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An Overview of Radiation Countermeasure Development in *Radiation Research* from 1954 to 2024

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Preparation for medical responses to major radiation accidents, further driven by increases in the threat of nuclear warfare, has led to a pressing need to understand the underlying mechanisms of radiation injury (RI) alone or in combination with other trauma (combined injury, CI). The identification of these mechanisms suggests molecules and signaling pathways that can be targeted to develop radiation medical countermeasures. Thus far, the United States Food and Drug Administration (U.S. FDA) has approved seven countermeasures to mitigate hematopoietic acute radiation syndrome (H-ARS), but no drugs are available for prophylaxis and no agents have been approved to combat the other sub-syndromes of ARS, let alone delayed effects of acute radiation exposure or the effects of combined injury. From its inception, *Radiation Research* has significantly contributed to the understanding of the underlying mechanisms of radiation injury and combined injury, and to the development of radiation medical countermeasures for these indications through the publication of peer-reviewed research and review articles.

INTRODUCTION

Radiation exposure has been known to be a double-edged sword since the discovery of radioactivity. The usefulness of diagnostic and therapeutic radiation had to be balanced against increased cancers among nuclear medicine and radiologic technicians. Deadly radiation exposures during warfare eventually translated to the development of life-saving treatments when the exposure could be limited to otherwise deadly cancerous tissues. Right alongside the drive to develop more powerful bombs was the drive to discover ways of limiting radiation-induced damage to biological systems.

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Initially the development of countermeasures was based on radiation physics. Shielding and distance were used to protect X-ray equipment operators. Some of the earliest countermeasures approved by the United States Food and Drug Administration (U.S. FDA) basically limited the time of the exposure by preventing retention of radioactive isotopes [e.g., potassium iodide, KI for ^{131}I exposure (*1–3*), Zn- and CA-DTPA (diethylenetriamine pentaacetate) for exposure to transuranic or rare earth radionuclides (*4–6*), and Prussian Blue for ^{137}Cs and ^{201}thallium exposure (*7*, *8*)]. These are useful but limited both in efficacy and in range of application.

The development of better countermeasures required extensive research into the complex and interactive mechanisms in play during radiation-induced damage. After more than 60 years of such research, more generalized countermeasures began to gain regulatory approval for treatment of the hematological disorder attributed to radiation, and gradually countermeasures for external radiation exposure injuries are being discovered and developed. Since its inception in 1954, *Radiation Research* has furthered this research mission.

This article focuses on work documenting the development of radiation injury (RI) and combined injury (CI) countermeasures published in *Radiation Research* with minimal citation of articles published in other journals (mostly for background material, U.S. FDA approvals and a few additional reviews). We have further limited our write-up to the more successful and widely tested countermeasures using animal models since it is impossible to cover all agents tested in all possible models. Searches were performed in PubMed and Google Scholar, using general radiation countermeasure terms and specific countermeasure names (in all fields for PubMed) with and without the additional qualification of *Radiation Research* as the journal of publication.

The authors recently performed a comprehensive literature search to systematically catalog the radiation-induced alterations of multi-omics profiles and biomarkers, and associated radiation countermeasures (*9*). Also, since 2005, the Radiation and Nuclear Countermeasures Program of the National Institute of Allergy and Infectious Diseases has funded countermeasures research and published many reviews, workshop proceedings, and details of regulatory approval for countermeasures in *Radiation Research* (*10–24*).

Radiation countermeasures currently fall into three categories as shown in Tables 1–3: Protective, mitigative and therapeutic (*25*, *26*). Currently approved radioprotectors (administered before exposure) are for very limited types of exposure or conditions. All currently approved mitigative agents (used shortly after exposure) are limited to decorporation of specific agents or address the hematopoietic acute radiation syndrome (H-ARS). More recently, therapeutic agents generally administered after radiation exposure, sometimes for an extended period and intended to address long-term effects, are being developed (Table 3). Countermeasures that have shown efficacy as mitigators may also have protective effects and vice versa, and many of the investigational drugs are being tested in all three capacities. *Radiation Research* has helped advance the development of most of these medical countermeasures, either through publication of the data that elucidated the mechanisms they target, or by publication of the data demonstrating their efficacy and safety.

