

**XXIII Chemistry of Radiation Protectors:**

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## Synthesis of radiation protectors

Exposure of eukaryotic cells to ionizing radiation (IR) results in part in direct ionization of biological molecules (direct damage by alpha particles, neutrons, ions or atoms) or indirect effects by secondary species. In particular, radiolysis of H<sub>2</sub>O generates a burst of species ( $e_{aq}^-$ ; H•, HO• and O<sub>2</sub><sup>-•</sup>) with high energy and indiscriminate reactivity; these react with biomolecules on the nano- to millisecond time scale, resulting in indirect damage by IR. Metabolic, post-IR amplification of the initial IR damage to critical biomolecules leads to anatomical lesions whose manifestation follow dose-dependent lag periods that can range from hours to years. While the initial radiolytic events happen instantaneously, protection of cells can be attained by radioprotectors (RP) present at the time of radiation exposure, but there is little or no protection if RP are administered after exposure to IR. Alternatively, radiation mitigators (RM) that counteract post-IR changes in the cellular homeostasis can be used to prevent radiation disease and long-term health defects.

Studies by Patt, Bacq, and others established that aminothiols such as cysteine and cysteamine protect mice from short-lived radiolytic intermediates.<sup>1,2,3</sup> Humans, however, do not tolerate the doses of aminothiols which would be required for analogous protection, and the initial hope that these compounds might be useful as RP therapeutics has not been realized. These early observations were followed by investigations from 1959 to 1978 under the auspices of the Walter Reed Army Institute of Research (WRAIR) which led to the synthesis of over 4,000 aminothiols as potential scavengers of HO• and to their assessment as RP in mice.<sup>4</sup> In the synthesis of these compounds, structural variables were the length and the branching of the carbon chain connecting NH<sub>2</sub>, SH and OH groups. It was found that compounds containing both thiol and amine groups exert maximal radioprotection.<sup>5</sup> Separation of the thiol and amine groups by more than three methylene units markedly decreases the protective potential of the corresponding compounds, and alkylation of either the thiol or the amine groups also yielded derivatives with marginal activity. From this library of aminothiols, only 2-(3-aminopropylamino)ethylsulfanyl phosphonic acid (amifostine; H<sub>2</sub>O<sub>3</sub>P-S-(CH<sub>2</sub>)<sub>2</sub>-NH-(CH<sub>2</sub>)<sub>3</sub>-NH<sub>2</sub>) has been approved for clinical use as a RP.<sup>6</sup>

Historically, the search for a suitable RP began prior to a comprehensive kinetic analysis of the reactions of HO• with biological molecules. In 1973, Dorfman and Adams reviewed reactions of HO• with several classes of organic compounds that proceeded in either acidic or alkaline milieu,<sup>7</sup> while kinetic analysis of reactions of HO• in biological systems began in the late 1970s.<sup>8</sup> Currently, HO• is considered to be one of the most reactive chemical species that can be formed in a biological environment; when generated, HO• reacts with any neighboring molecule at first collision, and thus cannot diffuse from its site of generation further than to its nearest neighboring molecule (diffusion-controlled reactions). This kinetic property implies that the intracellular concentration of a RP that efficiently scavenges HO• should be at least one order of magnitude higher than that of protein thiols, which represent a larger redox pool than glutathione (GSH).<sup>9</sup> In the high mM range, however, most RP are toxic to humans. Alternatively, high RP concentrations at the site of the most damaging reactions of HO• can be attained via their binding to critical target molecules, as shown for amifostine, which binds to nuclear and mitochondrial DNA.<sup>10, 11</sup>

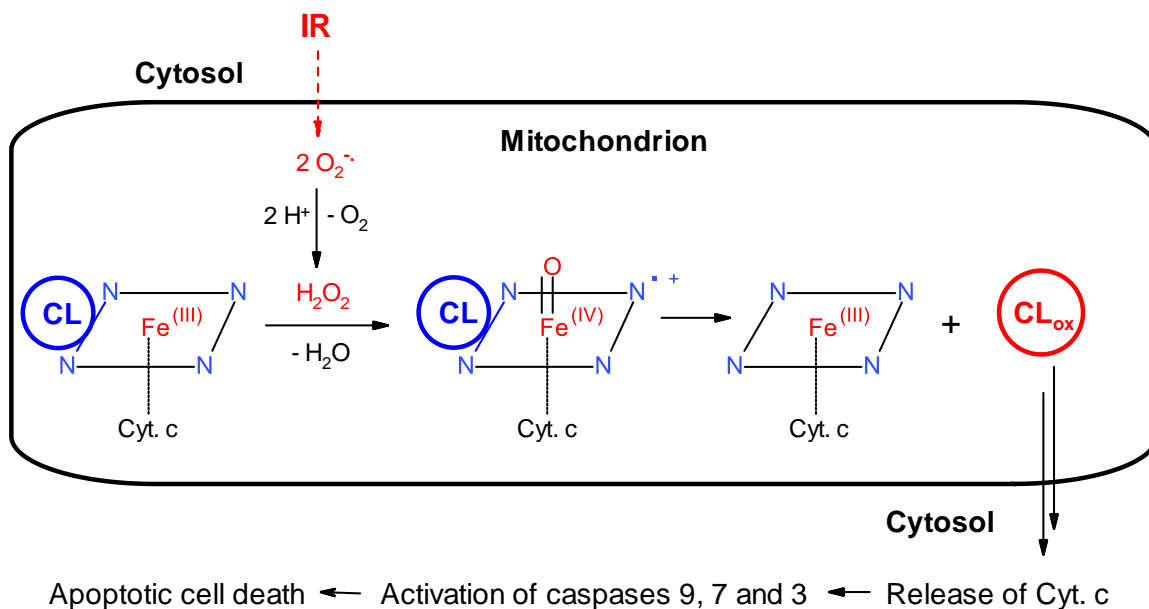
Superoxide radical anion, O<sub>2</sub><sup>-•</sup>, which is mainly generated by secondary reactions during IR,<sup>12</sup> is also potentially highly damaging to cells. Although O<sub>2</sub><sup>-•</sup> is a rather inert radical (i.e., it is a poor H-abstractor), it readily inhibits proteins containing iron-sulfur clusters in their active sites, such as ferredoxins, NADH dehydrogenases and aconitase (protein-[4Fe-4S]<sup>2+</sup> + O<sub>2</sub><sup>-•</sup> → protein-[3Fe-4S]<sup>+</sup> + Fe<sup>2+</sup> + H<sub>2</sub>O<sub>2</sub>).<sup>13,14</sup> Mitochondrial aconitase has been shown to be particularly sensitive to IR-induced inactivation.<sup>15</sup> In the presence of traces of iron salts, O<sub>2</sub><sup>-•</sup> reacts with H<sub>2</sub>O<sub>2</sub> to give •OH, OH<sup>-</sup> and O<sub>2</sub> in a Haber-Weiss reaction, generating lipid peroxides and increasing oxidative stress (Benov, L., How superoxide radical damages the cell. *Protoplasma* **2001**, 217, 33-36).

