# RADIOPROTECTOR DEVELOPMENT PROGRAM IN DAE: PAST, PRESENT AND FUTURE

**Rahul Checker, R.S. Patwardan, D.K. Maurya,
Deepak Sharma, Santosh K. Sandur and Tapan Kumar Ghanty**

Radiation Biology & Health Sciences Division, Bio-science Group,
Bhabha Atomic Research Centre, Trombay, Mumbai-400085, India.
Homi Bhabha National Institute, Anushaktinagar, Mumbai-400094, India.

E-mail: tapang@barc.gov.in

**Abstract:**

Ionizing radiations (IR), which includes X-rays and gamma-rays, interact with critical cellular biomolecules indirectly via radiolysis of water molecules to generate free radicals in the cytoplasm or directly through interaction with cellular components like DNA, lipids and proteins. The degree of cellular damage is governed by the dose of IR and it can have two major outcomes namely cell cycle arrest that allows the cell to repair radiation induced damage or cell death.

Radiotherapy is one of the key treatment modalities for solid tumors and it is often associated with severe side effects due to exposure of surrounding normal tissue to radiation. Despite technical advancements in the delivery of radiation, damage to normal cells and tissues is a limiting factor in radiotherapy. In addition to this, there are chances of fortuitous exposure of IR to workers and general public during accidents at nuclear power plants or as a result of nuclear warfare. In view of the aforementioned scenarios, it is essential to identify novel approaches and drugs to prevent injury to normal tissues and increase the therapeutic ratio of radiotherapy and also to protect human lives during unplanned exposure settings.

This chapter chronicles the recent advancements in radioprotection research worldwide and underscores the evolution of radioprotector development program at DAE, India over the last three decades.

## 1.0 Introduction:

Henri Becquerel, in 1896, proclaimed the discovery of radioactivity to the Academy of Sciences in Paris and the term "radioactivity" was later coined by Marie Curie in 1898. Subsequent to the discovery of radiation, extensive research has been carried out for more than a century in the field of radiation. It has allowed scientists to garner immense understanding of the health effects of radiation exposure and the mechanisms by which radiation interacts with biological systems (Fig 1) and the environment. Radiation exposure can damage critical biomolecules like deoxyribonucleic acid (DNA) by direct interaction or indirectly via radiolysis of water molecules. This damage can induce cell death by a variety of mechanisms such as apoptosis (programmed cell death), necrosis, autophagy or interphase death [1]. The type and mechanisms of radiation induced cell death is governed by several factors such as radiation dose, dose rate, cell type (normal or tumour cell) and the stage of cell cycle [2]. If a large number of cells are killed due to radiation exposure, it may lead to organ dysfunction and sometimes even death of the organism. Direct interaction of radiation causes damage to DNA which can be repaired by DNA repair enzymes or signals for cell death can be induced if the damage is not repaired. However, in some cases, erroneous or unrepaired DNA damage can lead to mutations and alteration in the genome. Double-strand breaks (DSBs) in DNA are the most pertinent lesions responsible for the deleterious effects of IR [3]. The genetic alterations or chromosomal aberrations are manifested when the cell divides and ultimately lead to neoplastic transformation i.e. the conversion of a normal cell to a cancer cell. Further, if genetic alterations occur in cells which are responsible for transmitting hereditary information, it may give rise to genetic disorders in the descendants.

Interestingly, the capability of radiation to induce cell death via DNA damage, especially double-strand breaks, has been applied deliberately to destroy cancer cells. This modality of cancer treatment is aimed at eliminating tumour cells or shrinking the tumour mass by exposing the tumour to ionizing radiation and is called as radiotherapy. It is a cornerstone of cancer therapy and widely used in conjunction with surgery, chemotherapy, immunotherapy and other targeted therapies. Radiotherapy treatment is given via two different modalities a) teletherapy or external radiotherapy wherein radiation source is outside the body of the patient; b) brachytherapy-wherein sealed radioactive sources are placed within the body of the patient either temporarily or permanently. Approximately 19 million new cases of cancer are detected every year globally, with 10 million deaths, and the projections indicate 26 million new cancer cases by 2030. Around 50% cancer patients receive radiation therapy [4]. The dose of IR required for the treatment of solid cancers depends on stage and type of cancer and typically ranges from 30 to 80 Gy. However, exposure of patients to a high single dose can cause severe toxicity. Hence, in order to limit the side effects, fractions of 2-4 Gy radiation dose per day are given over a period of several weeks. The fractionated dose regime induces extensive DNA damage in rapidly dividing tumour cells leading to their preferential killing as compared to normal cells. The time between consecutive radiation doses allows repair of the DNA damage in slow dividing / non-dividing normal cells. However, radiation exposure of surrounding normal tissue inevitably leads to multiple short and long -term side effects such as nausea, diarrhoea and hair-loss [5]. Recent technological advancements have enabled clinicians to reduce the side effects associated with radiotherapy [6]. However, the

complex relationship between tumour and normal tissue makes it difficult to completely avoid normal tissue toxicity.

