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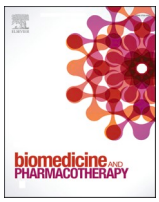

Review

Nanotechnology meets radiobiology: Fullerenols and Metallofullerenols as nano-shields in radiotherapy

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ABSTRACT

Despite significant advances in the development of radioprotective measures, the clinical application of radioprotectors and radiomitigators remains limited due to insufficient efficacy and high toxicity of most agents. Additionally, in oncological radiotherapy, these compounds may interfere with the therapeutic effectiveness. Recent progress in nanotechnology highlights fullerenols (FulOHs) and metallofullerenols (Me@FulOHs) as promising candidates for next-generation radioprotectors. These nanostructures possess unique antioxidant properties, demonstrating greater efficacy in rediucing oxidative stress compared to conventional agents. Moreover, their potential to minimize pro-oxidative risks depends on the precise identification of cellular environments and irradiation conditions that optimize their radioprotective effects. In parallel, Me@FulOHs serve as powerful theranostic tools in oncology. Their strong imaging signals enable high-resolution PET and MRI, facilitating early detection and accurate localization of pathogenic alterations. This dual functionality positions Me@FulOHs as key components in advanced radiotherapy. By integrating these nanomaterials with modern theranostic approaches, it is possible to enhance the precision of treatment while minimizing side effects, addressing a critical need in contemporary oncology. This review emphasizes the importance of systematic evaluation of context-dependent effects of Me@FulOHs, particularly in pre- and post-irradiation scenarios, to optimize their clinical relevance. The dual role of Me@FulOHs as both radioprotectors and diagnostic agents distinguishes them from traditional compounds, paving the way for innovative practical applications. Their use in radiotherapy represents a significant step toward the development of safer and more effective strategies in radiation protection and cancer treatment. We also review ionizing radiation effects, classifications, cancer radiotherapy applications, and countermeasures.

1. Introduction

The main components of the cell are proteins and water (proteins make up about 20 % and water about 70 % of the total mass of a mammalian cell). Ionizing radiation (IR) primarily triggers chemical reactions within these cellular components and DNA. In the first stage, water is mainly ionized, resulting in the formation of: hydroxyl radicals ($HO^{\bullet}$), hydrated electron (e^{-}_{aq}), hydrogen atom ($H^{\bullet}$), and H_2O_2 molecules [1]. These products, referred to as primary water radiolysis products, react mainly with nucleic acids, and also with proteins, lipids, and other biomolecules, leading to cell damage and metabolic disorders and can also induce cell death. Due to the huge variety of molecules present in the cell, other subsequent radical reactions are possible, *e.g.*, primary products of water radiolysis react with proteins to form protein radicals,

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which in turn react with nucleic acids leading to their damage [1–3]. They can also entrain high-molecular protein aggregates, such as cross-linked proteins or lipid-protein complexes [4–6]. The foundation of modern oncoradiotherapy lies in the harmful consequences of IR, which induces oxidative stress and DNA damage within cancer cells [7]. Besides tumor cells, during radiation therapy, a significant portion of normal cells are also exposed to radiation and consequently damaged. Acute effects of radiation can be observed immediately or shortly after exposition, while others may manifest over weeks, months, or even years later. Common side effects, which typically diminish over time, include fatigue, weakness, loss of appetite, vomiting, and radiation-induced complications [8,9]. Ideal radioprotective compounds should scavenge free radicals reducing oxidative stress in healthy cells near cancer cells undergoing radiotherapy.

Studies have suggested that certain molecules, such as polyphenols, ascorbate, melatonin, and others can selectively act as radiosensitizers for cancer cells while providing radioprotective benefits to normal tissues [10–12]. However, the limitations of existing radioprotective agents underscore the need for novel compounds that are both effective and free from undesirable side effects [13–16].

In recent years, nanomaterial technology has emerged as a promising approach, with nano-radioprotectants/radiosensitizers demonstrating high efficacy, low toxicity, and prolonged blood retention [17,18]. In 2023, Guo et al. [19] published a systematic review on nano-radioprotectors and discussed various types of nanomaterials aimed at protecting against radiation, with a conclusion that the development of nano-radioprotectors will greatly benefit from an interdisciplinary approach to fully realize the potential of these innovative materials. The development of nano-radioprotectors is of critical importance not only in protecting healthy cells during radiotherapy but also in preventing healthy cells damage in situations involving uncontrolled radiation exposure, such as radiation accidents or exposure to radiation sources due to industrial or military disasters or spaceflights [20]. Nano-radioprotectors, due to their unique ability to adapt to the cellular microenvironment, offer the potential to protect healthy cells against various types of IR-induced damage, including DNA-level injuries, which can lead to the development of cancers, or other radiation-related diseases [21,22]. Fullerenes are a relatively new group of compounds and represent a class of sphere-shaped molecules made exclusively of carbon atoms. Fullerenes are hydrophobic molecules; therefore, potential biomedical applications are restricted by their extremely poor solubility in water and polar solvents [23]. The chemical modification of fullerene C_{60} molecule by the attachment of hydroxy groups is an easy and straightforward method to synthesize water-soluble fullerenes, namely fullerenols ($C_{60}(OH)_n$, n = 2–48; FulOHs) [24]. Notably, FulOHs have gained significant attention for their ability to generate and scavenge reactive oxygen species (ROS) [23,25]. Metallofullerenols (Me@FulOHs) represent another promising class of compounds in radiobiology, with their radioprotective function dependent on factors, such as the type of metal introduced into the carbon cage, the number and distribution of -OH groups on the surface, particle size, preparation method, and compound concentration [26]. Understanding the mechanisms of action of FulOHs and Me@FulOHs in various experimental systems is crucial to recognizing conditions where their protective properties prevail and where pro-oxidative properties may manifest. The differential behavior of fullerene water-soluble derivatives in normal *vs.* cancer cells holds promise for their application in radiotherapy.

In our recent paper, we discussed the biomedical applications of Me@FulOHs [26]. To broaden this aspect, herein we address the novel question of whether these nanocompounds could serve as a promising alternative for protecting healthy cells during radiotherapy. By creatively integrating nanotechnology with radiological protection, we aim to open the door to novel solutions not only in cancer treatment but also in broader aspects of nuclear safety [17]. This review aims to thoroughly explore the potential of FulOHs and Me@FulOHs as new radioprotective agents, offering effective protection for healthy tissues from the harmful effects of radiation while minimizing the side effects associated with current protective therapies.

2. Understanding radiation dose effects: biological responses and biophysical considerations

The interaction of IR with living matter depends on many factors: absorbed dose, dose rate, radiation quality factor (Q), as well as the type and properties of irradiated objects and their surrounding environment. The biological effects induced by IR are proportional to the absorbed dose. As the dose of IR increases, oxidative damage to cells intensifies, leading to damage to proteins, lipids, and DNA. Higher radiation doses exceed the cell's defense capabilities, potentially causing cell death and increasing the risk of biological damage [27]. An important key element influencing the effect of IR on a biological system is the dose rate. Post-radiation damage to the plasma membrane manifests in the oxidation of lipids and proteins, exhibiting an inverse dose-rate effect (IDRE), defined as an increase in the degree of peroxidation at a constant radiation dose and a decrease in dose rate. The final products of lipid and protein oxidation are formed more efficiently after irradiation of biological membranes or entire cells with low dose rates compared to irradiation with high dose rates. This phenomenon is associated with the indirect effect of the activity of lipid and protein peroxidation products. The direct effect of free radical activity is characterized by increased damage with increasing dose rate [28–30].

Another important factor determining the impact of IR on biological systems is the radiation quality factor. This dimensionless factor represents the Relative Biological Effectiveness (RBE) of high-LET radiation in comparison to low-LET radiation at low exposure levels. RBE is a measure that defines the ability of different types of radiation to produce biological effects at the same absorbed dose. It is used in radiobiology to compare the biological impacts of various types of IR, such as alpha, beta, gamma rays, and neutrons [31]. RBE increases along with the rise in the radiation quality factor for DNA damage; however, for cell membrane damage, low-quality radiation proved to be more effective [32]. The radiation quality factor measures the number of ionizations induced in a medium per unit distance traveled by the radiation. Alpha particles and protons cause enhanced ionization, and both are examples of a high LET radiation $>$ 40 keV/μm. On the other hand, gamma rays, X-rays, and beta particles, which cause weak ionization, are forms of radiation with low linear energy transfer (LET), typically less than 2 keV/μm [29,33]. The mechanisms of damage development vary depending on the radiation quality factor. High LET radiation directly damages macromolecules, while most cellular damages induced by low LET radiation are attributed to oxidative stress induced by free radicals formed during the radiolysis of water [32].

To sum up, it should be emphasized that IR-induced alterations in living cells and organisms depend on many physical factors and conditions. These key aspects including the absorbed dose, dose rate, radiation quality, and the chemical properties of the environment in which irradiation occurs, are essential for accurately assessing the post-radiation effect.

3. Living matter and IR: general biochemical effects

The biochemical effects of IR on living matter depend on the radiation source, linear energy transfer (LET) of the radiation, amount of radiation energy absorbed (radiation dosage), and, importantly, the genetic and epigenetic makeup of the exposed individual [6,34]. Health effects from radiation doses can be grouped into two categories: deterministic and stochastic [35]. Deterministic effects occur when radiation doses exceed a specific threshold and become more severe as the dose increases. Typical for such kinds of effects is dose-related severity, predictability, reproducibility both in animal and human studies, tissue-specific damage, and short-term manifestation. Examples of

deterministic effects include a variety of cellular and tissue reactions such as skin burns, necrosis, acute radiation syndromes (described later in detail), cataracts, and radiation sickness [35,36]. In contrast, stochastic effects are random, have no threshold dose, are not severity-dependent on dose, and often involve a long latency period. Radiation consequences are probabilistic, but they may result in malignancies such as cancer and hereditable changes [35,36].

IR induces cellular damage through direct and indirect mechanisms. The interaction of IR with the DNA has for many years been considered the primary mechanism responsible for the genotoxic effects of radiation (Fig. 1) [6].

High-energy radiation can directly ionize and break chemical bonds, leading to significant DNA structural damage, including single-strand breaks (SSBs), double-strand breaks (DSBs), nucleotide base damage, glycosyl damage, and DNA cross-linking [38,39]. DSBs are most importantly involved in IR-induced damages, and in fact, less than two unrepaired DSBs are sufficient to cause cell death in humans [40]. Indirectly, IR interacts with water molecules, producing reactive oxygen species (ROS) such as $HO^{\bullet}$, hydrogen peroxide (H_2O_2), and superoxide anions ($O_2^{\bullet -}$). The overall amount of ROS generated from primary ionization events is further propagated *via* the intracellular activation of endogenous ROS-producing systems such as nicotinamide adenine dinucleotide phosphate and the mitochondrial electron transport chain [41]. Irradiation (2–50 Gy) also increases the expression of inducible nitric oxide synthase (iNOS), leading to a dose-dependent increase in nitric oxide (NO) levels along with the increased occurrence of nitrated tyrosine residues *in vivo* [42]. Additionally, ROS can react with NO to form other reactive nitrogen species (RNS), which enhance oxidative/-nitrosative stress and the inflammatory response contributing further to cellular injury. NO and proinflammatory cytokines are implicated in the bystander/abscopal effects—where unirradiated cells exhibit damage due to signaling from irradiated neighbors [43,44]. Thus, the interplay of oxidative and nitrosative stress and inflammation enhances the overall detrimental impact of IR on cellular integrity and function [45].

The first reaction to DNA damage caused by IR, or induced by ROS and RNS, is the activation of the cell cycle checkpoint system. This is a highly regulated network of sensors, transducers, and effector proteins that detect DNA damage and trigger a protective mechanism known as the DNA damage response (DDR) pathway [6]. This involves checkpoint proteins that halt the cell cycle, allowing time for repair. Transducer proteins then amplify the signal, recruiting DNA repair enzymes to the damaged sites. Effector proteins carry out the repair processes, restoring DNA integrity and preventing mutations or cell death [40]. Cells activate several DDR pathways, such as non-homologous end joining (NHEJ) and homologous recombination, to rectify this damage. Efficient DNA repair is crucial for maintaining genomic integrity and preventing cell death. If DNA damages are not repaired, they can lead to mitotic or apoptotic cell death or mutations that can affect somatic or germinal cells, increasing the cancer risk and risk of teratogenicity for the offspring, respectively [46–49]. In addition to DNA repair mechanisms, cells rely on antioxidant defenses, including enzymes like superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), to neutralize ROS and limit oxidative damage [49–53]. These antioxidant systems are essential for reducing the harmful effects of IR on cellular components beyond DNA.