### **Mechanism-based target identification and development of RM**

Post-IR responses in humans represent a multifaceted disease state caused by DNA damage and a surge of reactive oxygen species (ROS) as well as pro-inflammatory molecules, including cytokines and chemokines. However, the mechanistic and derivative biochemistry of IR damage in cells remains incompletely understood.

To depict the biochemical pathways that are perturbed by IR, considerable research effort has been directed toward the assessment of the radiomitigative properties of enzyme inhibitors, hormones, and novel chemical entities. In the context of the “IR-induced mitochondrial oxidative stress” hypothesis,<sup>16</sup> the authors of this chapter have carried out the targeted synthesis of mitochondria-specific RM.

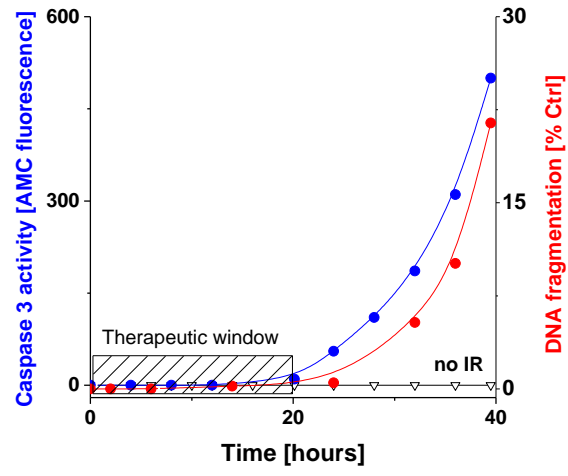
The immediate burst of radicals upon exposure to IR is followed by a dose-dependent and continuous mitochondrial overproduction of reactive oxygen species (mROS), which ultimately induce intrinsic apoptosis in radio-sensitive cells (Figure 1).<sup>15</sup> Among these secondary oxidants, H<sub>2</sub>O<sub>2</sub> and lipid hydroperoxides (LOOH) are most prominent in setting-up the “peroxide tone” and perpetuating enzymatic and nonenzymatic oxidations of critical biomolecules with signaling functions. In particular, peroxidase functions of the mitochondrial intermembrane space hemoprotein cytochrome c (cyt c) toward a mitochondria-specific phospholipid, cardiolipin (CL), have been associated with the accumulation of CL hydroperoxides, which are required for the execution of mitochondrial apoptosis.<sup>17</sup> Both the toxicological and the therapeutic significance of the production of mROS are underlined by findings that overexpression of either mitochondrial superoxide dismutase or catalase increases radioresistance *in vitro* and *in vivo*.<sup>18</sup> Furthermore, overexpression of mitochondrial glutathione peroxidase 4 (GPx4) impedes both the oxidation of CL and mROS-induced apoptosis.<sup>19, 20</sup>



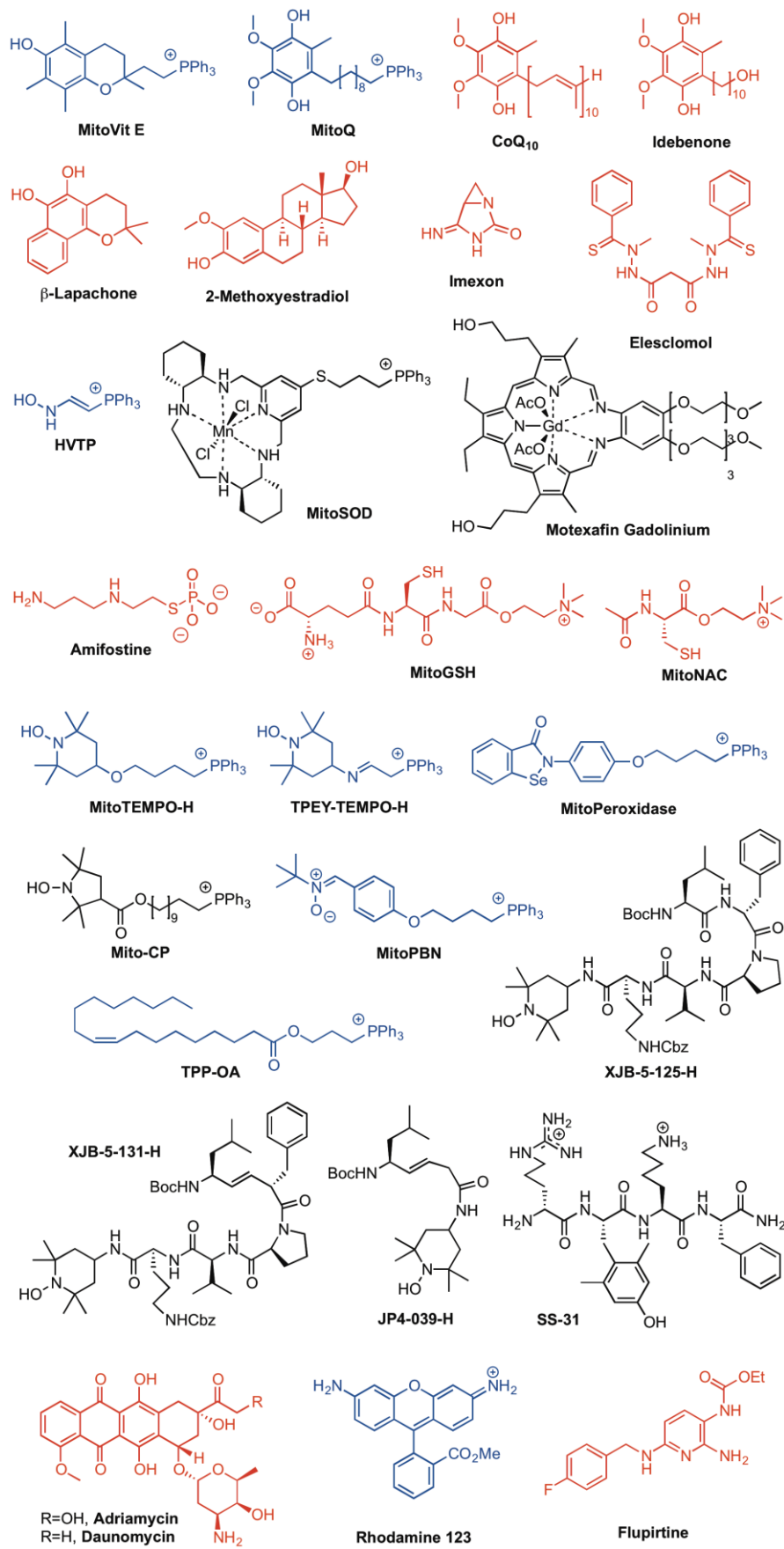
**Figure 1.** In radiosensitive cells, IR triggers continuous production of mROS, oxidation of CL and intrinsic apoptosis.