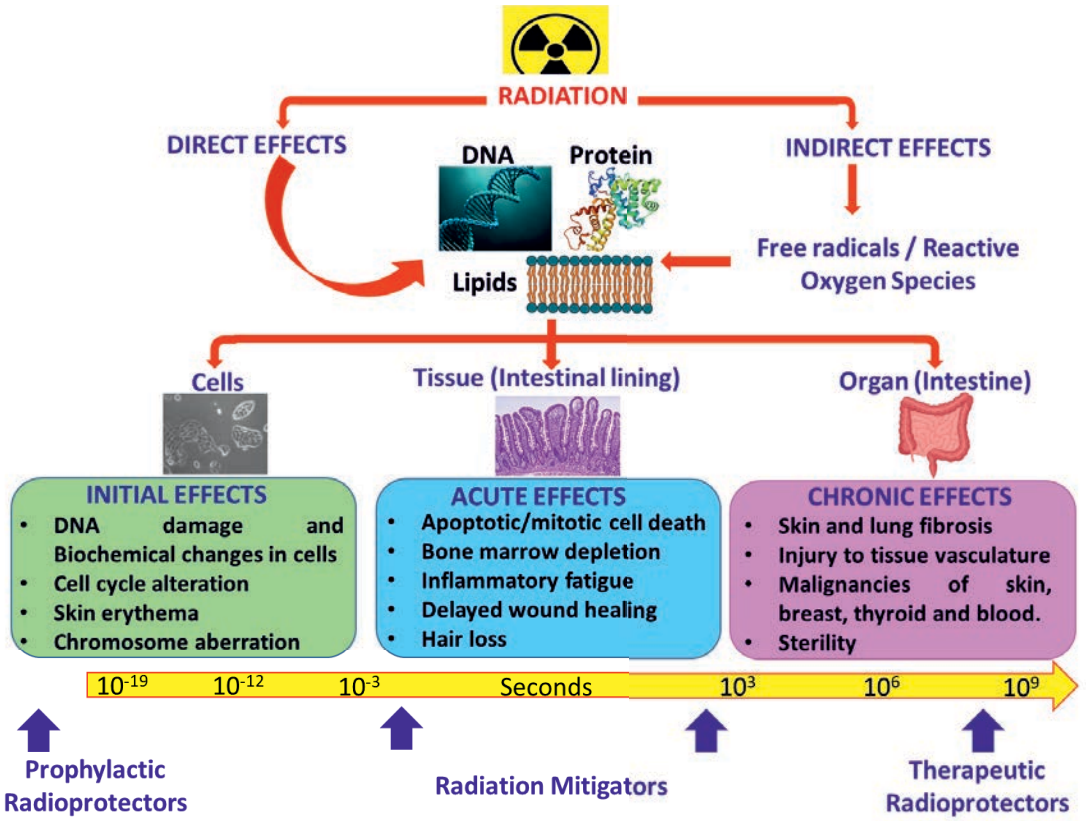



**Figure 1: Timeline for Biological Effects of Radiation**

Further, unplanned exposure of nuclear workers or general public to radiation is possible during accidents at nuclear facilities such as the 1986 Chernobyl accident, 1999 Tokai-Mura in Japan and the 2011 Fukushima-Daiichi accident or as a consequence of "dirty bombing. Furthermore, a broad spectrum of industrial applications employ radiation sources such as sterilization of medical/pharmaceutical products, food preservation, insect sterilisation, industrial radiography, oil or gas exploration. Although industrial applications of IR cause very low levels of exposure, in case of any accident there is possibility of localized contamination and high levels of radiation exposure.

In view of the above mentioned planned as well as unplanned radiation exposures, there is an unmet need to identify novel "radioprotective agents" to prevent toxicity of normal tissues. An ideal radioprotector has the following characteristics:

1. It should protect normal tissues against acute and chronic effects of radiation.
2. It should not protect tumour tissues from radiation.

3. It should be easy to administer (preferably oral) and rapidly absorbed and distributed in the body.
4. It should not be associated with side-effects.
5. It should be readily available and inexpensive
6. It should be chemically stable to allow easy handling and storage
7. It should have a long shelf life.

Further, based on the administration time, radioprotectors are classified as [7] (Fig 1)

1. **Prophylactic agents:** These agents are administered prior to IR exposure to prevent damage. Most prophylactic radioprotectors are free radical scavengers and anti-oxidants[8].
2. **Radiation mitigators:** These agents are given during or immediately after IR exposure to prevent or reduce the effect of IR on tissues before symptoms appear.
3. **Therapeutic agents:** These agents are given after development of clinical symptoms of exposure to IR. They reverse the deleterious effects of radiation and boost tissue recovery.

**2.0 Approved radiation countermeasures and molecules at advanced stages of development:** Research on radioprotectors started in 1949 and during the last seven decades only few agents have received US FDA approval for mitigating radiation injury, and several drugs are currently under clinical trials. To date, there is no US FDA approved radioprotector which can specifically prevent or treat hematopoietic acute radiation syndrome (H-ARS) and gastrointestinal ARS (GI-ARS). As shown in Table 1, amifostine is approved for preventing xerostomia in head and neck cancer patients undergoing radiotherapy. In addition, four growth factors have been repurposed as radiomitigators: Neupogen (filgrastim), Neulasta (PEGylated filgrastim), Leukine (sargramostim), and Nplate (romiplostim) [9].