MEDICAL COUNTERMEASURES FOR RADIATION INJURY PUBLISHED IN *RADIATION RESEARCH*

The detrimental effects of radiation are known to be dependent on the dose, dose rate, quality, shielding, and the distance from the radiation source. Different syndromes, including acute, delayed, late, and chronic syndromes, are manifested depending on radiation exposure time, duration, and radiation quality. ARS was further classified by organs involved which were sensitive to radiation, namely H-ARS, gastrointestinal (GI-ARS), and neurovascular (NV-ARS) sub-syndromes. In some tissues such as lung, kidney, and skin, acute exposure results in delayed effects (delayed effects of acute radiation exposure, DEARE).

1954–1980

The first publication on cysteine as a radioprotector was published in 1949 in *Science* (*27*). From its outset in the mid-50s, *Radiation Research* published many articles on the molecular effects of ionizing radiation and on *in vivo* effects (*28*, *29*), studies which were necessary for the rational design of radiation medical countermeasures. The idea of reducing radiation damage by preventing the internal deposition of plutonium was published in 1955 (*30*) and the use of DTPA as a radiation countermeasure for retained monomeric plutonium was published in *Radiation Research* 12 years later (*5*). In 1961, the idea of dietary supplementation as a radiation countermeasure was proposed (*31*), and beginning in 1975, amifostine (originally designated WR-2721) was also advanced as a radiation countermeasure against H-ARS (*32*).

TABLE 1

Countermeasures: Protection

Agent	Activity/limitations	Refs.
U.S. FDA approved: limited use radioprotectors		
Potassium iodine	For ^{131}I exposure, blocks uptake, narrow window	(*2*)
Amifostine (thiol scavenger) Ethyol®, WR-2721, WR-1065	Used to prevent xerostomia when salivary glands are irradiated, high tox., narrow delivery window, IV	(*45*, *48*, *117*)
Palifermin (n-truncated KGF, KGF is part of the FGF family), Kepivance™	Used to prevent oral mucositis in patients receiving stem cell ablation/transplantation, epithelial repair/protection	(*36*)
Experimental drugs - protection		
BIO 300 (genistein) IND FT	Polyphenolic estrogen receptor β agonist	(*56*, *83*, *118*)
Gamma-tocotrienol IND	Vitamin E, antioxidant	(*41*, *42*, *60*, *61*, *119*)
EX-RAD IND	Small molecule kinase inhibitor, chlorobenzylfulfone derivative	(*40*)
5-androstenediol (steroid) IND	Development discontinued then initiated again.	(*37*)
CBLB502 (truncated flagelin) NDA entolimod, KMRC011, GP532 IND	TLR5 binder, induces G-CSF, hold 2019 (immunogenicity), now lifted, reengineered GP532, lacks immunogenicity	(*75*)
HOPO ligands IND hydroxypyridinonate ligand	Decorporation of actinides	(*4*, *77*)
CBLB613	TLR 2/6 agonist	(*120*)
N-acetylcysteine	Free radical scavenger	(*44*)
Alpha-tocopherol succinate	Vitamin E, antioxidant	(*52*)
Nutraceuticals	Increases in antioxidants	(*31*, *43*, *59*)
Probiotics	Regrow and promote good microbiota	(*82*)
Statins	Lower LDL cholesterol in blood	(*46*, *47*, *67*)
Metformin, biguanides	Diabetes control	(*70*, *74*)