Due to the immense diversity of molecules present in the cell, other subsequent radical reactions with proteins are possible, forming secondary protein radicals, which in turn react with nucleic acids, causing their damage [2]. Proteins exposed to the hydroxyl radical ($HO^{\bullet}$) or the combination of $HO^{\bullet}$ plus the superoxide radical and oxygen ($HO^{\bullet} + O_2^{\bullet -} + O_2$) exhibit changes in their primary structure and become more susceptible to proteolytic degradation. Negative changes in the secondary and tertiary structure of proteins are caused by the modification of amino acid residues, alterations in the overall charge, and phenomena such as aggregation and fragmentation. It has been suggested that the denaturation and increased hydrophobicity of proteins make irradiated

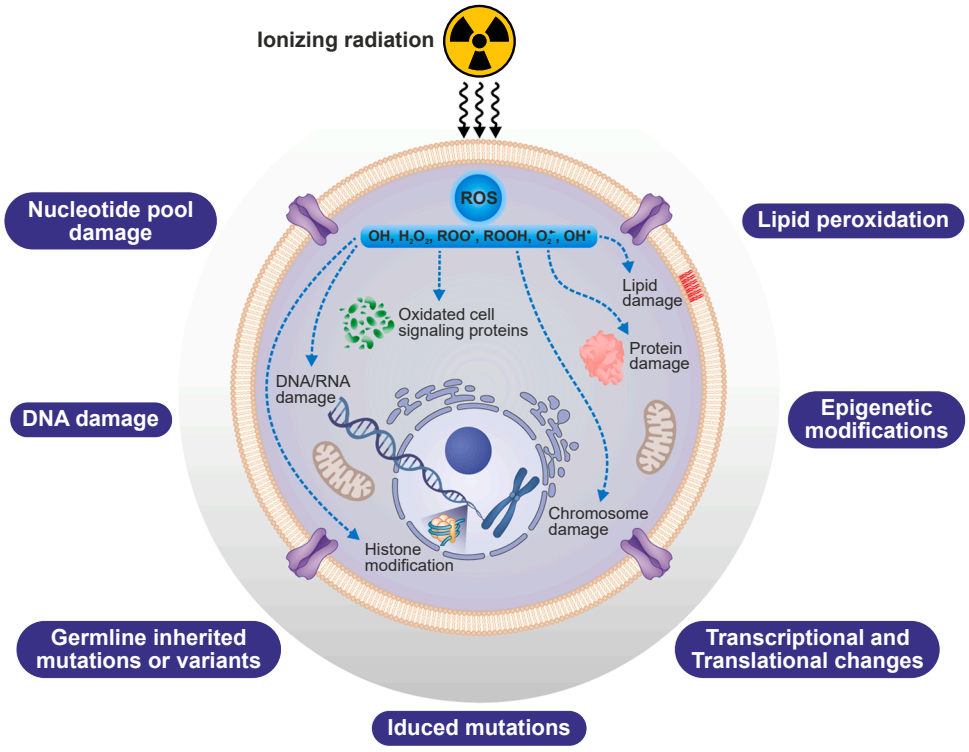

Fig. 1. Radiation-induced damage in cellular components and classification of radiation biomarkers. The illustration depicts various types of cellular damage induced by radiation, including DNA damage (*e.g.*, single/double-strand breaks), protein damage (*e.g.*, oxidative modifications), and lipid damage (*e.g.*, lipid peroxidation). Different biomarkers are used to assess the extent and type of cellular damage caused by radiation, which is significant in diagnostics and therapy. Adapted from a book chapter by Ainsbury et al. [37] and used under CC BY 4.0.

proteins significantly more susceptible to degradation by proteolytic enzymes compared to non-irradiated proteins [54]. A comprehensive understanding of the primary reactions of water radiolysis products and secondary organic radicals with biomolecules not only provides better insight into the IR effects on organisms but also reveals the mechanisms underlying the bioactivity of free radicals.

4. The use of radiotherapy for cancer treatment

In cancer treatment, the primary goal of radiotherapy is to eradicate cancer cells while minimizing damage to healthy cells [55]. The DNA damages induced by radiotherapy can result in the arrest of cancer cell proliferation or even cell death through mechanisms such as apoptosis, necrosis, and senescence. Damage to several organelles including the cell membrane, endoplasmic reticulum, lysosome, and mitochondria has been also implicated in the effects of radiotherapy-induced tumor cell death [56]. Radiotherapy can contribute to tumor vasculature remodeling increasing chemotherapy distribution and efficacy [57]. In addition, radiotherapy can be applied as palliative care to alleviate pain associated with advancing disease [58]. In radiotherapy, techniques enhancing the radiosensitivity of abnormal cells are often employed. Oxygen levels inside cancerous tumors are typically lower compared to peripheral areas, leading to reduced sensitivity to radiation. Fractionated radiation allows for some recovery of the tumor's blood supply between treatment sessions. This recovery leads to better oxygen delivery in subsequent doses, which in turn can increase the sensitivity of tumor cells to radiation and improve immune response [59]. Radiosensitizers are chemicals or pharmaceutical agents that make cancer cells more sensitive to radiation therapy, trying not affect normal cells [55]. Some examples of radiosensitizers that have already been incorporated into clinical practice are: cisplatin, 5-fluorouracil, tirapazamine nimorazole, and poly-ADP ribose polymerase (PARP) inhibitors [56,60]. Several phytochemicals such as paclitaxel, curcumin, genistein, and papaverine are being assayed as radiosensitizers in clinical assays [61]. However, clinical applications of natural products in radiotherapy are scarce, which may be related to their low bioavailability.

Conventional (60–400 keV) and megavolt (1.25–25 MeV) radiation therapy are distinguished based on the energy utilized [62,63]. Conventional radiotherapy, employed for treating skin cancer, utilizes only low-penetrating X-rays. In contrast, megavolt radiotherapy utilizes gamma radiation produced by the ^{60}Co isotope (at 1.17 and 1.33 MeV energy lines), high-energy X-rays generated through linear acceleration (ranging from 4 to 25 MeV), and electrons (ranging from 6 to 22 MeV). Two fundamental types of radiotherapy can be distinguished based on the irradiation method: brachytherapy (BTH), which involves treatment using a radiation source in direct contact with the tumor, and teleradiotherapy (RTH), where the source is positioned at a certain distance from the tumor [55,64]. One of the latest advancements is so-called intensity modulated radiotherapy, offering the capability to adjust the dose distribution to the shape of the irradiated area. Nevertheless, despite employing these advanced techniques, certain side effects become inevitable, such as damage to normal tissues located within the irradiated area, particularly skin fibroblasts that are mostly exposed. Often, these side effects entail a compromise between the dose offering the highest probability of tumor eradication and the dose posing the lowest risk for normal tissues. Such dilemmas must be considered and decided upon based on data, such as that presented in Fig. 2 [65]. The relationship curves between tumor control probability (TCP) and normal tissue complication probability (NTCP) should be empirically determined through retrospective studies. The distance observed for these two curves indicates the therapeutic index, which can be enhanced by shifting the TCP or NTCP curve. Increasing the dose per fraction raises the probability of toxic effects in late-responding normal tissues, which are more sensitive to fractionation than early responding tissues.

As these curves closely converge, the complete avoidance of complications in normal tissues through radiotherapy becomes infeasible

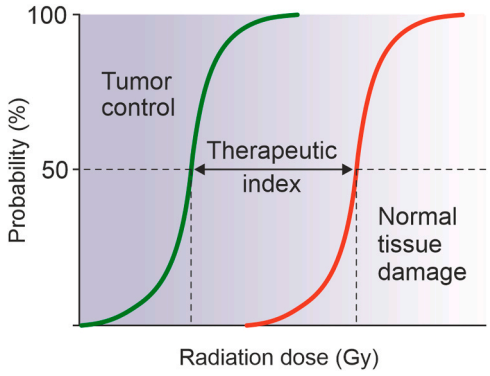

Fig. 2. Sigmoid curves represent the probability (%) of tumor control (green curve) and the probability (%) of normal tissue damage (red curve). The dashed lines indicate values of 60 % TCP and 5 % NTCP for a specific dose. These curves are crucial in radiotherapy for assessing the effectiveness of cancer treatment and the risk of complications in healthy tissues, enabling the adjustment of therapeutic strategies to maximize tumor control while minimizing side effects. Adapted from a book chapter by Sminia et al. [9] and used under CC BY 4.0.

(Fig. 2). Therefore, achieving therapeutic effects inevitably involves the risk of certain complications. The side effects of irradiation vary depending on tissue sensitivity, the area irradiated, and the absorbed radiation dose. Bone marrow, lymphatic tissue, reproductive cells, and intestinal epithelial cells are among the most sensitive to radiation [62]. Muscle cells, parenchymal organs like the liver, nervous tissue, and connective tissue exhibit lower sensitivity [66].

5. Classification of IR Induced Effects

The majority of the IR-evoked biological effects in cells, tissues, organs, and systems can be assigned to oxidative damage of crucial biomolecules, however, direct damage also occurs [34,67,68]. Among factors determining the effect of IR, the dose, time of exposure, and the type of targeted cells are considered critical (Fig. 3) [69]. Rapidly dividing cells (such as hematopoietic, reproductive, and intestinal stem cells) are more sensitive to radiation than their differentiated counterparts or other differentiated cells [70]. Consequently, acute radiation syndrome (ARS) typically manifests in hematopoietic, gastrointestinal, and neurovascular subsyndromes normally preceded by non-specific prodromal symptoms [71,72].

Classically, the threshold dose for ARS is a whole-body or significant partial-body irradiation of greater than 1 Gy delivered at a relatively high dose rate. Doses less than 0.5 Gy are not expected to cause acute symptoms, whereas doses of 4.5 Gy are lethal to 50 % of exposed persons [73]. Patients exposed to at least 0.7 Gy can develop hematopoietic syndrome (H-ARS) characterized initially by lymphopenia and immunodeficiency or pancytopenia (at higher doses) associated with an increased risk of infection, hemorrhage, and anemia [71]. Exposition to a dose over 10 Gy, causes endothelial and epithelial cell dysfunction and increased vascular permeability involved in the development of gastrointestinal syndrome (GI-ARS) in addition to bone marrow damage. GI-ARS symptoms include vomiting, diarrhea, electrolyte imbalance, and dehydration, which is often lethal, especially at higher exposure levels [74]. A cardiovascular/central nervous system syndrome (CNS-ARS) will develop in addition to the damage to the hematopoietic and GI systems when patients are exposed to whole-body radiation over 20 Gy. Individuals with this level of injury die within a few days, and no treatment options are currently available [75]. Cutaneous radiation syndrome characterized by erythema, dry and moist desquamation, ulcerative lesions, or even tissue necrosis is the fourth sub-syndrome of ARS [76].