**Table 1: US-FDA approved radioprotectors and radiomitigators**

| S.No | Name | Chemical/biological name | Indication | Mechanism of action |
|---|---|---|---|---|
| 1 | Amifostine (WR-2721) | 2-[(3-aminopropyl)amino] ethanethiol dihydrogen phosphate | To decrease xerostomia in radiotherapy for head and neck cancers | Free radical scavenger |
| 2 | Neupogen (Filgrastim) | Granulocyte-colony stimulating factor (G-CSF) | Patients exposed to myelo-suppressive doses of radiation | Stimulates the proliferation and differentiation, of neu- |

| | | | | |
|---|---|---|---|---|
| | | | | trophil precursors and maturation and function of neutrophils. |
| 3 | Neulasta (PE-Gylated filgrastim) | PEGylated G-CSF | Patients exposed to myelo-suppressive doses of radiation | Similar mode of action as G-CSF. Longer half-life requiring less frequent administration |
| 4 | Leukine (Sargramostim) | Granulocyte-macrophage colony-stimulating factor (GM-CSF) | Patients acutely exposed to myelo-suppressive doses of radiation | Stimulates the proliferation, differentiation, and function of Neutrophil, monocytes/ macrophages and hematopoietic progenitors |
| 5 | Nplate (Romiplostim) | Chimeric recombinant biologic (thrombopoietin receptor agonist) | Patients exposed to myelo-suppressive doses of radiation | Thrombopoietin receptor agonist increases platelet production. |

**3.0 Radioprotector development program in DAE:** Radioprotective agents find applications in diverse areas of human endeavours such as protection of normal cells during radiotherapy, as a protector for astronauts during spaceflight and countermeasures during nuclear warfare. The radioprotector development program started in DAE during the 1990s with the aim of identifying novel targets and molecules that can be appropriately applied in different planned and unplanned radiation exposure scenarios. Some of the studies are at pre-clinical stages of research whereas one is in advanced stages of clinical translation as detailed in the chapter and summarised in Table 2.

**3.1 Prophylactic radioprotectors:**

Biological effects of radiation involve generation of ROS that damage critical biomolecules inside the cells such as DNA, lipids and proteins resulting in the death of normal cells and also depletion of stem cells. In 1949, Patt et al, demonstrated for the first time that antioxidants can protect against radiation injury after which several investigators have shown that anti-oxidants and free radical scavengers such as N-acetyl cysteine, glutathione, vitamin E and vitamin C can protect mice against lethal radiation. However, several limitations have led to failure of clinical translation of anti-oxidants as radioprotectors such as low bioavailability and undesirable tissue distribution. Further, it is vital to recognize that cellular antioxidant defence is primarily regulated by antioxidant enzymes using their specific

substrates to reduce oxidants. Hence, it will be a prudent strategy to upregulate the cellular antioxidant defence machinery to limit ROS production. Studies carried out in Radiation Biology & Health Sciences Division, Bio-Science Group showed that contrary to the widespread concept of antioxidants as radioprotectors, pro-oxidants can also function as radioprotectors. Three pro-oxidants, 1,4-naphthoquinone (NQ), Plumbagin and Withaferin A have been shown to act as radioprotectors via activation of cellular antioxidant machinery. These pro-oxidants protect normal cells against IR injury via their ability to activate anti-oxidant and pro-survival signaling inside the cells.

### 3.1.1: Anti-oxidants as radioprotectors:

**a) Baicalein (BCL):** Baicalein, isolated from an Ayurvedic medicinal plant *Terminalia arjuna* is a lipoxygenase (12-LOX) inhibitor with multiple health beneficial effects (Fig. 2). Baicalein was shown to reduce radiation induced oxidative stress and protected mouse lymphocytes from radiation-induced cell death via inhibition of MKP3 and activation of ERK/Nrf2 pathway. Baicalein protected mice against whole body irradiation (WBI) induced death (Fig 4A) [10]. Oral administration of solid lipid nanoparticles of baicalein (SLNB; 25mg/kg twice a day) prevented radiation-induced mortality and morbidity in mice [11]. Baicalein has been shown to be safe and well tolerated by healthy human subjects. Owing to radioprotective, anti-leukemic and immune-suppressive properties, use of baicalein as an adjuvant during radiotherapy of lymphoma appears to be a precocious approach to increase the therapeutic gain.