The lag period for induction of apoptosis by IR<sup>21</sup> can be viewed as a post-irradiation therapeutic window (Fig. 2) during which clearance of mROS is expected to inhibit apoptosis, and thus to provide additional opportunities for the cellular repair system to counteract the deleterious effects of radiation.

As an alternative to gene therapy, we and others have synthesized and assessed as RM small molecules that have the potential to react with mROS and to impede intrinsic apoptosis (Figure 3). An important aspect of this design is the identification of pharmacophores that are capable to inhibit apoptosis prior to CL oxidation, release of cytochrome c in cytosol, and activation of the executioner caspase 3. While sufficient concentrations of antioxidants at the sites of generation of reactive metabolites are critical to protection from oxidative damage under conditions of oxidative stress, we attained mitochondrial delivery of redox-sensitive pharmacophores via their attachment to gramicidin derivatives and to acyclic hydrocarbons carrying the triphenylphosphonium group ((Ph)<sub>3</sub>P<sup>+</sup>; TPP; Figure 3; Schemes 1, 3 and 4; Table I).



**Figure 2.** Time-course of caspase 3 activity (blue circles) and DNA fragmentation (red circles) in U937 cells exposed to X-ray irradiation (5 Gy).<sup>21</sup>

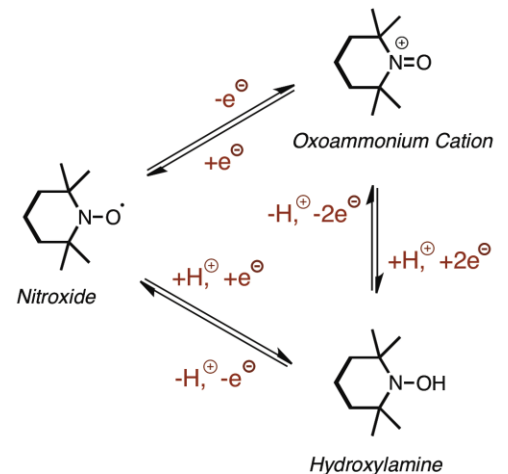


**Figure 3.** Small molecule agents that have the properties to potentially scavenge mROS and act as RM.<sup>22</sup> Some compounds are shown in their reduced (redox-active) oxidation state. Compounds that have not been reported to act as

RM are colored. Blue compounds should compartmentalize in mitochondria; red compounds will most likely exhibit a random intracellular distribution. MitoSOD and motexaflin gadolinium were shown to protect some tissues from IR-induced damage but the authors are not aware of any data on mitigation by these complexes in mice exposed to total body irradiation (TBI). Accelerated wound healing can be attained by most chelators of metal ions, incl. EDTA. The authors tested ebselen and MitoPeroxidase for RM properties; however, in cells, both compounds were inactive as RM, and MitoPeroxidase caused considerable toxicity.<sup>46</sup>

By virtue of their three redox states, nitroxides are particularly effective at scavenging ROS at low concentrations. They are small molecule mimetics of SOD, and can also trap escaping electrons from the oxidative phosphorylation as well as many types of free radicals at diffusion-controlled rates (Figure 4). At a dose of 275 mg per kg body weight, TEMPOL protects mice exposed to lethal doses of radiation.<sup>23</sup> This protective effect should be considered with caution because, at such doses, nitroxides reduce arterial blood pressure in rodents<sup>24,25</sup> to levels that cannot be tolerated by humans. When directed into mitochondria, however, TEMPO acts as a RM at ~ 500 times lower dosage and without causing any apparent toxicity.

- Nitroxides can act as SOD mimics; i.e. catalyze the dismutation of superoxide radical anions into H<sub>2</sub>O<sub>2</sub>:
  - Nitroxide radical + O<sub>2</sub><sup>-•</sup> + H<sup>+</sup> → Hydroxylamine + O<sub>2</sub>
  - Hydroxylamine + O<sub>2</sub><sup>-•</sup> + H<sup>+</sup> → Nitroxide Radical + H<sub>2</sub>O<sub>2</sub>
- Nitroxides can act as electron scavengers; i.e. trap escaping electrons from OXPHOS:
  - Nitroxide radical + e<sup>-</sup> + H<sup>+</sup> → Hydroxylamine
- Nitroxides can act as free radical scavengers; i.e. trap radicals formed by hydrogen atom abstractions of superoxide radical anions:
  - R-H + O<sub>2</sub><sup>-•</sup> + H<sup>+</sup> → R<sup>•</sup> + H<sub>2</sub>O<sub>2</sub>
  - Nitroxide radical + R<sup>•</sup> → O-Alkyl Hydroxylamine



**Figure 4.** Nitroxides, hydroxylamines, and oxoammonium cations are effective shuttles for electrons and protons and can scavenge ROS in a diffusion-controlled manner. Conversion of the nitroxide function into other oxidation states is reversible and can contribute to catalytic ROS turnover.

The one-electron reduction of the nitroxide generates a hydroxylamine, which can undergo a two-electron oxidation to the oxoammonium cation (Figure 4). Single-electron reduction of the oxoammonium cation



regenerates the nitroxide, and therefore all of these N-O oxidation states are in an equilibrium determined by the redox state of the environment.<sup>26</sup> In a conversion resembling that of superoxide dismutase (SOD), superoxide radical anion as well as reactive nitrogen species can react with nitroxide and hydroxylamine species, generating hydroxylamine and nitroxide radical, respectively.<sup>27</sup> The nitroxide radical is also a superb single-electron acceptor and both the resulting hydroxylamine as well as the parent nitroxide can quench radicals originating, among other pathways, from hydrogen atom abstraction by HO•.<sup>28,29</sup>

### **Delivery of pharmacological agents into mitochondria through the use of peptides**

The mitochondrially targeted nitroxides XJB-5-131 and JP4-039 have shown considerable therapeutic potential *in vitro* and *in vivo* mitochondrial disease models, including IR-induced toxicity, traumatic brain injury, hemorrhagic shock, Huntington's disease, post-ischemic recovery of cardiac function in aged rats, and cerebral ischemia-reperfusion.<sup>22</sup> Based on the sequence of the antimicrobial cyclopeptide natural product Gramicidin S, these agents are uniquely mitochondrial-targeted and enriched in the inner mitochondrial membrane, but they are not charged and do not lower the mitochondrial membrane potential. In spite of the absence of a positively charged moiety, XJB-5-131 enriches 600-fold in mitochondria over the cytosol and rapidly partitions into the brain.<sup>30</sup> JP4-039 is more uniformly distributed in cytosol and mitochondria and only ca. 20-30-fold enriched in the latter, and this compound shows significant protective effects in models of radiation-induced injury.<sup>31,32</sup> The targeting mechanism of XJB-5-131 and JP4-039 distinguishes them from the large number of simple antioxidants, such as ascorbate, that are not enriched in cells and/or mitochondria due to adverse polarity or rapid metabolism.