Many survivals of ARS often develop delayed effects of acute

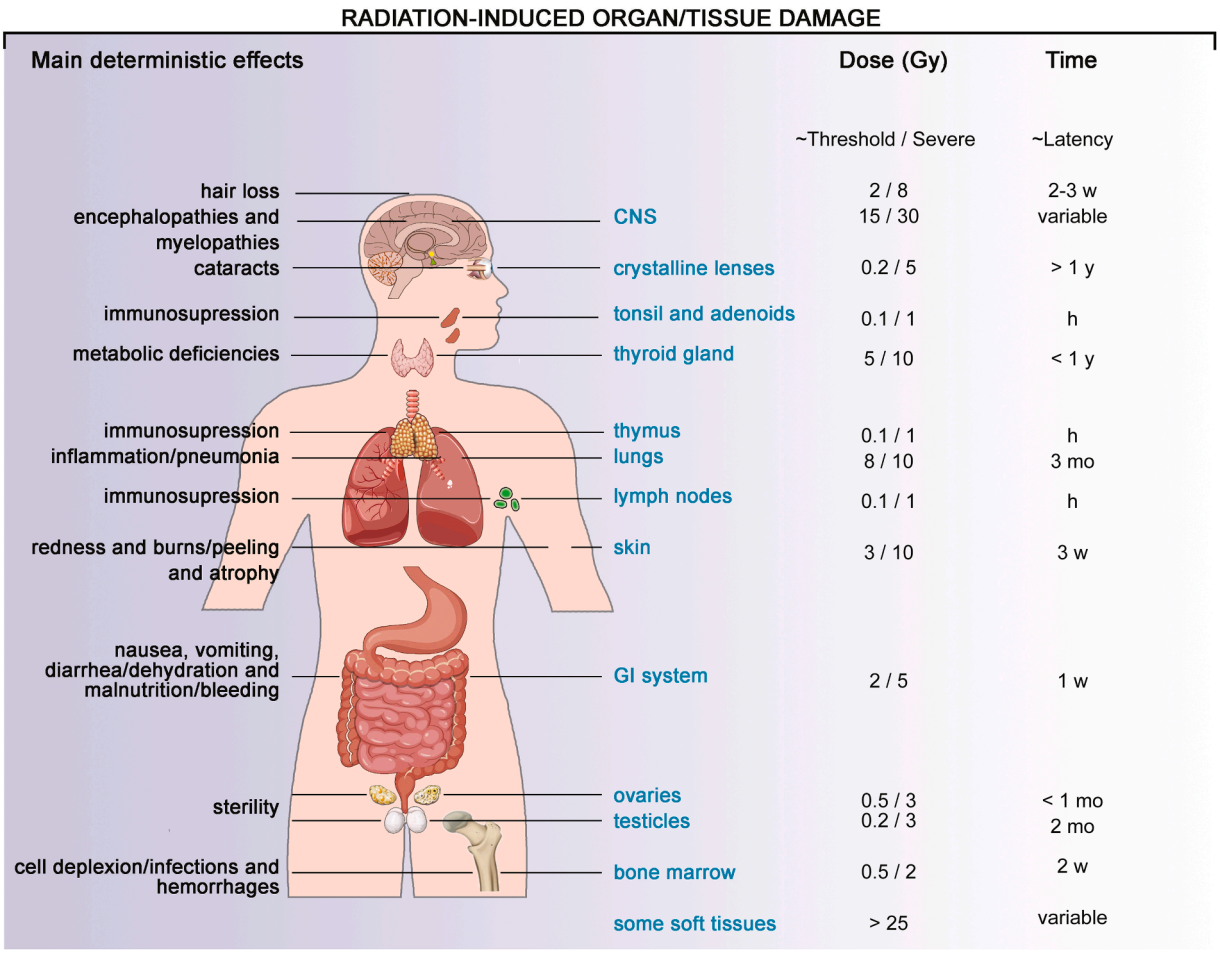

Fig. 3. General biological effects induced by ionizing radiation, highlighting the relationship between dose and exposure time. Biological effects are classified according to the threshold dose (the minimum dose causing noticeable effects) and the dose leading to serious damage. Adapted and refined from review [69] and used under CC BY 4.0.

radiation exposure (DEARE) that are largely associated with chronic inflammation and may display a multi-organ disease syndrome with a shortened life span [77]. DEARE encompasses a diverse spectrum of pathologies observed in survivors exposed to high doses of IR, which includes: cancer (*e.g.*, leukemia, thyroid, breast, and lung cancer), cardiovascular diseases (*e.g.*, atherosclerosis and heart diseases), pulmonary diseases (*e.g.*, fibrosis, pneumonitis), cataracts (due to radiation damage to the eyes), gastrointestinal complications (*e.g.*, malabsorption, dysmotility, peptic ulcers), bone marrow suppression (leading to anemia, immune deficiencies) and neurological disorders (*e.g.*, cognitive decline, neurodegenerative changes) [78–82].

Chronic Radiation Syndrome (CRS) is a condition that arises from long-term exposure to low but repeated doses of IR, typically in the range under 1 Gy. It primarily affects individuals in environments with sustained radiation exposure, such as nuclear workers, and is frequently developed over months or years. Unlike ARS, CRS develops gradually, and its progression is tied to cumulative radiation exposure. Initial CRS symptoms are nonspecific and can be reversible if there is a break in radiation exposure. If exposure continues, the initial symptoms (granulocytopenia, thrombocytopenia, and moderate anemia) grow progressively worse, and others may appear (olfactory and vestibular excitability decline, taste fatigue, tendency to hypotension, asthenic syndrome, *etc.*) [83]. Deterioration of morphology (fibrosis, hypoplasia, atherosclerosis, *etc.*) and functional activity of tissues and organs is latter evidenced (cardiovascular insufficiency, pulmonary pathologies, increased cancer risk), and used to be complicated by infections of respiratory and digestive systems. Causes of death in the late period of CRS are sepsis and hemorrhages resulting from inhibition of hematopoiesis and immunity, malignant solid tumors, and especially leukemia [84]. In most cases it seems that as the dose exposure increases, the threshold of absorbed dose for the development of clinical manifestations decreases [85,86]. In many articles CRS manifestations are considered as part of DEARE complications but this association is incorrect because mechanisms involved in DARE and CRS are significantly different [84,87].

The exposure of organisms to IR evokes a complex cascade of damaging reactions and induces cellular responses at different molecular levels [88,89]. Typically, these reactions include an arrest of the cell cycle, repair of DNA damage, or activation of the apoptotic response. At a level of tissues or organs, post-radiation changes involve acute or chronic inflammation, proliferative response, tissue remodeling, or necrosis. For that reason, more detailed classifications of IR effects (*i.e.*, Table 1) have also been proposed [90].

6. Medical IR countermeasures: radioprotection, radiomitigation and therapeutic interventions

The development of new radioprotective strategies is crucial due to the increasing exposure to IR from medical procedures, nuclear accidents or terrorism attacks, and environmental sources. In recent decades, several compounds, including natural [100–102], recombinant, and synthetic [16] substances have been tested for their radioprotective effect. However, many of these attempts have failed, mainly due to unsatisfactory efficiency or high toxicity in animal/clinical trials. It is assumed that an ideal radioprotector should meet a few basic criteria, *i.*

Table 1
Classification of effects caused by IR, dependently on the time of occurrence and type of tissue [90].

Effects of exposure to IR	Time of occurrence	Impact on cells/tissues/ organs	Ref.
Type I- (early) acute radiation reaction	Hours to weeks	Hematopoietic, gastrointestinal, and neurovascular syndromes. Programmed/necrotic death of rapidly proliferating cells, accompanied by a proliferative response of stem cells to recover cell population.	[71, 91–93]
Type II- early radiation reaction	Weeks to months	Cellular/tissue dysfunction associated with an inflammatory response at different levels: activation of genes responsible for the inflammatory post-radiation response, production of cytokines and growth factors, increase of vascular permeability, enhancement of macrophage recruitment, angiogenesis, inhibition of apoptosis/dysfunction of the connective tissue stromal pool.	[45,94]
Type III- late radiation reaction	6 months to even 2–3 years	Swelling, fibrosis, and tissue disorganization, including permanent organ dysfunction. In the case of extensive damage to connective tissues (fibroblasts and vessels), edema, interstitial, arteriocapillary, and septal fibrosis, telangiectasia, and other pathological changes.	[95–97]
Type IV- stochastic effects	Years	Genome mutations in irradiated somatic cells, including their accumulative effects and ability to pass to offspring. Development of hematological malignancies or solid tumors. Genotoxicity of radiation on germ cells.	[35,98]
Type V- bystander/ abscopal effects	Accompanying early and late effects	Biological effects in cells that have not been directly exposed to IR but are in close proximity to cells irradiated. ROS, RNS, and pro-inflammatory cytokines (*i.e.*, TGFβ, IL6, TNFα), epigenetic modulators are involved in mediating the processes leading to DNA damage leading to cancer development.	[44,99]

e., provide effective protection against the damaging action of radiation, have a protective effect on most organs, have an appropriate toxicity and stability profile, oral or topic administration is preferred, selectively protect normal cells without reducing the radiosensitivity of cancer cells and not interfere with other drugs used during therapy [90]. Although the two latter criteria strictly refer to radiotherapy, the remaining features of radioprotectors are universal and relevant also for compounds dedicated to protecting from radioactivity derived from other sources, including the environment.

A general categorization of medical IR countermeasures should consider the use of radioprotectors, radiomitigators, supportive/palliative measures, or interventions to reduce the internal accumulation of radioisotopes (Fig. 4A). The high reactivity of free radicals makes the presence of a radioprotector necessary to attenuate damage to DNA or other organic molecules. For this purpose, radioprotectors are administered previously or during IR exposition with the aim to prevent or reduce radiation-induced damage. At a difference of radioprotectors, radiomitigators can alleviate/attenuate these damages even when they are administered after IR exposition [103,104]. Therapeutic interventions may serve supportive or palliative purposes, aiming to reduce damage that has already occurred. Some examples include fluid or electrolyte replacement, transfusions, bone marrow transplant, antibiotics to treat infections, anti-inflammatory (AINEs or corticoids), antipyretic agents or sedative agents to alleviate pain, mechanical ventilation, *etc*. [105]. The FDA has approved the use of potassium iodide (KI), Prussian Blue (ferric hexacyanoferrate), trisodium zinc, or calcium diethylenetriaminepentaacetate (Zn/Ca-DTPA) to prevent uptake or treat individuals exposed to radionuclides [106]. Radionuclides may be internalized through ingestion, inhalation, and wound contamination.

Despite not being specifically approved by the FDA, additional agents are used with similar purposes [87]. Due to the complexity of biochemical processes triggered by different types of radioprotectors and the timing of their application in treatment, it is challenging to create one universal and plain classification of these compounds. Thus, other possible categorizations are presented in Fig. 4. Radioprotectors and radiomitigators can also be classified according to their biochemical structure or the mechanism of action involved in their protective effects (Figs. 4B and 4C). Another more flexible approach can consider the five types of effects caused by radiation (Table 1) as well as a whole pool of radiation-induced damage [90]. As it is exposed in Fig. 4D, the first group includes general radioprotectors, such as free radicals scavengers, antioxidants and substances that reduce oxygen consumption or enhance DNA repair. The second group encompasses compounds protecting against the effects of type I radiation (modulators of cell death pathways and growth factors). The third class includes substances protecting against the type II and III radiation effects, *i.e.* inhibitors of inflammatory processes and chemotaxis as well as blockers of autocrine/paracrine pathways. The fourth group is represented by compounds responsible for antimutagenic action and genome integrity protection (*i.e.* DNA protectors or substances with the capacity to enhance DNA repair). The fifth group includes substances with the capacity to block bystander effects (protecting against the type V of radiation effects).

Despite the almost 90 % failure rate of drugs in late-stage trials, many experimental therapeutic agents are actively under study. Advances in nanomaterials are expected to enhance the efficacy of radioprotectors [19], and the next milestone will be the development of nanoparticles capable of simultaneously protecting normal tissues from radiation and increasing the radiosensitivity of cancer cells [107]. Notably, Me@FulOHs induce cell cycle arrest and modulate gene expression. These compounds exhibit distinct effects on cancerous and healthy cells, paving the way for combination therapies that selectively target tumors while sparing normal tissues [26,108,109]. Furthermore, Me@FulOHs hold a unique position as potential radioprotectors with simultaneous imaging capabilities, enabling visualization of radiotherapy target areas, potentially increasing treatment precision and effectiveness [26], and this dual functionality makes them promising candidates for theragnostic applications, combining therapy and diagnostics in a single platform.

Among promising radioprotective agents, fullerenols and metallofullerenols stand out as potential universal radioprotectors due to their broad spectrum of action. These compounds demonstrate exceptional free radical scavenging abilities and possess antioxidant properties. In most studies evaluating protective effects against damage induced by IR, fullerenols and metallofullerenols have been administered either before or at the time of radiation exposure [25,110–117]. The prevention of oxidative stress mediated by free radical scavengers has been shown to

Fig. 4. Classifications of radioprotective agents. Abbreviations: **ACEi**: angiotensin-converting enzyme inhibitors; **bFGF**: basic fibroblast growth factor; **COX-2**: cyclooxygenase-2; **DTPA**: diethylenetriaminepentaacetic acid; **EDTA**: ethylenediaminetetraacetic acid; **EPO**: erythropoietin; **FSL-1**: fibronectin-synthetic lipopeptide-1; **G-CSF**: granulocyte colony-stimulating factor; **GM-CSF**: granulocyte-macrophage colony-stimulating factor; **KGF**: keratinocyte growth factor (fibroblast growth factor 7, FGF-7); **NAC**: N-acetylcysteine; **NSAIDs**: non-steroidal anti-inflammatory drugs; **SOD**: superoxide dismutase; **TPO**: thrombopoietin. Compilation of data [69,102,106,125–127].

attenuate most of harmful effects induced by radiation (Table 1). For this reason, we consider that FulOHs and Me@FulOHs should be considered general radioprotectors (Fig. 4D). Preclinical *in vivo* studies evidence that fullerenols protect erythrocytes from biochemical and molecular changes induced by ionizing radiation [115,118] and exert radioprotective effects on the microcirculation system [113], myocardium [119], colon [120], mucosal epithelial cells [121], as well as the small intestine, lungs, and spleen [25], contributing to the prevention of ARS. More recent studies have demonstrated that topically applied fullerenols effectively prevent radiation-induced dermatitis [112,122,123], making them excellent "free radical sponges" [124,132].