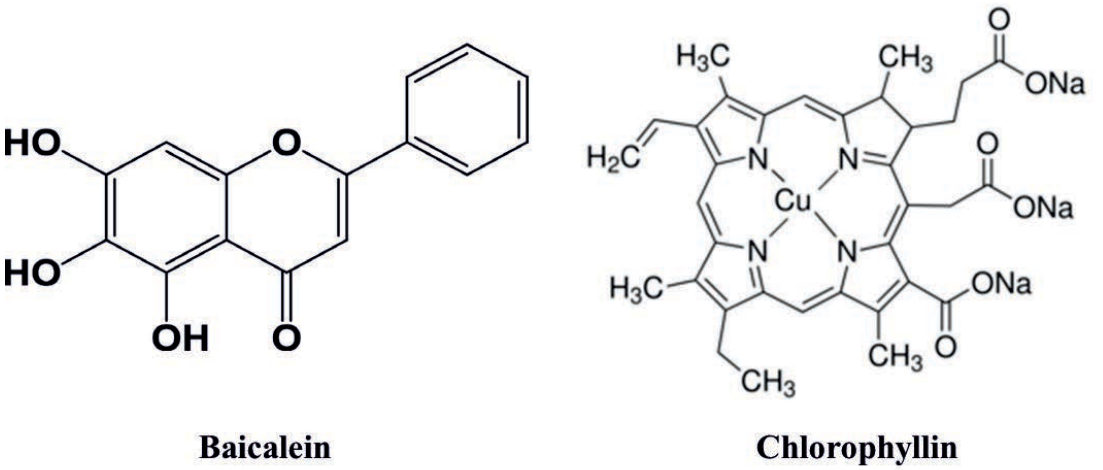



**Figure 2: Structures of Baicalein and Chlorophyllin.**

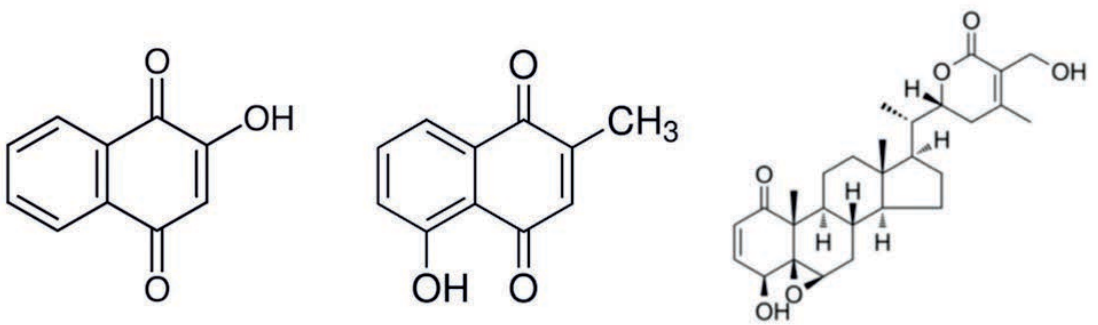
**b) Chlorophyllin (CHL):** It is a water-soluble derivative of chlorophyll derived by replacing the central magnesium atom with copper or sodium (Fig. 2B). CHL is used as a food colorant known as natural green 3 (E141). Chlorophyllin reduced 6 Gy gamma radiation induced single-strand breaks (SSBs) in pBR322 plasmid DNA. CHL inhibited the formation of 5,5-dimethyl-1-pyrroline-N-oxide adduct with hydroxyl radicals (DMPO-OH adduct) generated by γ-radiation [12]. Moreover, CHL significantly inhibited whole body irradiation induced lipid

peroxidation and apoptosis in lymphocytes. CHL significantly augmented the abundance of stem cells in bone marrow and expedited the recovery from whole body irradiation (4 Gy WBI) induced aplasia. CHL upregulated antiapoptotic genes and the antioxidant defence mechanisms by activating prosurvival transcription factors Nrf-2 and NF-κB. Prophylactic administration of CHL significantly mitigated radiation-induced mortality in mice following exposure to lethal radiation (Fig. 4A). Further, CHL administration provided significant protection against WBI damage to intestine and lungs [13]. Most importantly, CHL enhanced IR-induced killing of human breast cancer cells in vitro and in xenograft tumor-bearing SCID mice [14]. In summary, CHL displayed promising radio-modifying effects in both normal and tumor cells, warranting further investigation regarding its potential use as an adjuvant to radiotherapy.

**c) Ferulic acid (FA)**: It is a monophenolic phenylpropanoid that naturally occurs in various plant-based sources. Our research demonstrated the remarkable radioprotective properties of FA in both in vitro and in vivo settings. FA effectively enhanced DNA repair processes in leukocytes of mice and demonstrated a notable preference for protecting normal tissues over tumor cells [15]. Our findings suggest that FA's radioprotective efficacy may be attributed to the early recovery of stem cells, facilitated by enhanced production at G-CSF and erythropoietin.

**3.1.2: Pro-oxidants as radioprotectors:** As mentioned previously, our **s**tudies showed that pro-oxidant can protect against IR mediated damage.

**a) 1,4-naphthoquinone (NQ):** It was shown that induction of mild oxidative stress using pro-oxidants such as $H_2O_2$ and NQ (Fig. 3) protected normal cells and mice against radiation-induced apoptosis (Fig 4B). A pro-survival signaling protein, called as Nuclear factor erythroid-2–related factor 2 (Nrf2), was activated in cells in response to low levels of oxidative stress as an adaptive response. Activation of Nrf2 signaling pathway upregulated the cyto-protective responses inside the cells and conferred protection against IR damage. NQ activated Nrf2 pathway and augmented the levels of protective gene hemeoxygenase-1 in normal cells. NQ also protected mice against whole-body irradiation induced mortality [16].




**1,4-naphthoquinone** **Plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone)** **Withaferin A**

**Figure 3: Structures of Prophylactic Radioprotectors**

**b) Plumbagin (PG):** In another study, Nrf2 activator molecule plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone) which is isolated from Ayurvedic medicinal plant *Plumbago zeylanica* (Chitrak) was used as a prooxidant (Fig. 3). PG protected normal cells, but not tumor cells, against IR mediated apoptosis. PG inhibited radiation induced activation of cell death activator protein called as caspase-3. PG also protected mice against radiation induced death (Fig 4B) [17].

