoma, tumor-enriched components may include circulating tumor DNA features (tumor-specific mutations, copy-number patterns, and methylation signatures), tumor-derived extracellular vesicle cargo, and nucleic-acid fragments (including mitochondrial DNA variants) that more directly reflect malignant origin. Therefore, the most clinically informative strategy is not to search for unique tumor-only DAMP species but to interpret canonical DAMP dynamics together with tumor-enriched molecular readouts and immune-context markers (e.g., adenosine-axis activity, myeloid polarization signatures), which together can increase specificity for tumor biology and treatment response [31].

6. From Measurement to Nanotheranostic Design

The ability to quantify DAMPs in blood, cerebrospinal fluid, extracellular vesicles, and through molecular imaging provides more than descriptive biological information; it offers actionable guidance for therapeutic development. Patterns of HMGB1 release, CRT exposure, cfDNA dynamics, and interferon-related signatures have been collectively shown to reflect whether radiotherapy is inducing immunogenic cell death or a more tolerogenic inflammatory state, as supported by studies such as Chen et al. and Wang-Bishop et al. [30,32]. When these circulating and imaging-based readouts are integrated longitudinally, they support the development of a biologically anchored framework capable of informing intervention timing, dose scheduling, and the rational selection of combination strategies [34].

Within this context, nanotheranostic platforms may be designed not only as carriers of therapeutic payloads but as responsive systems engineered around measurable biological cues. Tumor hypoxia, redox status, and inflammation—all processes linked to DAMP signaling—are already used to trigger the release of radiosensitizers, immunomodulatory compounds, or DNA-damage amplifiers in preclinical glioma models [7,10,22,28]. Incorporating imaging components into these platforms enables simultaneous visualization of nanoparticle distribution and correlation with DAMP-derived biomarkers, thereby linking delivery, biological effect, and outcome within the same system.

Such alignment between measurable DAMP biology and bioresponsive nanosystems establishes a closed diagnostic–therapeutic loop: Radiotherapy initiates damage signaling; DAMP assays and molecular imaging quantify the resulting immune and microenvironmental response; nanotheranostic agents are deployed or adjusted according to these signals [31]. This iterative, data-driven model represents a realistic pathway by which biological monitoring can be translated into precision radiotherapy strategies, particularly in tumors like glioblastoma, where conventional imaging alone provides limited insight into treatment biology [22,33].

7. Conclusions

DAMPs released during radiotherapy represent an important biological interface between tumor cell damage and the immune system [3]. Radiation-induced immunogenic cell death is characterized by the exposure and release of molecules such as CRT, HMGB1, ATP, and extracellular DNA, which have been shown to activate dendritic cells and trigger adaptive immune responses through pattern-recognition receptors and type I interferon pathways, as reported in experimental and translational studies [10]. These responses underpin the emerging understanding of RT not merely as a cytotoxic modality but as a therapy that has been shown to modify the tumor microenvironment and systemic immunity, thereby potentially enhancing antitumor effects beyond direct DNA damage, as supported by studies such as Wang-Bishop et al. [30]. These responses underpin the emerging understanding of RT as not merely a cytotoxic modality but as a therapy that modifies the tumor microenvironment and systemic immunity [32].

A major translational implication of DAMP biology is their potential role as biomarkers of treatment response [7,28]. Serial measurement of DAMPs and downstream signals could provide real-time insight into whether RT is eliciting effective ICD within an individual patient [10]. For example, studies have identified HMGB1, CRT exposure, and interferon signatures as correlates of immunogenic responses in irradiated tumors, suggesting that such markers might predict which patients mount meaningful antitumor immunity following RT [1,6]. Because these signals may change dynamically over the course of treatment, monitoring DAMPs could inform adaptive strategies, such as interplay with immunotherapy or modification of dose/fractionation [7].

Moreover, DAMP levels might reflect intrinsic tumor radiosensitivity [10]. Tumors that fail to generate robust DAMP signals after irradiation may be less likely to engage immune effector mechanisms, contributing to resistance [24]. While genomic radiosensitivity indices such as RSI and GARD integrate gene expression patterns to predict intrinsic radiosensitivity [21], DAMP profiling offers a complementary, functionally grounded approach that directly measures the immunogenic consequences of irradiation at the cellular level.

Critically, the impact of DAMPs is context-dependent. Excessive or persistent DAMP release can drive chronic inflammation and immune suppression, recruiting myeloid-derived suppressor cells and regulatory networks that blunt antitumor immunity and promote tumor regrowth [6]. This dualism underscores the need not only to measure DAMPs but to interpret their temporal patterns and biological context.

From a clinical personalization standpoint, integrating DAMP biomarkers into RT planning could enable stratification of patients according to their baseline and early treatment immunobiology [29,31]. For example, a TME with pre-existing immunogenic signaling might benefit from conventional dose schedules combined with immune checkpoint inhibitors, whereas a poorly immunogenic TME might require strategies to enhance ICD, such as high-LET particles or immune modulators [25]. Similarly, DAMP signatures could inform decisions about combining RT with targeted agents or nanomedicines designed to modify the TME, as supported by radiobiological studies such as Onishi et al. [33].

Our insights show that in glioblastoma, radiotherapy-induced DAMP signaling emerges as a decisive but inherently ambivalent determinant of treatment outcome. While acute DAMP release can transiently convert irradiated tumors into immunologically active sites, the dominant trajectory in GBM is frequently one of rapid immune adaptation, characterized by myeloid reprogramming, adenosine accumulation, and checkpoint reinforcement that ultimately favors immune escape and radioresistance. These dynamics underscore that DAMPs should not be viewed as static indicators of immunogenicity, but as temporally and contextually regulated signals for which their biological meaning depends on dose, modality, tumor state, and host immunity, as supported by studies such as Wang-Bishop et al. [30]. Future progress in GBM radiotherapy will therefore require integrating longitudinal DAMP profiling with immune and molecular stratification in prospective clinical studies [35]. Such an approach has the potential to transform DAMPs from descriptive correlates into actionable biomarkers, enabling rational personalization of radiotherapy and its combinations and offering a pathway toward overcoming the long-standing immunological barriers that define GBM.

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