At the current stage of research, precisely categorizing FulOHs and Me@FulOHs into specific groups remains a challenge due to the lack of comprehensive studies elucidating their mechanisms of action and range of applications. Addressing these gaps requires further investigation to fully understand their versatility and efficacy across various biomedical contexts. Integrating these materials into future therapeutic strategies could bridge the gap between protecting healthy tissues and enhancing cancer treatment effectiveness, contributing to advancements in radioprotection and cancer therapy.

7. Fullerenols: potential applications in radiotherapy

The protection of healthy cells during radiotherapy and the prevention of IR-induced damage are significant challenges in biomedicine. A potential solution to these issues may lie in the rapidly advancing

research on nanomaterials, including FulOHs, which have gained attention for their ability to interact with free radicals and protect cells from oxidative damage [23,128]. Their radioprotective properties have been demonstrated in various experimental models, showing promise in reducing IR-induced cellular damage. An early study by Zhao et al. [129] investigated $C_{60}(OH)_x$ on *Stylonychia mytilus* cells exposed to γ-rays at doses of 100–2000 Gy for 90 min, providing valuable insights into the concentration-dependent action of $C_{60}(OH)_x$. By introducing a $C_{60}(OH)_x$ stock solution (0.06–0.25 mg/mL) to cell cultures 2 hours prior to irradiation, they observed that a concentration of 0.10 mg/mL resulted in the maximum surviving fraction—four times higher than in control conditions—attributed to fullerenol's radical-scavenging abilities, as shown by increased superoxide dismutase and catalase activities, and reduced oxidative stress markers. Interestingly, higher concentrations (0.25 mg/mL) led to oxidative damage through peroxidation, indicating a threshold at which FulOH shifts from antioxidant to pro-oxidant. A similar investigation aimed to evaluate the radioprotective potential of the water-soluble fullerene derivative, $C_{60,70}O_y(OH)_x$, $(x + y = 24–28)$, under low-dose tritium radiation (< 0.05 Gy) [130]. The effects were assessed using the luminescent bacterium *Photobacterium phosphoreum*, with the intensity of its luminescence serving as an indicator of physiological functions. Exposure to tritium increased luminescence, but the addition of FulOH ($< 3{\cdot}10^{-3}$ g/L) reduced this effect, restoring control values, and suggesting its radioprotective properties. The study examined the impact of $C_{60,70}O_y(OH)_x$ on ROS content and enzymatic luminescence intensity. Tritium exposure caused alterations in these parameters, but $C_{60,70}O_y(OH)_x$ restored them to control levels, indicating its action involves modifying the ionic balance in the aqueous medium, which in turn influences bacterial cell membrane functions. These findings suggest that fullerenol's radioprotective effects may be related to its modulation of cellular oxidative stress and membrane functions.

Another *in vitro* study [110] demonstrated that $C_{60}(OH)_n$ $(n = 12–26)$, significantly enhanced the radioprotective efficiency in whole-body irradiated mice exposed to X-ray irradiation of 6, 7, and 8 Gy (X-ray energy of 8 MV). The first group of animals was given 10 mg/kg FulOH intraperitoneal (*i.p.*) and the second group was given 100 mg/kg FulOH (*i.p.*), both 30 minutes before the irradiation. An optimal dosage of 100 mg/kg of FulOH markedly improved the survival metrics, notably increasing both the LT_{50} (from 11.15 to 16.72 days) and LD_{50} in irradiated male mice. The LT_{50} in the 100 mg/kg group was significantly higher than in both the control ($p < 0.05$) and the 10 mg/kg group ($p < 0.05$), highlighting the radioprotective effects of fullerenol at the higher dose.

This increase in radioprotection was particularly pronounced at higher radiation doses, indicating a dose-dependent protective effect. Bogdanovic et al. [131] investigated the radioprotective effects of $C_{60}(OH)_{24}$ pre-treatment (10 μM) on human erythroleukemia K562 cells against X-ray irradiation (24 Gy). The key findings showed that FulOH pre-treatment significantly enhanced the survival and proliferation of irradiated K562 cells which was accompanied by a notable increase in the activity of antioxidant enzymes such as SOD and GPx in irradiated cells. The authors observed 2-fold elevated SOD and GPX levels in FulOH pretreated irradiated cells 24 h post-irradiation time and a significant reduce in gamma-glutamyltransferase (GGT) levels 48 h post-irradiation. Interestingly, FulOH alone used in the concentration of 10 μM did not exhibit cytotoxic effects. The authors suggested that the radioprotective mechanism probably involves ROS and nitrogen species (RNS) modulation and maintaining redox homeostasis. The findings underscored the potential of FulOH as a therapeutic agent for reducing radiotherapy-induced toxicity in cancer treatment. Grebowski et al. [132] employed pulse radiolysis to determine the rate constants for the reaction of highly hydroxylated fullerene $C_{60}(OH)_{36}$ with $HO^{\bullet}$ (2×10^9 $M^{-1}s^{-1}$) and with hydrated electrons (2.5×10^9 $M^{-1}s^{-1}$). The likely reaction pathway for $C_{60}(OH)_{36} + HO^{\bullet}$ involves initial π-complex formation, potentially dissociating back to reactants or rearranging into a σ-complex, suggesting a competitive reaction process. This research underscored the significance of the hydroxy groups attached to the carbon cage of fullerene, which not only preserves but potentially enhances the capability to act as a scavenger of radicals. These findings suggested that $C_{60}(OH)_{36}$ may serve as a potent antioxidant in radiobiology. The study of the same group investigated the radioprotective effects of $C_{60}(OH)_{\sim 30}$ on alcohol dehydrogenase (ADH) activity when exposed to X-radiation under aerobic conditions. At a concentration of 75 mg/mL, $C_{60}(OH)_{\sim 30}$ reduced the radiation-induced inactivation of ADH by 20 %. It was postulated that during irradiation, approximately 50 % of $HO^{\bullet}$ interacted with FulOH, and the remaining 50 % with ADH, suggesting that the mechanism of protection against the radiative inactivation of ADH predominantly involves the scavenging of $HO^{\bullet}$ radicals. Radioprotective mechanism of $C_{60}(OH)_{\sim 30}$ action involves the preservation of –SH groups in ADH through electrostatic, dipolar, or Vander Waals interactions, emphasizing the potential of $C_{60}(OH)_{\sim 30}$ particles in mitigating radiation-induced protein damage [133]. Fullerenol $C_{60}(OH)_{36}$ at a concentration of 150 μg/mL exhibited also protective effects on human erythrocyte membranes against damage induced by high-energy electrons of 6 MeV. Fullerenol significantly reduced radiation-induced hemolysis of erythrocytes, prevented the decrease in membrane fluidity, and protected -SH groups in erythrocyte membranes from oxidation. Specifically, in comparison with erythrocytes irradiated without $C_{60}(OH)_{36}$, there is a 30 % and 39 % increase in protection from hemolysis at irradiation doses of 0.65 and 1.3 kGy, respectively, and the protection of -SH groups against oxidation was about 25 % (0.65 kGy) and about 42 % (for a 1.3 kGy dose). Fullerenol also attenuated the radiation-induced release of potassium ions (K^+) from erythrocytes. Administration of 150 μg/mL of $C_{60}(OH)_{36}$ to erythrocytes exposed to a 1.3 kGy irradiation resulted in a roughly 32 % reduction in K^+ release compared to non-treated erythrocytes. Moreover, the study also revealed that while $C_{60}(OH)_{36}$ alone did not alter acetylcholinesterase enzyme activity, its presence at 150 μg/mL significantly decreased the enzyme-substrate affinity in erythrocytes during irradiation. The authors concluded that the protective effects of $C_{60}(OH)_{36}$ were attributed to its antioxidant properties, by ROS scavenging, and providing a barrier to oxidative damage possibly due to physical interactions with membrane components [115]. A continuation of this study was the investigation of the ability of $C_{60}(OH)_{36}$ to protect the endogenous antioxidant system of erythrocytes against high-energy electrons [118]. At a concentration of 150 μg/mL $C_{60}(OH)_{36}$ significantly reduced radiation-induced damage in human erythrocytes by maintaining the activity of antioxidant enzymes. Specifically, it attenuated the depletion of -SH groups caused by irradiation and preserved erythrocyte microviscosity. Interestingly, $C_{60}(OH)_{36}$ did not affect CAT activity directly but enhanced GPx and reductase (GSR) activities. The presence of $C_{60}(OH)_{36}$ also led to a decrease in glutathione transferase (GST) activity after irradiation, which could be due to the reduction of oxidative stress or direct interaction with the enzyme's active site [118].

In vitro study by Nowak et al. [134] observed minimal protective effects against radiation-induced cell death on X-ray irradiated human peripheral blood mononuclear cells (PBMCs) both untreated and pre-treated with $C_{60}(OH)_{36}$ at concentrations of 75 and 150 μg/mL subjected to substantial doses of IR (10, 30, and 50 Gy). $C_{60}(OH)_{36}$ increased cell granularity and affected membrane fluidity of lymphocytes 24- and 48 hours post-irradiation without causing cytotoxic effects. Interestingly, $C_{60}(OH)_{36}$ did not influence DNA damage in both non-irradiated and X-irradiated PBMCs. A similar cellular model was used in a confocal microscopy study by Lichota et al. [135] on the internalization of $C_{60}(OH)_{36}$ nanoparticles into PBMCs and focused on cytotoxicity under oxidative stress induced by IR. After 24-h and 48-h incubation with FulOH at the concentrations of 75 μg/mL and 150 μg/mL the uptake of $C_{60}(OH)_{36}$ nanoparticles by PBMCs was concentration-dependent with a more efficient absorption observed at higher FulOH concentrations. Moreover, $C_{60}(OH)_{36}$ was accumulated in PBMCs without significantly affecting cell survival or

phosphatidylserine distribution, suggesting low cytotoxicity of those nanoparticles. JC-1 fluorescent probe indicated that $C_{60}(OH)_{36}$ decreased mitochondrial membrane potential in PBMCs in a dose-dependent manner. The most significant reduction, comparable to the effect of 10 Gy IR, was observed at the highest concentration used (150 μg/mL), with a decrease in the mitochondrial potential to about 80 % of untreated control. Fullerenol displayed no protective effects against IR at any concentration tested (50–150 μg/mL), nor did it amplify radiation effects. Despite the ability of FulOH to enter the cell and affect mitochondrial membrane potential, it did not exhibit radioprotective properties under the studied conditions.

Subsequent studies on FulOHs were focused on in-depth analysis of their radioprotective effects on tissues and organs, alongside validation in animal models. An extensive examination of the radioprotective effects of $C_{60}(OH)_{24}$ on mice subjected to IR comes was performed by Cai et al. [111]. They systematically investigated the survival rates, immune function, mitochondrial activity, and oxidative stress markers post-exposure, demonstrating the ability of $C_{60}(OH)_{24}$ to significantly enhance survival, preserve immune and mitochondrial functions, and reduce oxidative damage in critical organs such as the liver and spleen. The animal survival rate was assessed 30 days after irradiation. Pretreatment of mice with $C_{60}(OH)_{24}$ (40 mg/kg in 0.5 mL saline *i.p.*) daily for 2 weeks decreased the radiation-induced mortality when compared with the radiation control group. Fullerenol $C_{60}(OH)_{24}$ enhanced immune function in the spleen possibly by guarding mitochondrial function and decreasing apoptosis. The study also confirmed earlier findings by showing that pre-treatment with $C_{60}(OH)_{24}$ counteracted the decrease in SOD activity and GSH levels caused by irradiation [131]. Additionally, $C_{60}(OH)_{24}$ significantly reduced the radiation-induced elevation of malondialdehyde (MDA), a marker of lipid peroxidation, indicating its protective role against oxidative stress. Further *in vivo* research by Trajkovic et al. [25] compared $C_{60}(OH)_{24}$ (10 and 100 mg/kg *i.p.*) and amifostine (300 mg/kg *i.p.*) in protecting rats against a lethal dose of X-rays (8 Gy) irradiation, revealing superior efficacy of $C_{60}(OH)_{24}$ used at 100 mg/kg in enhancing survival rates and body mass post-irradiation. Hematological analysis showed that $C_{60}(OH)_{24}$ significantly alleviated white blood cell count reduction in the first and the second week post-irradiation, surpassing the effectiveness of amifostine. Histopathological assessments indicated stronger protective effects of $C_{60}(OH)_{24}$ on the spleen, small intestine, and lungs, whereas amifostine better shielded the heart, liver, and kidneys. Vesna et al. [136] aimed to evaluate the protective efficiency of $C_{60}(OH)_{24}$ nanoparticle versus amifostine in rats subjected to whole-body X-ray irradiation (7 or 8 Gy). Each of the compounds: $C_{60}(OH)_{24}$ (100 mg/kg) and amifostine (300 mg/kg *i.p.*) administered 30 minutes before exposure demonstrated similar effectiveness in enhancing survival rates following a lethal dose of radiation. Histopathological analysis post-sublethal irradiation revealed a superior protective effect on the intestines, lungs, and spleen, whereas amifostine more effectively protected from lesions of the tissue of the heart, liver, and kidneys, establishing $C_{60}(OH)_{24}$ nanoparticles comparable cytoprotective compound to the well-established amifostine.