**c) Withaferin A (WA):** It is a steroidal lactone isolated from roots of *Withania somnifera* (Ashwagandha) (Fig. 3). Ashwagandha is used in Ayurvedic, Siddha, and Unani medicine as a medicament for multiple disorders [18]. Withaferin A was shown to protect normal cells against IR-induced apoptosis. Withaferin A also activated pro-survival protein Nrf2 and increased the levels of anti-oxidant proteins in normal cells. Administration of Withaferin A to mice reduced IR-induced DNA damage and mortality (Fig 4B) [19].

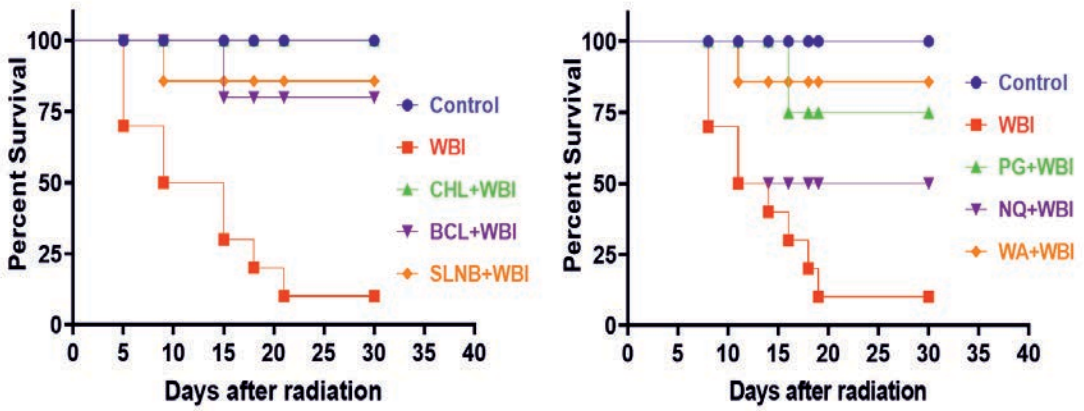



**Figure 4: Administration of (A) Chlorophyllin, Baicalein and SLNB or (B) 1,4-naphthoquinone, Plumbagin and Withaferin A reduced radiation-induced mortality in mice.**

**Table 2: Radioprotectors being developed in DAE**

| S. No | Name | Source | In vitro model system and concentration | In vivo model system and dose | Mechanism of action |
|---|---|---|---|---|---|
| **Prophylactic Radioprotectors** | | | | | |
| **1** | Baicalein | Natural (isolated from roots of *Scutellaria baicalensis*) | Murine splenocytes and 50 μM | Mice and 10 mg/kg body weight | Increase in HSPCs abundance in bone marrow and activation of ERK/Nrf2 |

| | | | | | |
|---|---|---|---|---|---|
| **2** | Chlorophyllin | Semi-synthetic (Copper-derivative of chlorophyll) | Murine splenocytes and 50 μM | Mice and 180 mg/kg body weight | Increase in HSPCs abundance in bone marrow and activation of Nrf2 |
| **3** | Ferulic Acid (4-hydroxy-3-methoxycinnamic acid) | Natural | Plasmid pBR322 and 500 μM | Mice and 50, 75 and 100 mg/kg body weight | Increased DNA repair pathways |
| **4** | 1,4-naphthoquinone | Synthetic | Murine splenocytes and 1 μM | Mice and 2 mg/kg body weight | Activation of Nrf2 signaling. |
| **5** | Plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone) | Natural (roots of *Plumbago zeylanica*) | Murine splenocytes and 1 μM | Mice and 2 mg/kg body weight | Activation of Nrf2 signaling and caspase inhibition |
| **6** | Withaferin A | Natural (isolated from roots of *Ashwagandha*) | Murine splenocytes and 10 μM | Mice and 10 mg/kg body weight | Activation of Nrf2 signaling |
| **Radiation Mitigators** | | | | | |
| **1** | Lectin | Recombinant proteins produced in *E. coli* | Murine splenocytes and 2.5 μM | Mice and 2 mg/kg body weight | Activation of T cells for production of regenerative cytokines, increase in HSPC and skin stem cells |
| **Therapeutic Radioprotectors** | | | | | |
| **1** | WJ-MSCs | Mesenchymal stem cells | Murine splenocytes | Mice and 0.25 to 1 million cells per mouse | Releasing soluble mediators to regenerate host HSCs and ISCs |
| **2** | WJ-MSCs-CM | Mesenchymal stem cells conditioned | Murine splenocytes | Mice and 100 to 200μl per mice | G-CSF present in CM help in stem cell |

| | | | | | |
|---|---|---|---|---|---|
| | | media | | | mobilization and enhances survival |
| **Selenium Based radioprotectors** | | | | | |
| **1** | Selenocystine (Diselenide amino acid;CysSeSeCys) | Naturally-occurring amino acid | | Mice and 0.5 mg/kg body weight | Cell cycle control and DNA repair |
| **2** | DSNA | Synthetic organoselenium compound | CHO cells and 25 μM | - | Increased intracellular GPx and GSH level |
| **3** | DHS | Synthetic Water soluble cyclic monoselenide | CHO cells and 25 μM | - | Elevated GPx activity, enhanced DNA repair. |
| **4** | DSePA | Synthetic (Selenocystine derivative) | - | Mice and 2 & 2.5 mg/kg body weight | NF-κB/IL-17/G-CSF/neutrophil axis activation |