TABLE 2
Countermeasures: Mitigation

Agent	Activity/limitations	Ref.(s)
U.S. FDA Approved Radiation Mitigation: Limited Use		
Prussian Blue Radiogardase®	For ^{137}Cs and ^{201}thallium exposure	(*121*)
Potassium iodine	For^{131}I exposure, blocks uptake, narrow window	(*2*)
Zn-DTPA; CA-DTPA (chelators)	Transuranic or rare earth radionuclide exposure	(*4*, *5*)
Silverlon	For radiation dermatitis and cutaneous radiation injury	(*10*)
U.S. FDA Approved Radiation Mitigation For H-ARS		
pegylated-G-CSF (Neulasta, Udenyca, Stimufend)	Neutrophil mobilization	(*53*, *89*)
G-CSF (Neupogen)	Neutrophil mobilization	(*13*, *16*, *54*, *55*, *62*, *78*)
GM-CSF (Leukine) Sargramostim	Granulocyte and macrophage mobilization,	(*13*, *16*, *78*)
Nplate (Romiplostim)	Platelet production	(*13*, *16*, *78*, *79*)
Experimental drugs - Mitigation		
BIO 300 (genistein) IND FT	Polyphenolic estrogen receptor beta agonist	(*56*, *83*)
gamma-tocotrienol IND	Vitamin E, antioxidant?	(*41*, *42*, *60*, *61*, *119*)
EX-RAD IND	Small molecule kinase inhibitor, chlorobenzylfulfone derivative	(*40*)
5-androstenediol (steroid) IND	Development discontinued then initiated again	(*37*)
myeloid progenitors IND	H-ARS, GI-ARS	(*49, 50*)
CBLB502 (truncated flagellin) entolimod IND	TLR5 binder, induces G-CSF, hold in 2019, now lifted, reengineered GP532, lacks immunogenicity	(*75*)
IL12, HemaMax, IK14800, Agrez IND	Induces IL12, IL2, IFNγ) ARS–24 h post; also, CI (burn), skin	(*73*, *122*)
HOPO ligands IND hydroxypyridinonate ligand	Decorporation of actinides	(*4*, *77*)
CBLB613	TLR 2/6 agonist	(*120*)
PEG-IL-11, Neumega® (BBT-059)	Platelet production, deemed too toxic post RAD, BBT-059 is a less toxic version	(*38*, *39*)
Thrombopoietin receptor agonists	Platelet production	(*88*)
ACE inhibitors	DEARE, off label, no clear approval process, lung, kidney	(*33*, *66*)
NLRP3 inhibitors/Nrf2 activators	GI-ARS, dermatitis Quercetin, Esomeprazole, triterpenoid RTA 408	(*63*, *72*, *76*, *123*, *124*)

1981–2000

Angiotensin converting enzyme (ACE) inhibitor CL242817 was reported in 1989 (*33*) to modify radiation-induced endothelium and lung fibrosis after a single dose of up to 30 Gy of gamma radiation to the hemithorax. Over the years, many more ACE inhibitors would be explored as mitigators of DEARE. Misoprostol, a prostaglandin E1 (PGE1) analog was explored as a radioprotector against fission spectrum neutron irradiation alone or in combination with amifostine in 1991 (*34*). Survival was improved in both mice and intestinal clonogenic cells, supporting the notion that misoprostol could be a potential treatment for GI-ARS. Later, misoprostol would be shown to reduce multiple organ DEARE in mice (*35*). In 1998, Savla and Waters found that keratinocyte growth factor protected the barrier function of epithelial cells in the irradiated animal airways, potentially reducing lung DEARE (*36*). Decorporation options were also expanded. Noteboom et al. published on the use of stable KI to protect against ^{131}I exposure (*2*, *3*).

2001–2010

As understanding grew about the effects of radiation on living systems, countermeasure research expanded. Steroids, cytokines and growth factors, antioxidants and statins were all explored as possible treatments. Whitnall and colleagues (2001) reported that a single subcutaneous (*sc*) dose of 5-androstenediol offered protection against H-ARS by stimulating myelopoiesis, increasing the numbers of circulating monocytes, neutrophils, natural killer (NK) cells and platelets for several weeks in CD2F1 male mice exposed to various doses of gamma radiation (*37*). In 2002, Van der Meeren et al. published data indicating that treatment with recombinant human interleukin-11 (IL-11) in combination with thrombopoietin (TPO) improved survival of C57BL/6J mice exposed to gamma radiation (10 or 15 Gy) compared to mice that received TPO alone due to the reduction of H-ARS effects (*38*). Hao et al. in 2004 demonstrated accelerated recovery of platelets, leukocytes and colony-forming bone marrow cells in non-human primates (NHP) exposed to 3 Gy of gamma radiation that were immediately treated