Most significantly, in a global lipidomics analysis in experimental traumatic brain injury (TBI), XJB-5-131 prevented the oxidative degradation of polyunsaturated CL, achieved a substantial reduction in neuronal death both *in vitro* and *in vivo*, and markedly reduced behavioral deficits and cortical lesion volume.<sup>30</sup> Using two-dimensional liquid chromatography mass spectrometry (2D-LC-MS), a global lipidomics analysis of phospholipids that revealed almost 190 individual molecular species of CL in normal rat brain, of which only ten were oxidized. Experimental TBI, controlled cortical impact (CCI), induced a burst of ROS and resulted in

oxidation of the majority of polyunsaturated molecular species of CL; the number of non-oxidized CL species decreased to ~100, whereas that of oxidized species increased to 166. Quantitatively, the content of oxidized CL species increased 20-fold at the expense of decreased amounts of non-oxidized CL. This oxidation effect was specific to CL, as other, more abundant polyunsaturated phospholipids, phosphatidylcholine and phosphatidylethanolamine, remained non-oxidized. Other cognitive performance tests, such as novel object recognition and balance beam tests, which are sensitive to motor and cognitive function and/or dysfunction, confirmed the efficacy of this treatment. L-band *in vivo* electron paramagnetic resonance imaging demonstrated the time- and dose-dependent distribution of XJB-5-131 in naïve rat brain after intraperitoneal injection. Overall, these agents therefore have distinct advantages over other classes of antioxidants, including mitochondrial enrichment, catalytic efficacy, direct acceptance of electrons from respiratory complexes to prevent production of ROS, as well as superoxide dismutase-, catalase- and peroxidase-mimetic effects

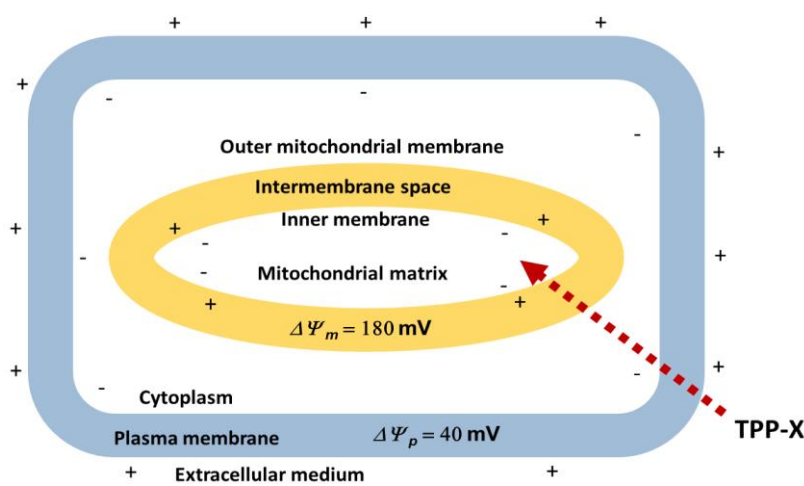
More recently, tetrapeptides containing basic residues, most often arginine and lysine, in combination with tyrosine or tyrosine analogs, have gained rapid acceptance in the field for their ability to promote the electron transfer mechanism over the peroxidase activity of cytochrome c.<sup>33,34</sup> Bendavia®, previously known as SS-31, protects mitochondria cristae, promotes ATP synthesis, and inhibits permeability transition. While this peptide is currently in clinical trials for skeletal muscle dysfunction in the elderly, ischemia reperfusion injury, age-related macular degeneration and mitochondrial myopathy,<sup>35</sup> it is quite possible that its beneficial effects also include protection from radiation injury.

### **Delivery of pharmacological agents into mitochondria through the use of TPP cations.**

Lipophilic organic cations, such as rhodamine 123, *N*-arylpyridinium and triphenylphosphonium cation are driven into cells and mitochondria by the negative plasma membrane ( $\psi_p \simeq -40$  mV) and mitochondrial ( $\psi_m \simeq -180$  mV) potentials.<sup>36</sup> Since mitochondria maintain potentials from -150 mV to -180 mV, positive cations drive attached molecules inside the mitochondrial matrix, towards diffusion equilibrium (Figure 3).<sup>37</sup> This process is

characterized by the Nernst equation (1), where  $z$  is the charge of the cation and where for a monocation, the factor  $F$  is 61 mV. For a mono-cationic carrier, a thousand-fold accumulation of an attached drug in the mitochondrial matrix vs. cytosol is theoretically achievable.<sup>38, 39</sup>

$$\Delta\psi_{(mV)} = \frac{2.303RT}{zF} \log \frac{[\text{cation}]_{in}}{[\text{cation}]_{out}} \quad (1)$$



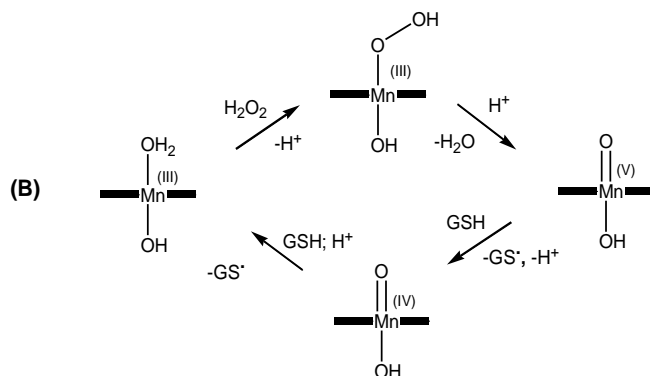
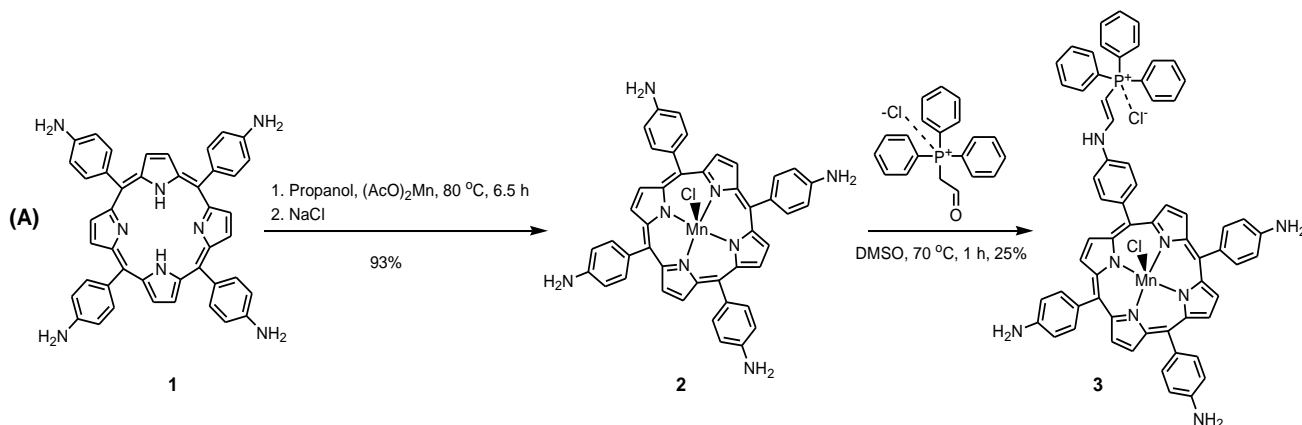
**Figure 3.** A schematic model for targeted delivery of TPP-derived pharmacophores in mitochondria. The model includes three compartments: extracellular medium, cytoplasm and mitochondrial matrix. TPP cations drive attached redox-sensitive pharmacophores (X) inside the mitochondrial matrix towards diffusion equilibrium.