Zhao et al. [122] evidenced for the first time the skin radioprotective properties of FulOHs *in vivo*, inspired by their good radioprotective performance *in vitro*. To be suitable for skin, fullerenol-sodium hyaluronate hydrogels (F-NaHA) nanoparticles were used as the active radioprotective ingredients to prepare hydrogels. These F-NaHA hydrogels, integrating the practicality and safety of sodium hyaluronate, exhibited significant film-forming properties, enabling effective skin protection against radiodermatitis. An X-ray tube positioned 2 cm from the back skin of a BALB/c mouse was used as the irradiation source to induce radiodermatitis. Before exposure, F-NaHA hydrogels and SOD salves were applied to the mouse skin to assess their radioprotective effects, with a control group receiving no treatment. Results demonstrated that F-NaHA hydrogels alleviated radiodermatitis more effectively than SOD salves, with observations of delayed onset, accelerated recovery, and hair regrowth. During 25 days of observation, in comparison to commercial SOD salves, there was a delayed emergence of radiodermatitis and a relatively short period from skin damage to recovery as well as quick hair restoration and hair growth in the X-ray + F-NaHA group. The histopathological analysis further confirmed the superior protective effects of FulOH on the epidermal stem cells in the basal layer, showing the ability of F-NaHA hydrogels to maintain skin tissue metabolism and regeneration by protecting collagen fibers. Additionally, the biosafety assessment confirmed the low systemic toxicity of FulOHs, highlighting their potential as a clinically viable radioprotectors for radiodermatitis prevention. Similar conclusions come from the study by Yin et al. [112] which provides comprehensive insights into the radioprotective effects of highly soluble FulOH on skin cells and radiation dermatitis (RD) in mice. Pretreatment with FulOH in the concentration of 12.5 and 25 mg/L notably scavenged intracellular ROS generated by X-rays safeguarding skin cells (HACaT) from X-ray-induced DNA damage and apoptosis. Inspired by the satisfying radioprotective performance of the FulOH on skin cells the authors evaluated its' effect on skin radioprotection *in vivo* by visually observing the irradiated skin area changes on the left hind legs of BALB/c mice and their corresponding histopathological phenomena. *In vivo* experiments revealed FulOH reduced radiation-induced skin injury in mice by decreasing epidermal thickening, collagen deposition, and damage to skin appendage damage, while also promoting hair regeneration. Furthermore, FulOH exhibited superior radioprotective effects compared to Trolamine cream, a conventional RD treatment, indicating a promising potential for FulOHs in enhancing skin repair mechanisms following radiation exposure.

Skin damage caused by head and neck radiation therapy is a significant clinical issue, markedly lowering patients' quality of life. Effective tissue repair requires adequate blood perfusion, yet current radioprotective agents primarily target specific organs and cells. Therefore, greater emphasis should be placed on microvascular protection. Radioprotective agents targeting the microcirculation could significantly improve patients' post-treatment quality of life [123]. Peng et al. [123] extensively analyzed the radioprotective effects of fullerenol, a key bioactive component in fullerenol emulsion, on vascular endothelial cells. Local application of fullerenol emulsion offers advantages over systemic administration by increasing drug concentration in the target tissue, improving local efficacy, and reducing systemic toxicity. Animal studies showed that fullerenol emulsion reduced radiation-induced vascular endothelial damage through antioxidant mechanisms, increasing VEGF expression, inducing endothelial cell proliferation, promoting angiogenesis, and significantly improving tissue perfusion in damaged areas. The emulsion also inhibited fibrosis by regulating collagen remodeling, which significantly alleviated skin damage in the irradiated head and neck regions of mice. Furthermore, cell studies confirmed the protective effects of fullerenol on vascular endothelial cells and indicated its mechanisms of action. Fullerenol reduced reactive oxygen species (ROS) levels, modulated TGF-β expression, minimized DNA damage, and inhibited the mitochondrial apoptosis. It also enhanced VEGF expression, stimulating endothelial cell proliferation, migration, and the formation of vascular structures. These findings suggest that fullerenol emulsion could be a promising agent for skin regeneration following radiation exposure.

Subsequent *in vivo* experiments revealed that fullerenol@nanomontmorillonite (FNMT) nanocomposite significantly reduced radiation-induced diarrhea, weight loss, and pathological damage in the duodenum of BALB/c male mice. Histopathological analysis revealed that FNMT nanocomposite treatment ameliorated radiation-induced changes in duodenal tissue architecture, indicating enhanced tissue repair and regeneration. The results from the above studies proved that radiation caused bad side effects, including radiation damage to the stomach and duodenum, accompanying cell apoptosis and necrosis, and shedding of gastric and duodenal mucosa cells. However, with the intervention of FulOH or FNMT nanocomposite, the radiation damage was alleviated to different degrees, and the FNMT nanocomposite

displayed the best effect [137].

The same authors [120] investigated the radioprotective effects of fullerenol@pectin@chitosan gel microspheres (FPCGM) on radiation-induced colon injury, focusing on their ability to alleviate damage, regulate intestinal flora, and enhance recovery in a mouse model. Encouraged by targeted colonic release and specific bioadhesion, the radioprotective effects were systematically evaluated in an animal model of radiation-induced colon injury. The administration of FPCGM notably reduced weight loss, diarrhea, and hematochezia, and decreased the mortality associated with intestinal damage in mice. Post-irradiation, all mice showed weight loss, yet those receiving FPCGM maintained a significantly higher weight compared to those exposed to radiation alone. Evaluations based on body weight, diarrhea, hematochezia, and constipation revealed that FPCGM treatment effectively improved symptoms of diarrhea and constipation induced by radiation. Furthermore, mice treated with FPCGMs had a 50 % higher survival rate following irradiation compared to the X-ray-only group. Another sign of intestinal damage, intestinal shortening, was also significantly reduced by pre-treatment with FPCGMs, preserving colon length. These findings suggest that FPCGMs hold considerable promise as radioprotective agents specifically for colon protection [120]. Oxidative stress, crucial in radiation-induced oral mucositis (RIOM) arises from a intensive ROS production leading to significant pathological lesions and oral microbiota imbalance. Therefore, a sprayable in situ hydrogel loaded with FulOH has been used as an antioxidant agent for RIOM radioprotection [121]. Findings from that study demonstrate the effectiveness of the hydrogel in both preventing and treating RIOM in mice models. The sprayable FulOH-enriched hydrogel exhibited strong muco-adhesion properties, ensuring sustained release and adherence in the oral cavity. It alleviated oral mucosal erythema, atrophic lesions, and symptomatic ulcers, while also maintaining the integrity of oral microbiota. These results emphasized the beneficial effect of FulOH-loaded hydrogels as a promising approach for RIOM prevention, showing their great potential in the protection of oral health during radiotherapy.

A very recent study from Zhang et al. [119] explored the protective effects of FulOH on adult human ventricular cardiomyocyte AC16 cells against radiation-induced injury. The study demonstrated that FulOH significantly reduced apoptosis in cardiomyocytes induced by X-ray irradiation. This was evident through decreased lactate dehydrogenase release in treated cells compared to controls. Fullerenol acted by scavenging ROS (measured with the use of CFH-DA probes) and thus mitigating mitochondrial oxidative stress leading to reduced cell damage and apoptosis. Inspired by the good radioprotective performance of FulOH *in vitro*, the authors decided to assess its potential as a protective agent against X-ray-induced heart damage *in vivo*. To this end, 90 healthy male BALB/c mice were selected as an animal model to investigate the radioprotective effects of the FulOH. Apart from the anti-apoptotic effect, the influence of FulOH on two indicators of myocardial damage (CKMB and cTnT) induced by X-ray in cardiac tissue was measured. Fullerenol was administered orally or by tail vein one week before and one week after X-ray treatment. After that, mice were sacrificed and vital organs were taken for subsequent experiments at 1st, 4th, and 12th week after X-ray irradiation. Fullerenol intervention

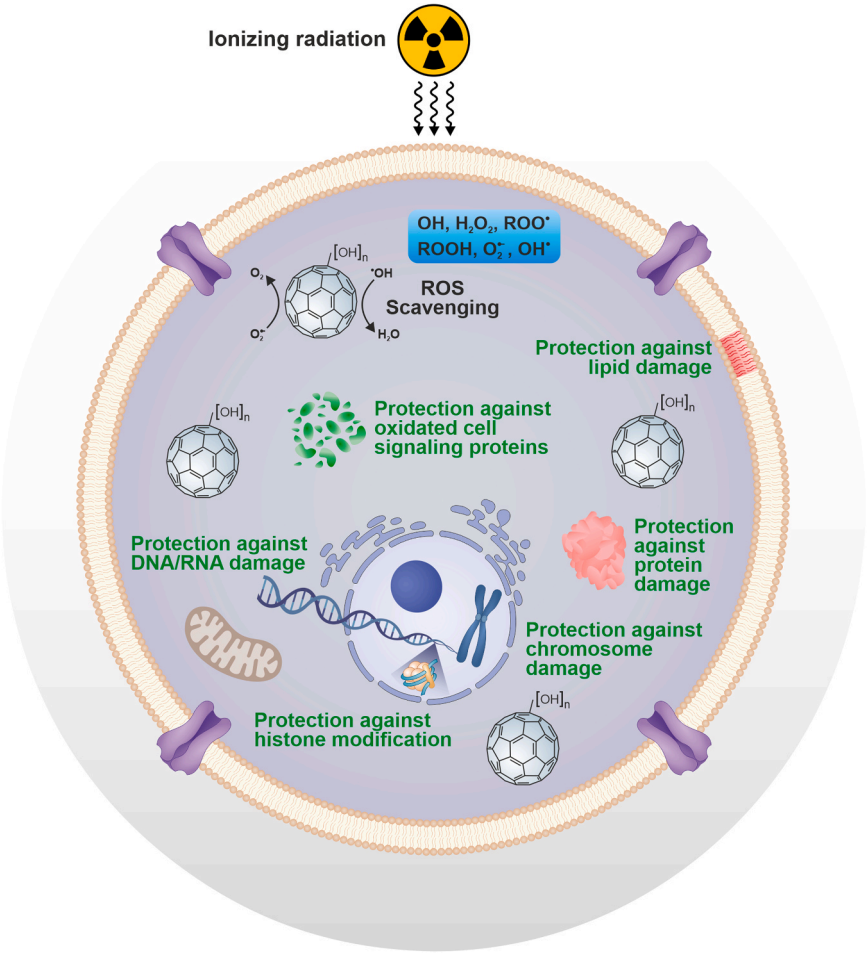

Fig. 5. The protective effects of FulOH against IR-induced cellular damages. FulOH scavenges free radicals and reactive oxygen species (ROS), thereby reducing oxidative stress in cells. It also helps in DNA repair by decreasing mutation frequency and promoting cell survival post-radiation exposure. The illustration depicts interactions of FulOH with the cell membrane, mitochondria, and the nucleus, highlighting its complex protective effects on various cellular components.

significantly attenuated injuries and apoptosis in heart tissue compared to the X-ray groups. Given its effective radioprotective properties and lack of side effects, FulOHs presented a promising therapeutic approach for protecting heart tissue during radiotherapy.