TABLE 3
Countermeasures: Therapeutic

Agent	Activity/limitations	
Experimental drugs	Therapeutic	Ref.(s)
BIO 300 (genistein) IND FT	Protection from DEARE-lung	(*83*, *118*)
AEOL 10150 (antioxidant) IND, FT	Protection from DEARE-lung	(*68*, *71*, *125*)
TGF-beta Receptor 1 inhibitor SKI2162, LY2109761, IPW-5371	Mitigate pulmonary and cardiac injury by increasing TGF-β signaling	(*69*)
ACE inhibitors	Protection from DEARE-lung, renal, optic	(*33*, *65*, *66*, *126*, *127*)
16,16 Dimethyl Prostaglandin E2 (PGE2)	Protection from DEARE lung, heart, optic	(*35*, *128*)
Statins	Protection from DEARE-heart	(*46*, *67*)

with IL-11 (*39*). This work would later progress using the pegylated version of IL-11, BBT-059. In 2009, Ghosh and colleagues demonstrated that Ex-Rad as a radioprotector increased survival in C3H/HeN mice after gamma irradiation (7.5 or 8 Gy) (*40*) and Berbee and colleagues reported that gamma-tocotrienol mitigated intestinal radiation injury and vascular oxidative stress in CD2F1 mice exposed to 8.5 Gy of gamma radiation (*41*). In 2010, Kulkarni et al. reported that pre-treatment with gamma-tocotrienol protected hematopoietic stem and progenitor cells in CD2F1 mice after 7 or 8 Gy of gamma irradiation (*42*). Both Ex-Rad® and gamma-tocotrienol appeared to have efficacy against both H-ARS and GI-ARS. 5-androstenediol, gamma tocopherol and Ex-Rad would all go on to receive U.S. FDA investigational new drug (IND) status.

Brown et al. found that an antioxidant supplementation diet containing L-selenomethionine, sodium ascorbate, N-acetyl cysteine, alpha-lipoic acid, alpha-tocopherol succinate and co-enzyme Q10, started 24 h after irradiation reduced mortality in C57BL/6 mice after 8 Gy total-body gamma irradiation, reducing both H-ARS and GI-ARS (*43*). Jia et al. found that the antioxidant N-acetyl-cysteine administered either before or immediately after irradiation improved the survival of C57BL/6 mice that received a 20 Gy abdominal X-ray exposure (*44*).

Research also continued into DEARE countermeasures. In 2002, Vujaskovic et al. reported that amifostine reduced both the accumulation of macrophages and the expression/activation of lung tissue growth factor beta 1 in Fisher 344 rats exposed to a single partial-body (right hemithorax) X-ray exposure of 28 Gy (*45*) demonstrating amifostine's role in reducing lung DEARE. Williams et al. published that lovastatin increased survival in C57BL/6 mice exposed to 15 Gy whole-lung gamma irradiation in 2004 (*46*). In 2005, pravastatin was shown to reduce radiation-induced (5 or 10 Gy of gamma radiation) microvascular activation in cultured human lung endothelial cells resulting in the reduction of inflammatory and thrombotic responses (*47*).

In other studies, Guo and colleagues used athymic nude mice irradiated with 30 Gy X rays to the head as a model for oral cavity mucositis and reported that pre-transfection of the superoxide dismutase 2 gene reduced radiation-induced epidermal thinning and ulceration in 2003 (*48*). In 2008, Otsuka et al. primed C57BL/6 mice with low-dose acute X rays (0.5 Gy) 2 weeks prior to a high-dose (6–8 Gy) challenge exposure and demonstrated that rapid myeloid recovery was a possible mechanism of the whole-body radio-adaptive response (*49*).

2011–2020

In this decade, the first growth factors were approved by the U.S. FDA for mitigation of H-ARS, and antioxidant research progressed. New treatment options such as ACE inhibitors, toll-like receptor (TLR) ligands, and myeloid progenitor cells were considered.

In 2012, myeloid progenitors were reported to improve survival in mice when administered several days after lethal doses of gamma rays or X rays (*50*). Administration of these cells mitigated death from H- and GI-ARS at radiation doses up to 15 Gy (gamma radiation, CD2F1 mice), which are doses that cause mice to succumb to multi-organ failure; the dose reduction factor of 5 million mouse myeloid progenitor cells (mMPC) administered 24 h postirradiation to CD2F1 mice was 1.73. Separately, using CD2F1 mice, CBLB613 (a naturally occurring mycoplasma-derived lipopeptide ligand for TLR 2/6) was assessed for toxicity, immunogenicity, radioprotection, radiomitigation, and pharmacokinetics (*51*). CBLB613 significantly protected mice against H-ARS after a lethal dose of gamma rays. Alpha-tocopherol succinate was also shown to protect mice against radiation-induced GI injury (total-body doses from 9.5 to 12 Gy) (*52*).