The sub-compartmentalization of TPP-X in mitochondria is dependent on their polarity;<sup>40</sup> more hydrophobic compounds are retained by membranes, while hydrophilic analogs tend to accumulate in the mitochondrial matrix. Thus, by using appropriate linkers between the TPP group and a pharmacophore, it is possible to control the polarity of the drug and thus to target clearance of mROSs produced within membranes or in the matrix. Following this strategy, TPP derivatives of small-molecule mimetics of glutathione peroxidase (GPx) and ligands for cytochrome c have been synthesized and assessed as RM.

*Triphenyl-[(2E)-2-[4-[(1Z,4Z,9Z,15Z)-10,15,20-tris(4-aminophenyl)-21,23-dihydroporphyrin-5-yl]phenyl]iminoethyl]phosphonium-Mn<sup>(III)</sup> (TPP-AAPP) reacts with H<sub>2</sub>O<sub>2</sub>, inhibits apoptosis, and acts as a RM in vivo.*

Porphyrin-Mn<sup>(III)</sup> complexes have attracted much interest as superoxide dismutase (SOD) and catalase mimics. While the reduction potentials of monocationic Mn<sup>(III)</sup>-porphyrin complexes preclude reactions with O<sub>2</sub><sup>-•</sup>, targeted synthesis of porphyrin-based SOD mimics has been achieved via conjugation of the porphyrin ring with charged groups.<sup>41</sup> This structural modification leads to decreased electron density on the Mn<sup>(III)</sup> center, thus increasing its ability to undergo one-electron reduction. Lee and Park have shown that, when preadministered for 14 days, the polycationic mimic of SOD Mn<sup>(III)</sup>-TMPyP<sup>5+</sup> increases the survival of mice exposed to whole-body irradiation.<sup>42</sup> However, the overall charge of Mn<sup>(III)</sup>-TMPyP<sup>5+</sup> reduces its ability to cross cell membranes (and thereby its bioavailability) and increases its toxicity due to intercalation into nucleic acids. To circumvent some of these limitations, at least in part, we synthesized TPP-AAPP (Scheme 1, **3**) as a potential RM with GPx-like activity (Scheme 1).<sup>43</sup> In aqueous solutions, the positive charge of TPP-AAPP that originates from the TPP group is delocalized in the conjugated porphyrin system, thus facilitating its uptake by mitochondria. We found that TPP-AAPP preferentially compartmentalizes into mitochondria in concentrations up to 3 mM versus 0.005 mM in the cytosol of mouse embryonic cells (MEC), reacts with mitochondrial H<sub>2</sub>O<sub>2</sub> (but not with O<sub>2</sub><sup>-•</sup>), and impedes  $\gamma$ -ray-induced mitochondrial apoptosis. Importantly, in contrast to complex **2**, TPP-AAPP proved a potent RM; when administered as a single dose (5 mg/kg body weight) 60 min after irradiation, TPP-AAPP markedly increased the survival of mice exposed to lethal doses of  $\gamma$ -rays.

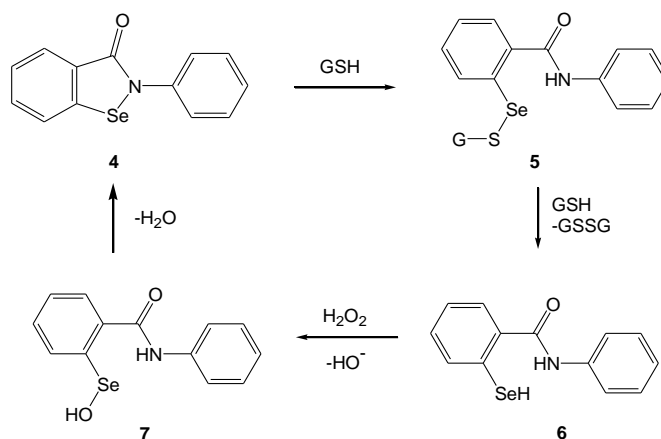
**Scheme 1.** Synthesis (A) and GPx-like activity (B) of TPP-AAPP.



Design and synthesis of a mitochondria-targeted mimic of glutathione peroxidase, MitoEbselen, as a RM

The central regulator of LOOH is the seleno-enzyme glutathione peroxidase 4 (GPx4). A deficiency of GPx4 leads to cell death, while overexpression in mitochondria has been shown to impede both oxidation of CL and mitochondrial apoptosis.<sup>19,20</sup> Recently, Tak and Park have reported that 2-phenyl-1,2-benzoselenazol-3-one (Ebselen; Scheme 2, compound 4), when administered for extended time periods prior to radiation, provides protection against killing and oxidative damage in mice exposed to whole-body irradiation (WBI).<sup>44</sup>

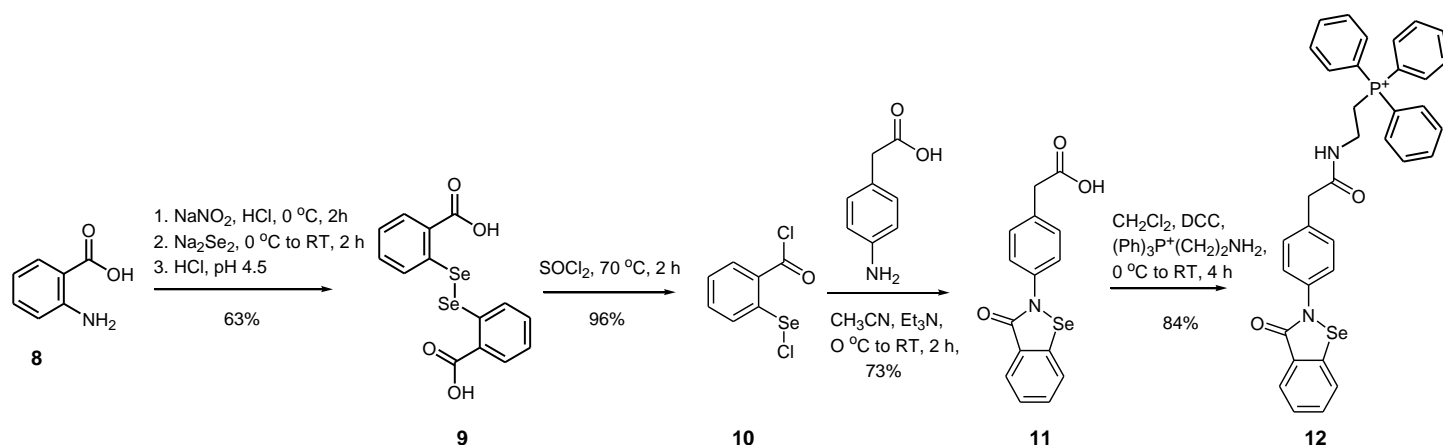
**Scheme 2.** Ebselen mimics the activity of GPx4.