**3.2 Radiation mitigators:** The molecules described in previous section perform well in prophylactic scenarios but may not provide substantial help in situations involving lethal or supra-lethal doses of radiation. In this direction, we have identified a plant lectin that can provide complete protection against mortality induced by WBI in mice. Lectin administration significantly enhanced the population of stem cells in the bone marrow and epithelial stem cells in the skin of mice potentially contributing to the observed therapeutic radioprotection. Mechanistic investigations have shown that lectin simultaneously activates pro-survival transcription factors Nrf2 and NF-κB, which may play a pivotal role in protection against high doses of ionizing radiation. Further investigations are ongoing to assess the safety of lectin and to enhance its efficacy in animal models.

**3.3 Therapeutic radioprotectors:** Therapeutic radioprotectors are pharmaceutical agents designed to protect healthy tissues from radiation damages caused during cancer treatment or nuclear accidents. These agents minimize the toxic side effects of radiation by enhancing cellular repair and through regenerative mechanisms. In this direction, stem cell therapy offers tissue regenerative capabilities for mitigating the toxic effects associated with Acute Radiation Syndrome (ARS). Recently, administration of human WJ-MSCs or their conditioned media (CM) to mice was shown to significantly enhance their survival following

exposure to lethal dose of radiation (Fig.5). WJ-MSCs migrated to radiosensitive tissues in irradiated mice and contributed to recovery from IR-induced damage. WJ-MSCs facilitated repair of damaged tissues and modulated the local microenvironment by secreting various cytokines and growth factors. Remarkably, therapeutic effect of WJ-MSCs was observed even when transplantation was performed 24 hours after whole-body irradiation (WBI). Interleukin-6 and G-CSF originating from WJ-MSCs and host (mouse) Nrf-2 were identified as essential mediators of radioprotection. Hence, WJ-MSCs can be a promising modality for facilitating recovery from toxicity caused by radiation exposure and in situations involving accidental radiation exposure.

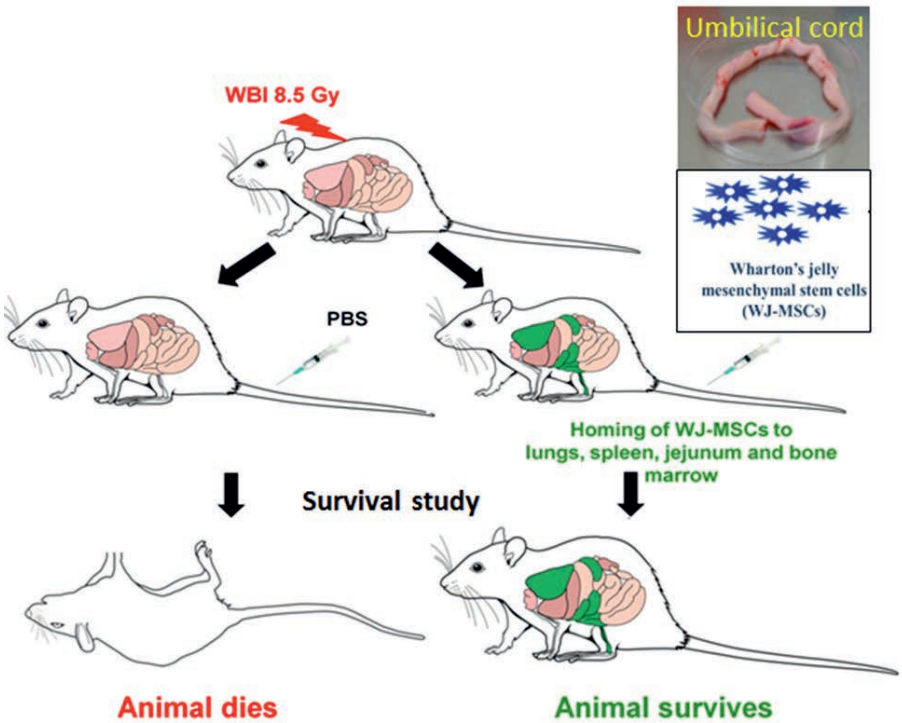



**Figure 5: Therapeutic radioprotection by WJ-MSCs**

**4.0 Selenium Based radioprotectors:** Selenium compounds play important roles in biology, primarily as trace elements essential for the proper functioning of various enzymes and proteins, including antioxidant defence and immune support. Some derivatives of selenium compounds, such as selenocystine, diselenonicotinamide (DSNA), dihydroxy-1-selenolane (DHS), and 3, 3'-diselenodipropionic acid (DSePA), were evaluated for their radioprotective properties. Selenocystine administration prior to radiation effectively mitigated DNA damage in hepatic tissue by accelerating repair mechanisms. Transcriptional analysis revealed its ability to modulate the expression of key genes involved in cell cycle control and DNA repair [20]. In another study, DSNA was found to significantly protect Chinese Hamster Ovary (CHO) cells from radiation-induced death, with a dose modification factor (DMF) of 1.26. Mechanistic insights revealed that DSNA increased the intracellular levels of GPx and GSH, mitigated radiation-induced DNA damage, and showed involvement of pro-survival pathways