Also in 2012, Farese et al. indicated that Neupogen® improved neutrophil recovery (*53*) and survival (*54*) in a NHP model after 6 and 7.5 Gy of X-ray radiation (respectively). These studies were pivotal in the U.S. FDA approval of Neupogen® and Neulasta® for H-ARS treatment under the animal rule in 2015. The kinetics of neutrophil recovery in mice after irradiation with gamma rays or protons (0.5, 1 or 2 Gy) and treatment with vehicle or Neupogen® were reported (*55*).

In 2013, Ha et al. reported that genistein protected the mouse hematopoietic system and prevented proinflammatory factor induction after 9.25 Gy gamma irradiation (*56*).

Li et al. found delta-tocotrienol protected mice from 11 Gy gamma-radiation-induced GI injury (*57*). In 2014, captopril, an ACE inhibitor, combined with gentamicin and levofloxacin exhibited its ability to increase survival in mice after 9.5 Gy gamma irradiation (*58*) and Roche and colleagues suggested that high levels of a dietary supplement with Vitamins A, C, and E protected GI transport against chronic low dose gamma radiation in mice (*59*).

In 2016, gamma tocotrienol was demonstrated to protect NHPs and improve complete blood count (CBC) when the animals were exposed to three different doses of total-body gamma irradiation (*60*). Using this agent in a murine model, proteomic changes in the spleen of animals exposed to 7 Gy of gamma radiation were examined to elucidate possible mechanisms of action of this promising prophylactic countermeasure (*61*). In 2017, Satyamitra et al. found that Neupogen® increased survival and hematopoietic recovery in 4 strains of irradiated mice with different radiosensitivities (using ^{60}Co gamma radiation at the $LD_{70/30}$ for each strain) and that a more limited dosing schedule was more effective across various strains (*62*).

Horton et al., studying DEARE in skin, indicated that adding quercetin, an antioxidant, to chow inhibited skin fibrosis in mice that received 35 Gy of X rays to the hind leg (*63*). Silverlon burn contact dressing was approved by the U.S. FDA for a limited range of cutaneous radiation injury and radiation dermatitis (*10*, *64*). Gao *et al*. published that enalapril (an ACE inhibitor) started 35 days after irradiation of the whole thorax with 13 Gy mitigated radiation-induced pneumonitis and pulmonary fibrosis (*65*). ACE inhibitors combined with a syngeneic bone marrow transplant were found to mitigate radiation-induced multiple organ injury in rats (11 to 11.5 Gy X irradiation) (*66*). Zhang *et al* found that atorvastatin mitigated cardiac fibrosis in rats after local heart irradiation given in 7 daily fractions of 3 Gy for a total of 21 Gy (X irradiation) (*67*). Murigi et al. reported that AEOL 10150 (a superoxide dismutase mimic) was a mitigator of radiation-induced lung injury in mice after whole-thorax lung irradiation (WTLI) (14.6 Gy of X rays) (*68*). Furthermore IPW-5371, a transforming growth factor (TGF)-beta receptor inhibitor, proved to mitigate radiation-induced late effects in mice that received a 5 Gy dose of total-body X irradiation immediately followed by a 6.5 Gy irradiation to the thorax (*69*). In 2017, Wang et al. reported that treatment with metformin attenuated radiation-induced pulmonary fibrosis in rats (20 Gy of X rays to the right thorax) (*70*) and AEOL 10150, an antioxidant, was found to mitigate radiation-induced lung injury in NHPs with 10.74 Gy (X irradiation) when the drug was administered for an extended period of time (*71*). AEOL 10150 would also go on to receive U.S. FDA IND status for lung DEARE.