Ebselen is a multifunctional drug that accumulates in the endoplasmic reticulum (ER),<sup>45</sup> reacts with cellular thiols, and mimics the activity of glutathione peroxidase (GPx) by clearing H<sub>2</sub>O<sub>2</sub> and lipid peroxides (Scheme 2); at  $\mu$ M concentrations of **4** and H<sub>2</sub>O<sub>2</sub> or LOOH, and mM GSH, the reaction cycle  $4 \rightarrow 7 \rightarrow 4$  closes in seconds.<sup>46</sup> Following recent clinical trials for the prevention and treatment of cardiovascular diseases, arthritis, stroke, and atherosclerosis, Ebselen has been included in the National Institutes of Health Clinical Collection, a chemical library of bioavailable drugs considered clinically safe.

To shift the cellular compartmentalization of Ebselen from ER to mitochondria, we have synthesized the TTP variant 4-[4-(3-oxo-1,2-benzoselenazol-2-yl)phenoxy]butyl-triphenyl-phosphonium chloride (Scheme 3, compound **12**; MitoEbselen).<sup>46</sup>

**Scheme 3.** Synthesis of MitoEbselen



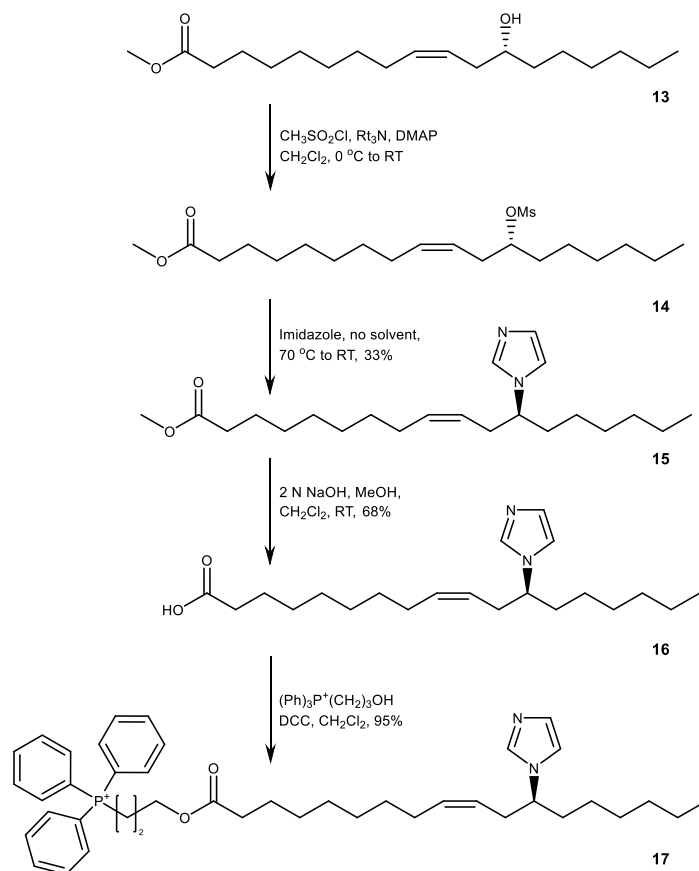
In the presence of GSH, we found that MitoEbselen readily reduces hydroperoxides of fatty acid to the corresponding alcohols, impedes (in contrast to Ebselen) IR-induced apoptosis in MEC, and acts as a RM when administrated as a single dose 24 h after irradiation.

*Mitochondria-targeted inhibitors of cytochrome c peroxidase mitigate IR-induced death*

In the sequence of mitochondrial reactions that lead to intrinsic apoptosis, the pro-oxidant enzymatic activity of [cytochrome c/CL] complexes represents a target for anti-apoptotic and radioprotective drugs. The peroxidase activity is due to a CL-induced partial unfolding of the protein in the complex resulting in a “loosened” liganding capacity of haem-iron by a distal Met80. This structural change allows H<sub>2</sub>O<sub>2</sub> to react with the heme center of cytochrome c, a reaction that is restricted for the native protein, and to trigger inner-sphere oxidation of CL with concomitant induction of apoptosis.

Atkinson et al. hypothesized that locking the haem-iron coordination bond with a strong ligand, such as imidazole, delivered through the hydrophobic channel to the immediate proximity of the catalytic site, will block the path for H<sub>2</sub>O<sub>2</sub> and thereby will inhibit both CL peroxidation and the progression of apoptosis (Figure 1).<sup>47</sup> Indeed, mitochondria-targeted TPP- and imidazole-conjugated fatty acids (Scheme 4) exerted strong and specific liganding of haem-iron in [cytochrome c/CL], suppressed the peroxidase activity and CL peroxidation, and prevented apoptotic cell death. Imidazole-derived fatty acids lacking the TPP group did not impede apoptotic cell death, which is in agreement with the notion that delivery of pharmacophores into the sites of redox reactions is required for protection from oxidative damage. Significant radiomitigative effects of TPP- and imidazole-derivatized oleic acid were observed after pretreatment of mice from 1 h before through 24 h after the irradiation.