The research findings presented in this review indicate that FulOHs can protect key cellular components (Fig. 5), which translates into the protection of organs such as the myocardium, colon, mucosal epithelium, small intestine, lungs, and spleen, contributing to the prevention of ARS. Current radiation protection strategies primarily focus on safeguarding target organs, often neglecting the microcirculation, which has significant implications for the long-term effects of radiotherapy [113, 138–140]. However, protecting the microcirculation is crucial, as its damage can lead to severe complications such as heart disease, dermatitis, or lung injury.

Fullerenol supports the proliferation of microcirculatory cells and angiogenesis by activating the PI3K/AKT pathway, facilitating vascular tissue regeneration [113]. Its dual mechanism—reducing oxidative stress and promoting angiogenesis—could significantly improve the prognosis of cancer patients undergoing radiotherapy. Although further studies are needed to determine the long-term effects and optimal administration methods, fullerenol represents an innovative approach to radiation protection.

Its stability and safety *in vitro* and *in vivo* studies further emphasize its potential clinical use (Table 2).

8. Metallofullerenols: a potential shift of paradigm in radioprotective therapy studies

Incorporating atoms or metal clusters into fullerene cages leads to the formation of metallofullerenes, which exhibit unique properties compared to pure carbon fullerenes. These properties can be attributed to the interaction of the metal atoms with the carbon cage, as well as the modification of the electronic structure arising from this interaction [141–143], which enhances the ability to donate electrons and scavenge ROS [26,144–146]. Metalofullerenol $Gd@C_{82}(OH)_{22}$ exhibits stronger antioxidant activity than $C_{60}(OH)_{22}$ [147], and this effect is likely due to the higher electron affinity of the endohedral fullerene $Gd@C_{82}$ (3.3 eV) compared to 3.14 eV for the empty-cage fullerene C_{82} (both parameters measured experimentally) [148]. Therefore, Me@FulOH with Me = Sm, Eu, Sc, Gd, Er, Lu appears to be a reasonable approach in modern radiotherapy due to both, high polarity (bioavailability) and the ability to effectively inhibit oxidative damage [26]. In our previous studies [114] we implemented the pulse radiolysis to evaluate the antioxidant properties of three water-soluble Me@FulOHs: $Sc_3N@C_{80}(OH)_{18}$, $Lu_3N@C_{80}(OH)_{18}$, and $Gd@C_{82}(OH)_{22}$, as potential candidates for radioprotectors. The obtained rate constants for reaction with $HO^{\bullet}$ were 2.24×10^9, 4.32×10^9, and 1.11×10^{10} $M^{-1}s^{-1}$, respectively. In a separate series of experiments the protective effect of Me@FulOHs against radiation-induced hemolysis in human erythrocytes was measured, indicating a correlation of degree of protection (expressed as hemolysis percentage) against attack by $HO^{\bullet}$ with the reactivity of Me@FulOHs (expressed as rate constants). The protective abilities of Me@FulOHs differ in oxygenated and anaerobic systems (Fig. 6A). Under anaerobic conditions, their activity correlates with reactivity towards $HO^{\bullet}$, whereas in the presence of oxygen, oxidative damage is primarily driven by lipid peroxidation mediated by $LOO^{\bullet}$ radicals. Thus, the ability of Me@FulOHs to scavenge these radicals is crucial. Since lipid peroxyl radicals are localized within the lipid membrane, this highlights the bimodal action of Me@FulOHs: they can act as preventive antioxidants (neutralizing $HO^{\bullet}$ before initiating peroxidation) and as chain-breaking antioxidants, effectively scavenging $LOO^{\bullet}$ radicals. Interestingly, all tested Me@FulOHs were found to be more effective than vitamin C and Trolox (a water-soluble analogue of tocopherol) in protecting erythrocytes against gamma radiation (Fig. 6A) [114]. Theoretical investigations of $Gd@C_{82}O_x(OH)_y$, (x = 0 or 3, and y = 8, 16, 24, 36, 44) suggest that antioxidant properties might be dependent on number of hydroxy groups [149]. For $y > 24$ an increased formation of intramolecular hydrogen bonds is preferred, thus, limiting their interactions with surrounding water molecules. Additionally, the aromatic character of the carbon core is diminishing for and increasing number of hydroxy groups. The authors concluded that $Gd@C_{82}O_x(OH)_y$ complexes with 24 hydroxy groups are the most promising candidates for biomedical applications [149]. Both FulOHs and Me@FulOHs interact with the cell membrane [114,150–153] and localize close to the lipid/water phase boundary, leading to membrane protection against oxidative damage caused by hydroxyl ($HO^{\bullet}$), alkylperoxyl ($ROO^{\bullet}$), and superoxide ($O_2^{\bullet -}$) radicals [114].

The potential applications of Me@FulOHs extend beyond their role as radical trapping agents used for minimizing ROS-induced damages. Due to the capsule-like shape of the carbon cage, Me@FulOHs have been recognized as carriers of active compounds and radiopharmaceuticals [26]. They also can be modified to attach specific ligands or contrast agents enabling their applications in advanced bioimaging [108,154, 155]. Metal embedded inside the carbon cage can generate strong signals in imaging techniques such as positron emission tomography (PET) or magnetic resonance imaging (MRI), allowing high-quality results in diagnostics [156]. The combination of these two areas can lead to the development of advanced diagnostic and therapeutic tools that utilize nano-technologies for disease detection and treatment at very early stages [157]. It is also crucial to note that Me@FulOHs exhibit high kinetic stability and resistance to metabolic processes, presenting them as viable alternatives to chelating agents with significant advantages over molecular carriers used for the transport of radioisotopes into the body [158,159]. Unlike chelating agents, Me@FulOHs do not release toxic compounds [26,159]. The high-resolution PET and MRI imaging capabilities of Me@FulOHs offer a theranostic advantage, enabling precise localization of tissues damaged by radiation or areas of heightened oxidative stress. This imaging precision informs treatment planning, allowing for the targeted administration of Me@FulOHs to areas most vulnerable to radiation-induced damage. Moreover, by facilitating real-time monitoring of the oxidative stress environment within tissues, these nanomaterials optimize the timing and dosage of radioprotective interventions, enhancing their efficacy while minimizing potential pro-oxidative risks. Additionally, the dual-functionality of Me@FulOHs aligns with the goals of contemporary oncology and radiotherapy to reduce side effects while ensuring rapid and precise diagnostics. By combining diagnostic imaging with radioprotection, Me@FulOHs tackle one of the key challenges in radiotherapy: balancing effective tumor treatment with the preservation of healthy tissues. This theranostic application distinguishes metallofullerenols from conventional radioprotectors and radiomitigators, which often lack such versatility.

Shultz et al. [160] presented an innovative concept of the synthesis of a metallofullerenol $^{177}Lu_xLu_{(3-x)}N@C_{80}$-based radiopharmaceutical coupled with IL-13 peptide (Fig. 6C). This advanced compound was designed to target the overexpressed receptor in multifocal glioblastoma tumors. Isotope ^{177}Lu enclosed in $Lu_3N@C_{80}$ is an emitter of β radiation and can be permanently embedded in the Me@FulOHs cage for at least one half-life period (6.7 days), indicating its promising potential in radiotherapy [160]. Such a type of platform with included radioactive isotopes raises questions about the stability of the proposed supramolecular systems. The impact of high-energy radiation on the structure and stability of Me@FulOHs has been investigated in several studies [161–164]. The stability (measured as the percentage of surviving species) of $Gd@C_{2n}(OH)_{38–40}$, when exposed to a neutron flux of 10^{16}-10^{19} neutrons/cm^2 with a broad energy range (from thermal to fast neutrons) in the WWR-M reactor, was an order of magnitude higher than that of the non-hydroxylated metallofullerene $Gd@C_{82}$. The increased stability of $Gd@C_{2n}(OH)_{38–40}$ is attributed to the fact that -OH groups are strongly solvated by water molecules. Thermal neutrons undergo highly effective incoherent scattering on protons in the -OH groups [161,162]. Studies comparing the radio-stability of endofullerenes and endofullerenols have shown differences, depending on the group of metal atoms in

Table 2
Characteristics of fullerenols (FulOH) as potential radioprotective agents.

Type of FulOH	Dose Concentration	Experimental Model	Radiation Source Energy	Absorbed Dose	Main results	Ref.
			Experiments on microorganisms			
$C_{60}(OH)_x$	0.06–0.25 mg/mL	*Stylonychia mytilus*	γ-rays ^{60}Co	100–2000 Gy	FulOH at conc. of 0.10 mg/mL provides the maximum survival effects; activates SOD and catalase, but exhibits pro-oxidative effects at higher concentrations (0.25 mg/mL).	[129]
$C_{60,70}O_y(OH)_x$ (x + y = 24–28)	$< 3{\cdot}10^{-3}$ g/L	*Photobacterium phosphoreum*	β⁻-radiation ^{3}H (5,7 keV)	< 0.05 Gy	FulOH reduces ROS, restores luminescence, and modulates ionic balance.	[130]
			***In vitro* studies**			
$C_{60}(OH)_{24}$	10 μM	Human erythroleukemia cells (K562)	X-rays (10 MV)	24 Gy	FulOH enhances survival, increases SOD and GPx activity, reduces GGT activity in irradiated K562 cells, and shows no cytotoxic effects.	[131]
$C_{60}(OH)_{36}$	150 μg/mL	Human erythrocytes	Electrons (6 MeV)	0.65–1.3 kGy	Protection against hemolysis. FulOH preserves -SH groups, reduces K+ release, and decreases acetylcholinesterase (AChE) affinity for its substrate, suggesting strong adsorption to plasma membranes. FulOH maintains erythrocyte microviscosity, boosts glutathione peroxidase (GPx), and glutathione reductase (GSR) activities.	[115, 118]
$C_{60}(OH)_{\sim 30}$	75 μg/mL	Alcohol dehydrogenase (ADH)	X-ray (195 kV, 18 mA)	20–100 Gy	FulOH protects ADH activity through $HO^{\bullet}$ radical scavenging and preserves the ADH thiol groups via electrostatic, dipolar, or van der Waals interactions.	[133]
$C_{60}(OH)_{36}$	75–150 μg/mL	Human PBMCs	X-ray (185 kV, 10 mA)	5–50 Gy	Decrease of mitochondrial membrane potential, no protection against IR at any tested concentration (50–150 μg/mL). No cytotoxic effects were detected. No protective effect against DNA damage.	[134, 135]
$C_{60}(OH)_{20}$	25 μg/mL	Human keratinocyte cells (HaCaT) and HUVECs	X-ray (160 kV, 25 mA)	2–8 Gy	A significant increase in survival rates (cell viability assays).	[122]
Fullerenol	12.5 or 25 mg/L	Human keratinocyte cells (HaCaT) and Human fibroblasts (HFF−1)	X-ray (160 kV, 25 mA)	25, 50, 100, 200 Gy	Scavenging of X-ray-induced ROS generation; mitigation of the X-ray-induced oxidative stress, inhibition of skin cell apoptosis, DNA damage.	[112]
Fullerenol	25 μg/mL	Human cardiomyocytes (AC16)	X-ray (160 kV, 25 mA)	9 Gy	FulOH mitigates cardiomyocyte damage, exhibiting biosafety, chemical stability, and radioprotective properties. It scavenges free radicals and inhibits mitochondrial oxidative stress by neutralizing ROS, stabilizing mitochondrial membrane potential, preventing cytochrome c leakage, regulating Bax/Bcl−2 expression, and reducing caspase−3 activity to inhibit apoptosis. FulOH also protects genetic material by reducing DNA strand breaks and modulating gene expression related to apoptosis and oxidative stress, including MAPK signaling pathways.	[119]
Fullerenol	25 μg/mL	HUVECs	X-ray	2–8 Gy	FulOH inhibits Caspase−3 activity and activates the PI3K/AKT signaling pathway (phosphoinositide 3-kinase/protein kinase B), along with its downstream proteins, such as eNOS and VEGF (endothelial nitric oxide synthase/vascular endothelial growth factor), reducing endothelial cell apoptosis and maintaining vascular proliferation potential and angiogenesis in response to radiation-induced damage.	[113]
Fullerenol (FNMT)	30 μg/mL	Rat small intestinal crypt epithelial cells (IEC−6)	X-ray (160 kV, 25 mA)	6 Gy	Radioprotection of the duodenum cells: good biosafety, high chemical stability, effective free radical scavenging, inhibition of mitochondrial oxidative stress pathway, reduction of radiation-induced DNA damage.	[137]
Fullerenol (FPCGMs)	25–50 μg/mL	Human normal colonic mucosal epithelial cells (NCM−460)	X-ray (160 kV, 25 mA)	6 Gy	FulOH protects cells from radiation-induced damage by scavenging ROS/RNS and reducing oxidative stress. It stabilizes the cell membrane, preventing damage, and limits mitochondrial dysfunction by preserving membrane potential, preventing cytochrome C release, and supporting ATP production. It reduces intracellular Ca^{2+} overload, minimizes DNA damage, and enhances cell regeneration, inhibiting apoptosis and necrosis.	[120]
Fullerenol emulsion	25 μg/mL	HUVECs	X-ray (50 kV, 75 μA)	6 Gy	FulOH Improves cell survival without affecting viability. Removes ROS, reduces oxidative stress, and minimizes DNA damage, it blocks the mitochondrial apoptosis pathway, downregulates pro-apoptotic	[123]