[21]. The radioprotective efficacy of DHS were explored and it was observed that treatment of CHO epithelial cells with DHS protected them from radiation-induced mitotic cell death [22]. Extensive studies have been carried out to study the radioprotective efficacy of DSePA, a selenium derivative. Administration of DSePA offered significant protection to hepatic tissue, spleen, and gastrointestinal (GI) tract and enhanced the survival of mice exposed to both sub-lethal and supra-lethal doses of radiation [23]. Further, oral administration of DSePA delayed the onset of radiation induced lung damage and improved asymptomatic survival [24]. In summary, various selenium derivatives have been tested and have shown potent radioprotective effects which underscores the potential of selenium compounds in mitigating the harmful effects of radiation.

**5.0 Future directions and perspectives:** Radioprotective agents are crucial for nuclear emergency medical plans and treatment of toxicities seen during cancer radiotherapy. Nevertheless, only a limited number of drugs have received approval for treating radiation injuries, and our understanding of their mechanisms remains incomplete. As the risk of radiation exposure increases in tandem with advancements in nuclear technology, developed nations have established nuclear emergency policies that focus on the creation of specialized radioprotective agents. Cytokines are central players in governing various biological processes, including hematopoiesis, immune responses, neural functions, inflammation, and the healing of wounds. Nplate (romiplostim), Leukine (sargramostim), Neulasta (PEGylated filgrastim), and Neupogen (filgrastim) have been included in US national stockpile for clinical use as radiomitigators for H-ARS. Despite significant progress made in the last six decades, we have not fully grasped the precise mechanisms underlying radiation injury, and a comprehensive strategy for treating it remains elusive. Future advancements in radioprotective agents should prioritize the following objectives. Firstly, we should explore the modification of existing drugs to enhance their effectiveness. Additionally, combining multiple radioprotective agents from diverse classes (pharmacological, biological, gene therapy, etc.) or incorporating immunomodulatory agents alongside standard therapy holds potential to enhance efficacy while mitigating the toxicity associated with individual agents. Furthermore, our research efforts should expand the clinical applications of approved drugs as radioprotectors. To gain a more comprehensive understanding, we must expand our understanding about different types of ionizing radiations, as previous research primarily centred on low linear energy transfer (LET) radiations, such as γ-rays or X-rays. The investigation of other types of radiation, particularly high-LET radiation like protons, neutrons, and heavy ions, is imperative, given the risks associated with human space activities and the need for radiation countermeasures. Finally, our studies should emphasize development of animal models for each sub-syndrome of Acute Radiation Syndrome (ARS) to mimic the mechanisms and pathophysiology of IR-induced injuries in humans. These models are essential to meet research and drug evaluation requirements effectively. In pursuit of these objectives, we have made notable strides. In this direction, we have repurposed bosutinib, a tyrosine kinase inhibitor initially approved for lymphoma treatment, as a promising radioprotective agent. Additionally, we have crafted a nanoformulation of potential prophylactic radioprotectors, and we intend to explore its efficacy in upcoming studies. In collaboration with the private pharmaceutical industry and Tata Memorial Hospital, we are actively engaged in joint efforts to assess the effectiveness of a chlorophyllin-based

radioprotector in the context of pelvic cancer radiotherapy [CLARITY Trial NCT05348239]. Furthermore, we are diligently working on the development of cell-based therapeutics for scenarios involving accidental radiation exposure. Concurrently, we are also investigating the combination of pharmacological and cell-based approaches to mitigate the adverse effects of radiation injury. By employing these multifaceted strategies, we aim to expedite the formidable task of advancing the development of radioprotective agents.

## 6.0 References:


1. Little, J. B., Cellular effects of ionizing radiation. *N Engl J Med* **1968,** *278* (7), 369-76 concl.
2. Surova, O.; Zhivotovsky, B., Various modes of cell death induced by DNA damage. *Oncogene* **2013,** *32* (33), 3789-97.
3. Hoeijmakers, J. H., Genome maintenance mechanisms for preventing cancer. *Nature* **2001,** *411* (6835), 366-74.
4. Laskar, S. G.; Sinha, S.; Krishnatry, R.; Grau, C.; Mehta, M.; Agarwal, J. P., Access to Radiation Therapy: From Local to Global and Equality to Equity. *JCO Glob Oncol* **2022,** *8*, e2100358.
5. Stone, H. B.; Coleman, C. N.; Anscher, M. S.; McBride, W. H., Effects of radiation on normal tissue: consequences and mechanisms. *Lancet Oncol* **2003,** *4* (9), 529-36.
6. Citrin, D. E., Recent Developments in Radiotherapy. *N Engl J Med* **2017,** *377* (11), 1065-1075.
7. Hosseinimehr, S. J., Trends in the development of radioprotective agents. *Drug Discov Today* **2007,** *12* (19-20), 794-805.
8. Weiss, J. F.; Landauer, M. R., Radioprotection by antioxidants. *Ann N Y Acad Sci* **2000,** *899*, 44-60.
9. Checker, R.; Patwardhan, R. S.; Jayakumar, S.; Maurya, D. K.; Bandekar, M.; Sharma, D.; Sandur, S. K., Chemical and biological basis for development of novel radioprotective drugs for cancer therapy. *Free Radic Res* **2021,** *55* (5), 595-625.
10. Patwardhan, R. S.; Sharma, D.; Checker, R.; Sandur, S. K., Mitigation of radiation-induced hematopoietic injury via regulation of cellular MAPK/phosphatase levels and increasing hematopoietic stem cells. *Free Radic Biol Med* **2014,** *68*, 52-64.
11. Joshi, H. A.; Patwardhan, R. S.; Sharma, D.; Sandur, S. K.; Devarajan, P. V., Pre-clinical evaluation of an innovative oral nano-formulation of baicalein for modulation of radiation responses. *Int J Pharm* **2021,** *595*, 120181.
12. Kumar, S. S.; Devasagayam, T. P.; Bhushan, B.; Verma, N. C., Scavenging of reactive oxygen species by chlorophyllin: an ESR study. *Free Radic Res* **2001,** *35* (5), 563-74.
13. Suryavanshi, S.; Sharma, D.; Checker, R.; Thoh, M.; Gota, V.; Sandur, S. K.; Sainis, K. B., Amelioration of radiation-induced hematopoietic syndrome by an antioxidant