**Scheme 4.** A general synthetic method for synthesis of TPP and imidazole conjugates of fatty acids.



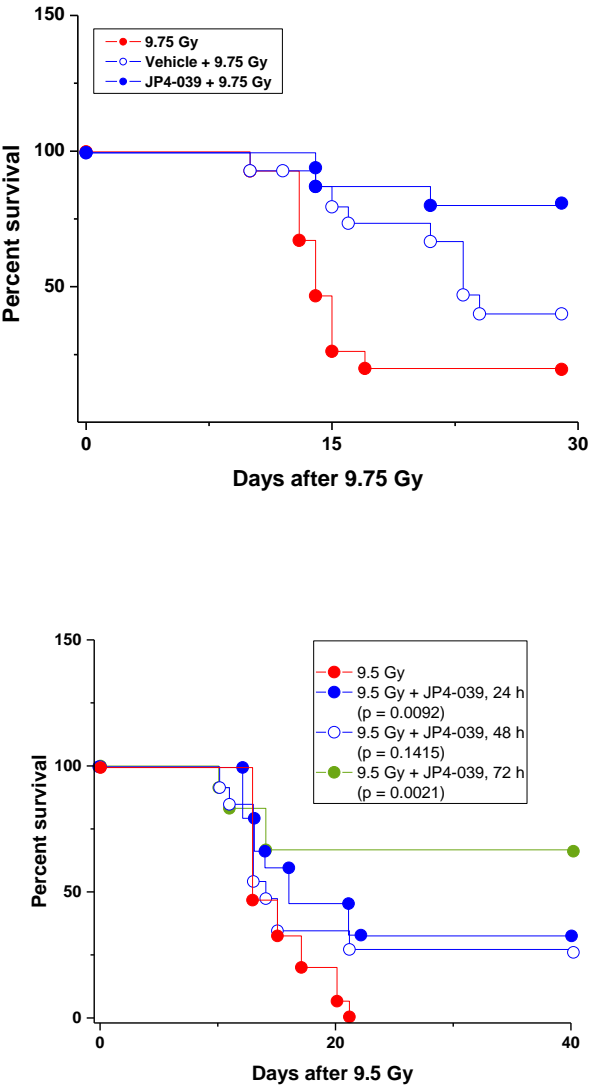
## Conclusions

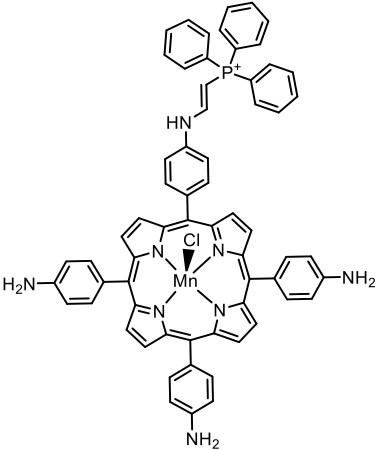
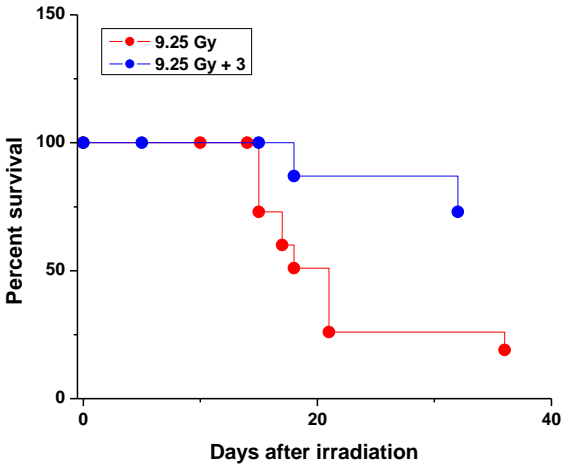
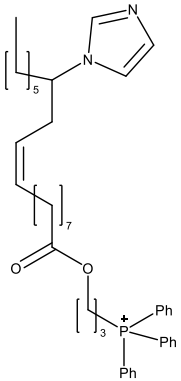
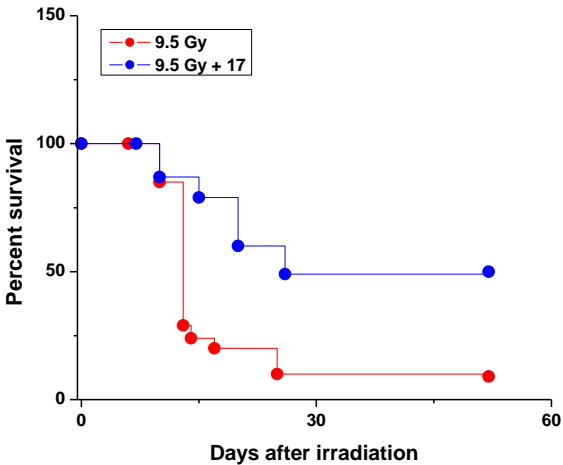
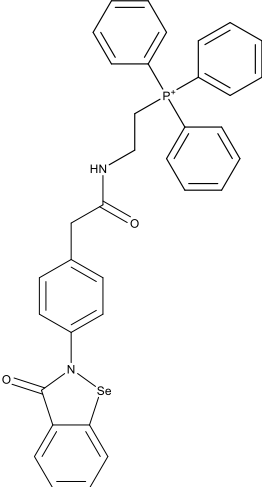
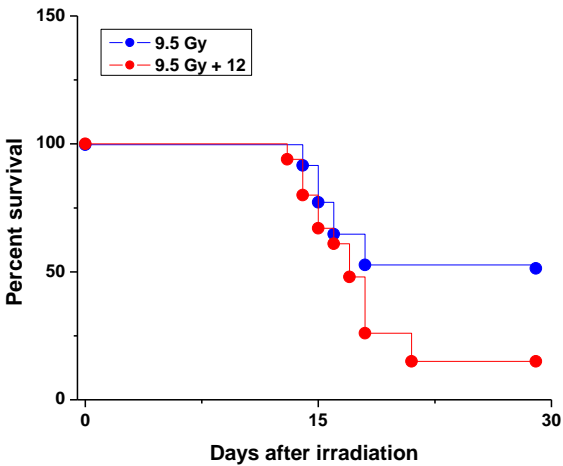
The development of RM is of considerable interest because IR damage to organisms is often the result of accidents, where post-IR treatment with RP is not a viable option. Furthermore, RM can be used for alleviation of post-IR toxicity in normal tissues following radiation therapy of cancer patients. Currently, however, the medicinal chemistry of RM is still in its infancy. A search in the Medline database with keywords “radiation mitigator” renders 48 entries, whereas the mechanism-based, targeted synthesis of RM is reported in 6 papers (Table I). To date, 4 pharmacophores with radiomitigative activities have been identified; namely, nitroxide,  $\text{Mn}(\text{III})$ -porphyrin, alkyl-imidazole, and benzoselenazol modalities have been found to exhibit RM properties. Importantly, it has been established that the targeted delivery of these pharmacophores into mitochondria is required for radiomitigation. Although these results are encouraging, further studies are needed to assess the druggability of newly identified RM. To this end, the findings reported herein provide a foundation for structure-activity relationship studies - including the analysis of drug potency as a function of mitochondrial sub-



compartmentalization - as well as for an expansion of the library of redox-sensitive pharmacophores with radiomitigative properties.

**Table I.** Radiomitigative properties of mitochondria-targeted small molecules administered after exposure to  $\gamma$ -rays in mice.