(*continued on next page*)

Table 2 (*continued*)

Type of FulOH	Dose Concentration	Experimental Model	Radiation Source Energy	Absorbed Dose	Main results	Ref.
					proteins (p53, BAX, cleaved-PARP, c-Caspase−3), and increases VEGF expression. It also stimulates angiogenesis and enhances proliferation, migration, and tube formation.	
Fullerenol (MGAF)	25 μg/mL	HaCaT cells	X-ray (160 kV, 25 mA)	6 Gy	FOH-loaded MGAF hydrogels exhibit strong radioprotective properties by scavenging excess ROS, reducing DNA double-strand breaks (lower γ-H2AX expression), and preserving mitochondrial integrity by maintaining membrane potential and limiting cytochrome C release. They also prevent calcium overload caused by radiation-induced oxidative stress, effectively breaking the cycle of ROS overproduction and calcium influx.	[121]
			Animal studies			
$C_{60}(OH)_n$ (n = 12–26)	10–100 mg/kg *i.p.*	Whole-body irradiated mice	X-rays (8 MV)	6–8 Gy	FulOH increases LT_{50} and LD_{50}; provides protection against oxidative damage, and modulates SOD and GPx activity.	[110]
$C_{60}(OH)_{24}$	40 mg/kg in 0.5 mL saline *i.p.* daily for 2 weeks	ICR mice	γ-rays ^{60}Co	8 Gy	Reduction of radiation-induced mortality compared to the control group, improvement of immune function in the spleen, protection of mitochondrial functions. Pre-treatment with FulOH countered the reduction in SOD activity and GSH levels, and reduced the radiation-induced elevation of malondialdehyde (MDA).	[111]
$C_{60}(OH)_n$ (n = 12–26)	10 and 100 mg/kg *i.p.*	Wistar rats	X-rays (8 MV)	7 and 8 Gy	FulOH (100 mg/kg) provided superior protection compared to both the lower dose (10 mg/kg) and amifostine, prevented radiation-induced reduction in white blood cell count (granulocytes and lymphocytes), radioprotective actions of FulOH on the spleen, small intestine, and lungs, histopathological analysis after sublethal irradiation showed better protective effects on the intestines, lungs, and spleen compared to amifostine.	[25, 136]
Fullerenol	0.02 % applied topically	BALB/c mice	X-ray (160 kV, 25 mA)	30 Gy	Reduction of skin oxidative stress, alleviation of radiation dermatitis (RD), enhanced skin antioxidant activity, promoting healing, reduced epidermal thickening, collagen deposition, and skin appendage damage, hair regeneration.	[112]
Fullerenol	4 mg/mL (oral)	BALB/c mice	X-ray (160 kV, 25 mA)	N/A	FulOH significantly reduced heart tissue damage and apoptosis compared to control groups just exposed to X-rays. It demonstrated strong radioprotective properties by protecting the heart from X-ray-induced damage, including reducing weight loss, minimizing structural changes in heart tissues (with lower levels of apoptosis and DNA damage), and lowering the levels of cardiac injury markers (CKMB, cTnT). No significant changes in heart function was reported. FulOH provided protection comparable to the efficacy of amifostine.	[119]
Fullerenol	25 mg/kg *i.p.*	BALB/c mice	X-ray	10 Gy	FulOH caused an increase in GPx activity and a decrease in MDA levels, reduced cell apoptosis markers, improved blood flow in the skin, and protected microcirculation from radiation-induced damage.	[113]
Fullerenols (F-NaHA)	1 mg/mL	BALB/c mice	X-ray (50 kV, 75 μA)	N/A	Better epidermal integrity and collagen fiber arrangement in the FuOH-NaHA group versus SOD or untreated controls.	[122]
Fullerenol (FNMT)	3 mg/mL taken orally	BALB/c mice	X-ray (160 kV, 25 mA)	6 Gy	Significant radioprotion by reducing body weight loss in mice and minimizing damage to the duodenum and stomach compared to the X-ray-only group. FNMT-treated mice had fewer tissue damages, with mild apoptotic cell loss in the upper mucosa of the duodenum and preserved crypt structure. Reduced oxidative stress was manifested as higher activity of SOD and GSH-Px, and reduction of lipid peroxidation (MDA assay). Biosafety assessments revealed no tissue damage or adverse effects after the administration of FNMT, confirming their biological safety.	[137]
Fullerenol (FPCGMs)	The concentration administered orally via gavage was not specified.	BALB/c mice	X-ray (160 kV, 25 mA)	8 Gy	FPCGMs' colon-targeted drug delivery, prolonged retention time, and cumulative release of FulOH in simulated gastrointestinal environments. Their radioprotective efficacy was demonstrated by a reduction in weight loss, diarrhea, hematochezia, intestinal shortening, and oxidative stress biomarkers (SOD, GPx, MDA), along with improved survival rates	[120]

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Table 2 (*continued*)

Type of FulOH	Dose Concentration	Experimental Model	Radiation Source Energy	Absorbed Dose	Main results	Ref.
					post-irradiation. FPCGMs preserved the intestinal barrier by maintaining tight junction proteins (occludin, claudin−1), promoting epithelial cell proliferation and migration, and reducing inflammatory cell infiltration and MPO expression. They effectively restored gut microbiota homeostasis, improving microbial diversity and reversing radiation-induced dysbiosis, including the Firmicutes/Bacteroides ratio. Finally, biosafety assessments confirmed no adverse systemic effects or harm to vital organs, highlighting the potential clinical applicability of FPCGMs.	
Fullerenol emulsion	applied topically	Mice	X-ray (50 kV, 75 μA	N/A	FulOH protects the skin by reducing blisters, eczema, and fibrosis through TGF-β regulation. It preserves hair follicles, enhances collagen synthesis, and maintains skin structure. FulOH improves blood flow, accelerates healing, and prevents vascular damage by reducing endothelial cell injury and apoptosis. Its antioxidant effects boost GSH-Px, upregulate VEGF, and promote angiogenesis, enhancing endothelial cell growth, blood vessel formation, and tissue repair.	[123]
Fullerenol (MGAF)	25 μg/mL	BALB/c mice	X-ray (160 kV, 25 mA)	15 Gy	MGAF hydrogels containing FulOH protected against radiation-induced damage, both before and after radiation exposure. FulOH eliminated excess of ROS and increased the levels of enzymatic antioxidants, protected cells from apoptosis and promoted tissue regeneration. The hydrogels adhered to the oral mucosa, enhancing the effective concentration of FOH. Additionally, they exhibit high biocompatibility and biological safety.	[121]

the cores. Endofullerenols with metal atoms of larger neutron absorption cross-sections, such as samarium (Sm) and europium (Eu), exhibited dominance in neutron capture in the initial irradiation phase, leading to gamma emission and expulsion of atoms from the molecule. This mechanism was not observed in the case of endofullerenols with metals of smaller neutron absorption cross-sections, such as Tm, Ho, or Co [164].

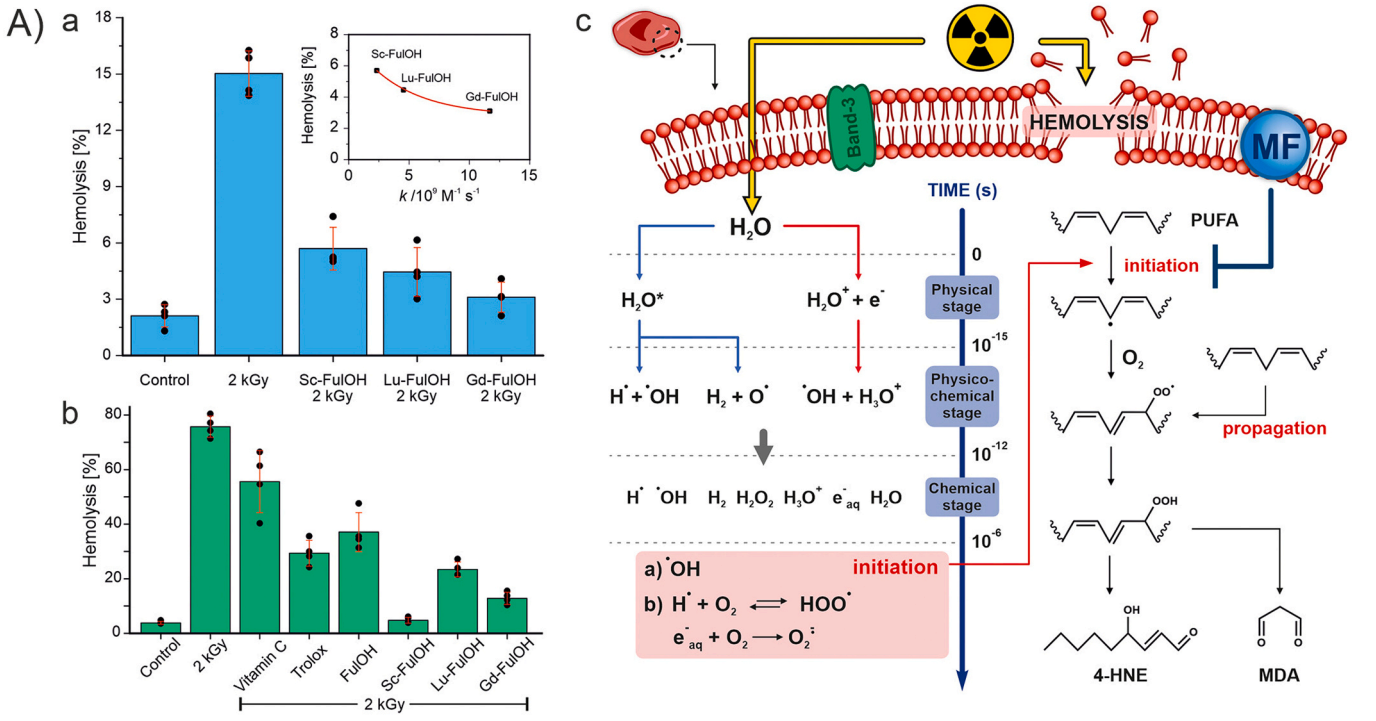
In many *in vitro* and *in vivo* studies, Me@FulOHs have shown significant therapeutic effects not only due to their antioxidant properties but also their ability to regulate gene expression involved in apoptosis, angiogenesis, and stimulation of the immune response [26,165–167]. Nanoparticles $[Gd@C_{82}(OH)_{22}]_n$, administered to tumor-affected mice, primarily accumulated in the bones, pancreas, spleen, kidneys, and liver, with fewer amounts in the H22 tumor and lungs, thus, the improvements in the functioning of damaged kidneys and livers in tumor-induced mice was observed in line with localization of antioxidant [116]. Such targeted delivery of nanoparticles represents a promising prospect for drug delivery systems [168].