chlorophyllin through increased stem cell activity and modulation of hematopoiesis. *Free Radic Biol Med* **2015,** *85*, 56-70.

14. Sharma, D.; Sandur, S. K.; Checker, R.; Patwardhan, R. S.; Gota, V.; Sundarraj, J.; Sasi, P.; Chattopadhyay, S. Method of adjuvant treatment with chlorophyllin containing therapeutic reparation including for radioprotection of normal tissues during radiation therapy and kit therefor. US 10,183,026 B2, 2019.
15. Maurya, D. K.; Nair, C. K., Preferential radioprotection to DNA of normal tissues by ferulic acid under ex vivo and in vivo conditions in tumor bearing mice. *Mol Cell Biochem* **2006,** *285* (1-2), 181-90.
16. Khan, N. M.; Sandur, S. K.; Checker, R.; Sharma, D.; Poduval, T. B.; Sainis, K. B., Pro-oxidants ameliorate radiation-induced apoptosis through activation of the calcium-ERK1/2-Nrf2 pathway. *Free Radic Biol Med* **2011,** *51* (1), 115-28.
17. Checker, R.; Pal, D.; Patwardhan, R. S.; Basu, B.; Sharma, D.; Sandur, S. K., Modulation of Caspase-3 activity using a redox active vitamin K3 analogue, plumbagin, as a novel strategy for radioprotection. *Free Radic Biol Med* **2019,** *143*, 560-572.
18. Tandon, N.; Yadav, S. S., Safety and clinical effectiveness of Withania Somnifera (Linn.) Dunal root in human ailments. *J Ethnopharmacol* **2020,** *255*, 112768.
19. Checker, R.; Bhilwade, H. N.; Nandha, S. R.; Patwardhan, R. S.; Sharma, D.; Sandur, S. K., Withaferin A, a steroidal lactone, selectively protects normal lymphocytes against ionizing radiation induced apoptosis and genotoxicity via activation of ERK/Nrf-2/HO-1 axis. *Toxicol Appl Pharmacol* **2023,** *461*, 116389.
20. Kunwar, A.; Jayakumar, S.; Bhilwade, H. N.; Bag, P. P.; Bhatt, H.; Chaubey, R. C.; Priyadarsini, K. I., Protective effects of selenocystine against γ-radiation-induced genotoxicity in Swiss albino mice. *Radiat Environ Biophys* **2011,** *50* (2), 271-80.
21. Raghuraman, M.; Verma, P.; Kunwar, A.; Phadnis, P. P.; Jain, V. K.; Priyadarsini, K. I., Cellular evaluation of diselenonicotinamide (DSNA) as a radioprotector against cell death and DNA damage. *Metallomics* **2017,** *9* (6), 715-725.
22. Verma, P.; Kunwar, A.; Arai, K.; Iwaoka, M.; Priyadarsini, K. I., Mechanism of radioprotection by dihydroxy-1-selenolane (DHS): Effect of fatty acid conjugation and role of glutathione peroxidase (GPx). *Biochimie* **2018,** *144*, 122-133.
23. Kunwar, A.; Bansal, P.; Kumar, S. J.; Bag, P. P.; Paul, P.; Reddy, N. D.; Kumbhare, L. B.; Jain, V. K.; Chaubey, R. C.; Unnikrishnan, M. K.; Priyadarsini, K. I., In vivo radioprotection studies of 3,3'-diselenodipropionic acid, a selenocystine derivative. *Free Radic Biol Med* **2010,** *48* (3), 399-410.
24. Gandhi, K. A.; Goda, J. S.; Gandhi, V. V.; Sadanpurwala, A.; Jain, V. K.; Joshi, K.; Epari, S.; Rane, S.; Mohanty, B.; Chaudhari, P.; Kembhavi, S.; Kunwar, A.; Gota, V.; Priyadarsini, K. I., Oral administration of 3,3'-diselenodipropionic acid prevents thoracic radiation induced pneumonitis in mice by suppressing NF-kB/IL-17/G-CSF/neutrophil axis. *Free Radic Biol Med* **2019,** *145*, 8-19.