RM	Radiomitigative properties of mitochondria-targeted pharmacophore in mice exposed to lethal doses of IR	Post-IR administration [hours]	Ref.
<div style="display: flex; flex-direction: column; align-items: center;"> <chem data-bbox="115 451 326 703">CC(C)CN(C)C(=O)C=CC(C)C(=O)NOC(=O)N1C(C)C(C)C(C)N1</chem>  <p><b>JP4-039</b></p> <chem data-bbox="183 835 544 1123">CC(C)CN(C)C(=O)C=CC(C)C(=O)NOC(=O)N1C(C)C(C)C(C)N1C(=O)NC(C)C(=O)N(C)C(=O)N2C(C)C(C)N2</chem>  <p><b>XJB-5-131</b></p> <chem data-bbox="183 1123 544 1417">CC(C)CN(C)C(=O)C=CC(C)C(=O)NOC(=O)N1C(C)C(C)C(C)N1C(=O)NC(C)C(=O)N(C)C(=O)N2C(C)C(C)N2</chem>  <p><b>XJB-5-125</b></p> <chem data-bbox="115 1430 586 1667">CC(C)CN(C)C(=O)C=CC(C)C(=O)NOC(=O)N1C(C)C(C)C(C)N1C(=O)NC(C)C(=O)N(C)C(=O)N2C(C)C(C)N2C(=O)N3C(C)C(C)N3</chem>  <p><b>XJB-7-75</b></p> </div>		<p>24</p> <p>24 – 78</p>	<p>48, 49, 50</p>

 <p>Chemical structure of a manganese porphyrin complex. The central manganese atom is coordinated by four nitrogen atoms in a porphyrin ring, with a chlorine atom coordinated above the plane. The porphyrin ring is substituted with three 4-aminophenyl groups and a 4-(dimethylamino)phenyl group. A phosphonium salt side chain is attached to the porphyrin ring via an imine linkage.</p>	 <p>Survival curve showing Percent survival (Y-axis, 0 to 150) versus Days after irradiation (X-axis, 0 to 40). Two data series are shown: 9.25 Gy (red circles) and 9.25 Gy + 3 (blue circles). The 9.25 Gy + 3 series shows significantly higher survival than the 9.25 Gy series.</p> <table border="1"> <thead> <tr> <th>Days after irradiation</th> <th>9.25 Gy (%)</th> <th>9.25 Gy + 3 (%)</th> </tr> </thead> <tbody> <tr><td>0</td><td>100</td><td>100</td></tr> <tr><td>5</td><td>100</td><td>100</td></tr> <tr><td>10</td><td>100</td><td>100</td></tr> <tr><td>15</td><td>100</td><td>100</td></tr> <tr><td>16</td><td>75</td><td>100</td></tr> <tr><td>17</td><td>60</td><td>100</td></tr> <tr><td>18</td><td>50</td><td>100</td></tr> <tr><td>19</td><td>25</td><td>90</td></tr> <tr><td>20</td><td>25</td><td>90</td></tr> <tr><td>30</td><td>25</td><td>75</td></tr> <tr><td>35</td><td>25</td><td>75</td></tr> </tbody> </table>	Days after irradiation	9.25 Gy (%)	9.25 Gy + 3 (%)	0	100	100	5	100	100	10	100	100	15	100	100	16	75	100	17	60	100	18	50	100	19	25	90	20	25	90	30	25	75	35	25	75	1	43						
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 <p>Chemical structure of a polymeric phosphonium salt. The main chain consists of a poly(vinyl ether) segment (indicated by a subscript 7) and a poly(ethylene glycol) segment (indicated by a subscript 5). A phosphonium salt group is attached to the main chain, consisting of a phosphorus atom coordinated to three phenyl groups and a nitrogen atom in a five-membered ring.</p>	 <p>Survival curve showing Percent survival (Y-axis, 0 to 150) versus Days after irradiation (X-axis, 0 to 60). Two data series are shown: 9.5 Gy (red circles) and 9.5 Gy + 17 (blue circles). The 9.5 Gy + 17 series shows significantly higher survival than the 9.5 Gy series.</p> <table border="1"> <thead> <tr> <th>Days after irradiation</th> <th>9.5 Gy (%)</th> <th>9.5 Gy + 17 (%)</th> </tr> </thead> <tbody> <tr><td>0</td><td>100</td><td>100</td></tr> <tr><td>5</td><td>100</td><td>100</td></tr> <tr><td>10</td><td>85</td><td>100</td></tr> <tr><td>15</td><td>25</td><td>80</td></tr> <tr><td>20</td><td>20</td><td>60</td></tr> <tr><td>25</td><td>10</td><td>50</td></tr> <tr><td>30</td><td>10</td><td>50</td></tr> <tr><td>40</td><td>10</td><td>50</td></tr> <tr><td>50</td><td>10</td><td>50</td></tr> </tbody> </table>	Days after irradiation	9.5 Gy (%)	9.5 Gy + 17 (%)	0	100	100	5	100	100	10	85	100	15	25	80	20	20	60	25	10	50	30	10	50	40	10	50	50	10	50	24	Error! Bookmark not defined.												
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 <p>Chemical structure of a selenium-containing porphyrin complex. The central selenium atom is coordinated by four nitrogen atoms in a porphyrin ring. The porphyrin ring is substituted with a 4-aminophenyl group and a 4-(dimethylamino)phenyl group. A phosphonium salt side chain is attached to the porphyrin ring via an imine linkage.</p>	 <p>Survival curve showing Percent survival (Y-axis, 0 to 150) versus Days after irradiation (X-axis, 0 to 30). Two data series are shown: 9.5 Gy (blue circles) and 9.5 Gy + 12 (red circles). The 9.5 Gy + 12 series shows significantly higher survival than the 9.5 Gy series.</p> <table border="1"> <thead> <tr> <th>Days after irradiation</th> <th>9.5 Gy (%)</th> <th>9.5 Gy + 12 (%)</th> </tr> </thead> <tbody> <tr><td>0</td><td>100</td><td>100</td></tr> <tr><td>10</td><td>100</td><td>100</td></tr> <tr><td>12</td><td>100</td><td>100</td></tr> <tr><td>13</td><td>100</td><td>95</td></tr> <tr><td>14</td><td>100</td><td>85</td></tr> <tr><td>15</td><td>95</td><td>75</td></tr> <tr><td>16</td><td>85</td><td>65</td></tr> <tr><td>17</td><td>75</td><td>55</td></tr> <tr><td>18</td><td>65</td><td>45</td></tr> <tr><td>19</td><td>55</td><td>25</td></tr> <tr><td>20</td><td>55</td><td>15</td></tr> <tr><td>25</td><td>55</td><td>15</td></tr> <tr><td>30</td><td>55</td><td>15</td></tr> </tbody> </table>	Days after irradiation	9.5 Gy (%)	9.5 Gy + 12 (%)	0	100	100	10	100	100	12	100	100	13	100	95	14	100	85	15	95	75	16	85	65	17	75	55	18	65	45	19	55	25	20	55	15	25	55	15	30	55	15	24	46
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