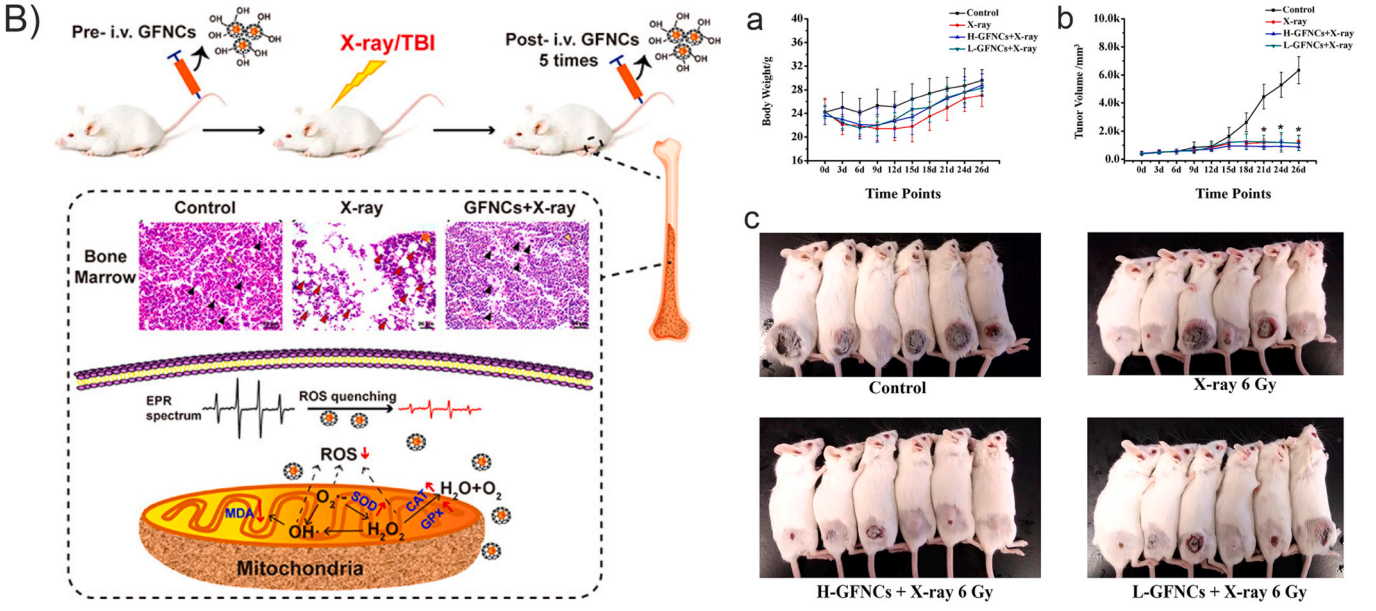
One of the serious side effects of radiotherapy is bone marrow suppression [169]. Damage to the bone marrow, characterized by abnormalities in stem cells, progenitor cells, and the stroma, often occurs after cytotoxic treatment. This damage can remain latent, not causing an immediate decrease in the number of mature functional cells in the blood, but it may manifest later as hypoplastic syndrome [170]. Jia et al. [117] demonstrated that administration of gadofullerene nanocrystals (GFNC) significantly reduced radiation-induced bone marrow suppression in mice without a negative impact on the anti-tumor efficacy of radiotherapy (Fig. 6B). Indicators such as MDA, SOD, GPx, and CAT demonstrate that GFNC decreased the level of oxidative stress. The same researchers investigated the effects of gadofullerene nanoparticles (GFNP) on myelosuppression (reduced activity of blood cell precursors in bone marrow) induced by radiotherapy [171]. Particularly, GFNP increased leukocyte production and alleviated pathological bone marrow conditions. GFNP stimulated the differentiation, development, and maturation of leukocytes (neutrophils, lymphocytes) in irradiated mice more effectively than growth factor (G-CSF). Additionally, GFNP showed minimal toxicity toward major organs such as the heart, liver, spleen, lungs, and kidneys, which is crucial since growth factors such as G-CSF exhibit multiple side effects including bone pain, liver damage, and lung toxicity, limiting their clinical application [171] (Table 3).

Finding Me@FulOHs capable of protecting normal cells from radiation-induced stress while being toxic to cancer cells would be of fundamental importance for the outcome of modern radiotherapy. The selective biodistribution of Me@FulOH and their ability to remove radicals and inhibit tumor growth [172] facilitates the protection of healthy tissues/organs. Future research should further investigate the synergy between metallofullerenol-based imaging and radioprotection, focusing on cellular contexts and environmental factors that optimize their protective effects. Integrating of these nanomaterials into theranostic frameworks holds great potential for enhancing both diagnostic precision and therapeutic efficacy in radiotherapy.

9. Challenges and opportunities in translating fullerenols and metallofullerenols into clinical translation as radioprotective agents

The clinical translation of FulOHs and Me@FulOHs as radiotherapy protective agents presents both significant challenges and promising opportunities. Key challenges include evaluating their long-term safety and biocompatibility, addressing concerns about potential toxicity, bioaccumulation, and variability in patient responses. Optimizing pharmacokinetics (*i.e.*, enhancing absorption, improving targeted delivery, and minimizing off-target effects) is also crucial. Scalable production of high-purity compounds poses another challenge, as does navigating complex regulatory pathways that require strong evidence of safety and efficacy. A deeper understanding of the molecular mechanisms is essential to refine their applications and maximize their benefits. Despite these challenges, FulOHs and Me@FulOHs offer exciting opportunities. Their potent antioxidant properties make them effective in scavenging reactive oxygen species, thereby protecting healthy





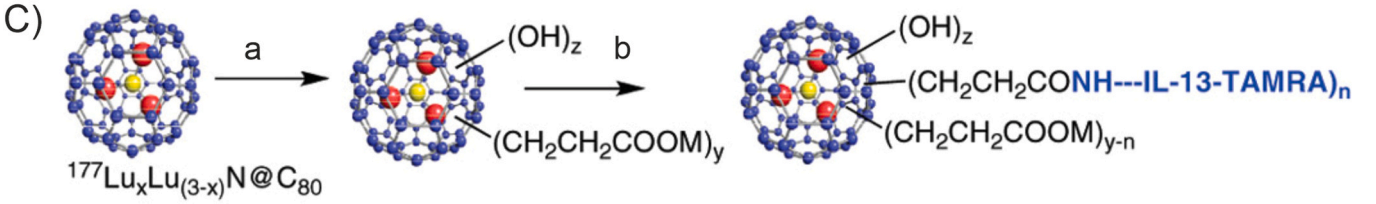


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Fig. 6. A) Impact of radiation and the role of metallofullerenols (Me@FulOHs) on erythrocyte hemolysis and potential protective applications. The subpanels illustrate: a) Degree of erythrocyte hemolysis after radiation-induced $HO^{\bullet}$ radicals in control conditions (non-irradiated), irradiated without Me@FulOHs, and with 25 μM Me@FulOHs. The inset shows hemolysis as a function of Me@FulOHs reaction rate with $HO^{\bullet}$. b) Chemical and biological effects of IR on erythrocytes, highlighting Me@FulOHs antioxidative properties (Me@FulOHs can act both as antioxidants - preventing lipid peroxidation and as interventional agents - neutralizing peroxyl radicals). c) Hemolysis after γ-radiation exposure in the presence of various Me@FulOHs (including Sc3N, Lu3N and Gd), showing superior protection compared to vitamin C and Trolox. Reproduced from Grebowski et al. [114] under CC BY 4.0 B) Schematic representation of the effect of gadolinium fullerene nanocrystals (GFNC) to protect mice from radiation damage without compromising cancer radiotherapy effectiveness: a) Plot of body weight changes. b) Plot of tumor growth inhibitory curves in different groups. c) Photos of tumors from different groups of treated mice after euthanasia. Reprinted with permission from [117]. Copyright ©2019 American Chemical Society. C) Synthesis of a metalofullerenol-based radiopharmaceutical coupled with IL-13 peptide for glioblastoma targeting, offering flexibility in radiotherapeutic and radiodiagnostic applications. Reprinted with permission from [160]. Copyright ©2010 American Chemical Society.

Table 3
Characteristics of metallofullerenol as potential radioprotective agents.

Type of Me@FulOH	Dose Concentration	Experimental Model	Radiation Source Energy	Absorbed Dose	Main results	Ref.
$Sc_3N@C_{80}(OH)_{18}$ $Lu_3N@C_{80}(OH)_{18}$ $Gd@C_{82}(OH)_{22}$	25 μM	Human erythrocytes (*in vitro*)	γ-rays[A] ^{60}Co or High energy electrons[B] (6 MeV)	2000 Gy	Me@FulOHs protected erythrocytes (RBCs) from hemolysis regardless of the type of radical generated. In an anaerobic environment (N_2O), their protective activity was correlated with reactivity toward the hydroxyl radical ($HO^{\bullet}$), while in the presence of oxygen, the mechanism of oxidative damage shifted to peroxidation mediated by lipid peroxyl radicals ($LOO^{\bullet}$). The ability of Me@FulOH to trap $LOO^{\bullet}$ radicals proved to be crucial in protecting against oxidative damage, especially in erythrocytes exposed to γ-radiation in an air atmosphere.	[114]
Gadofullerene nanocrystals (GFNCs)	8 μmol/kg (low dose), 16 μmol/kg (high dose)	Mice (tumor-bearing and healthy models) (*in vivo*)	X-rays	3, 4.5, 6 Gy	Protection against myelosuppression, normalization of oxidative stress markers (MDA, SOD, GPx, CAT); protection of bone marrow without interfering with the anticancer effects of radiotherapy. GFNP enhanced leukocyte production and alleviated bone marrow pathology, promoting differentiation, development, and maturation of leukocytes (neutrophils, lymphocytes) in irradiated mice more effectively than G-CSF, with minimal toxicity to major organs such as the heart, liver, spleen, lungs, and kidneys.	[117, 171]

[A] System 1: High Energy Electrons, Anaerobic, under N_2O.
[B] System 2: γ-rays, under Air Atmosphere.

tissues from radiation-induced damage. Metallofullerenols offer high-resolution PET and MRI imaging, enabling precise localization of radiation-damaged tissues or areas of oxidative stress. This precision supports targeted treatment planning and real-time monitoring of oxidative stress, optimizing radioprotective interventions while minimizing risks. Their dual functionality aligns with modern oncology goals by integrating diagnostics with radioprotection, addressing the challenge of treating tumors while preserving healthy tissue. Unlike conventional radioprotectors, metallofullerenols combine imaging and therapeutic capabilities, promising significant advancements in radiotherapy. Future research should refine their protective effects in specific cellular and environmental settings. Advances in nanotechnology will improve targeted delivery, improving therapeutic outcomes while minimizing systemic toxicity. Promising preclinical results provide a solid foundation for advancing these compounds to clinical trials. Beyond radiotherapy, their potential applications extend to other areas, such as protection against accidental radiation exposure and use in space exploration. In conclusion, while the road to clinical translation is complex, the unique properties and therapeutic potential of FulOHs and Me@FulOHs underscore their promise as radiotherapy protective agents. Addressing these challenges through multidisciplinary research and innovation will be crucial in realizing their full clinical potential.

10. Conclusion

Despite the fact that many preclinical studies have demonstrated significant advances in the development of radioprotective measures, the clinical use of most radioprotectors/radiomitigators remains very limited due to their lack of efficacy and/or the high toxicity of the evaluated agents. Furthermore, in the case of oncological radiotherapy, the use of these agents may compromise the effectiveness of the treatment. Future studies should focus on systematic evaluation of the context-dependent effects of FulOH and Me@FulOH, with emphasis on the identification of specific cellular environments and conditions (*e.g.* before and after irradiation) that optimize their radioprotective effects while minimizing pro-oxidative risks. Given the advancements in theranostic techniques, contemporary oncology, and radiotherapy should focus on minimizing the side effects while ensuring rapid and precise diagnosis. Metallofullerenols can serve as an effective diagnostic tool enabling high-quality diagnostic imaging to be used in PET and MRI. Their ability to generate strong signals in imaging techniques allows for precise localization of pathogenically altered areas, which is crucial in diagnostics. Furthermore, the properties of Me@FulOHs make them better candidates for next-generation compounds reducing oxidative stress compared to those currently in use. The combination of Me@FulOHs with theragnostic techniques paves the way for the development of advanced diagnostic and therapeutic tools, utilizing nanotechnologies for disease detection and treatment at very early stages. The dual use of Me@FulOHs, especially in radiotherapy, distinguishes these nanomaterials from other radioprotectors/radiomitigators, potentially leading to further research and innovative clinical applications.

CRediT authorship contribution statement

Grebowski Jacek: Writing – review & editing, Writing – original draft, Visualization, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization. **Litwinienko Grzegorz:** Writing – review & editing, Investigation. **Kazmierska-Grebowska Paulina:** Writing – review & editing, Writing – original draft, Investigation. **Obrador Elena:** Writing – review & editing, Writing – original draft, Investigation. **Jankowski Maciej M:** Writing – review & editing,

Visualization, Investigation. **Kolodziejczyk-Czepas Joanna:** Writing – review & editing, Writing – original draft, Investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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