In 2014, topical application of the synthetic triterpenoid RTA408, which activates the antioxidative transcription factor Nrf2 and inhibits nuclear factor kappa B (NF-kappa B) was shown to protect mice from radiation-induced dermatitis after repeated 10 Gy irradiations (X rays) limited to an area of skin on the back (*72*). Gerber et al. found IL-12 preserved the cutaneous physical and immunological barrier function after irradiation (a combination of 6 Gy total-body gamma irradiation and 40 Gy beta irradiation from a strontium-90 applicator to the skin) of C57BL/6 hairless mice (*73*). Pretreatment or post-treatment with metformin alone or in combination with several other drugs exhibited a significant improvement after irradiation (7 Gy X irradiation) as measured by spleen nodule formation 13 days postirradiation (*74*). TLR5 agonist entolimod mitigated radiation-induced epithelial damage in mice during fractionated head and neck irradiation (5 fractions of 5, 6 or 7 Gy of X radiation) when used immediately before or after irradiation (*75*). In 2019, topical esomeprazole (a proton pump inhibitor) mitigated radiation-induced dermal inflammation and fibrosis in mice that received 2×15 Gy X irradiation to the left flank (*76*).

A safety and efficacy study for two new decorporation agents, 3,4,3-LI(1,2-hydroxypyridinone (HOPO) and 5-LIO-(Me-3,2-HOPO), was published in 2013 (*77*). These agents would also receive U.S. FDA IND status.

2021–2024

Granulocyte-macrophage colony-stimulating factor (GM-CSF), used alone, was found to be effective in mitigating H-ARS in NHP exposed to 6.55 or 7.13 Gy of gamma radiation (*78*), or in combination with multi-cytokine therapy in humans who received accidental heterogenous doses of ^{60}Co or ^{192}Ir gamma rays (*79*). A pediatric model for H-ARS was established in mice and used to demonstrate that Neulasta® (pegylated G-CSF) was an effective countermeasure for H-ARS but not for DEARE after irradiation with 550 to 875 cGy of gamma radiation (*80*, *81*). Oral administration of the probiotic *Lactobacillus reuteri* expressing IL-22 was shown to facilitate intestinal radioprotection after a single fraction of 9.25 Gy total-body irradiation (TBI), 15 Gy partial-body irradiation with one hind limb shielded (5% of bone marrow), or 19.75 Gy whole-abdominal irradiation (X rays) (*82*). Using a murine model and total-body gamma radiation (7.75 Gy of ^{60}Co gamma radiation), BIO 300, a nanosuspension of genistein, in combination with Neulasta®, was found to protect against DEARE lung damage in mice, reducing both inflammation and fibrosis, neither of which was significantly affected by Neulasta® alone (*83*). BIO 300 has received U.S. FDA IND status.

Countermeasures on the Horizon

As stated above, the U.S. FDA has approved a limited number of radiation countermeasures for very specific exposures/circumstances (see Tables 1 and 2). For ARS, however, only H-ARS has limited radiation mitigation options. The options include Leukine®, Neupogen®, and

Neulasta® [and the Neulasta® biosimilars Stimufend® (peg-fpgk), and Udenyca® (peg-cbqv)], all of which stimulate recovery of neutrophils, and Nplate® which improves platelet recovery. These H-ARS radiomitigators are recombinant growth factors or growth factor receptor agonists that were developed for other indications, and recently repurposed to treat H-ARS (*84*). No radioprotector for H-ARS or mitigator for GI-ARS or for DEARE has yet been approved by regulatory agencies for human use (*85*, *86*). Several radiation countermeasures developed for external exposure, such as entolimod/CBLB502, Ex-Rad/Recilisib, BIO 300 (genistein), myeloid progenitors/CLT-008, 5-androsteinediol, and PLX-R18 (placental-derived cellular therapy) have received U.S. FDA IND status and as shown above, *Radiation Research* publications have disseminated critical information regarding the development of most of them (*85*). Some of these agents have shown promise as protectors, mitigators and/or therapeutic countermeasures, with effects on multiple tissues. Several additional agents have shown great promise as countermeasures and are currently being tested in pre-clinical animal studies (*87*).

The majority of the countermeasures investigated have been studied as single therapies. As more countermeasures with different mechanisms of action have become available, the use of multiple drugs either simultaneously or in sequence has also become an attractive treatment option, known as the polypharmacy approach. Examples of a few combination therapies that have been tested so far include Neulasta® (or a biosimilar) combined with: ALXN4100TPO (TPO receptor agonist) (*88*), L-citrulline (*89*), ghrelin (*90*), ciprofloxacin (*91*) or BIO 300 (*83*). Other combinations include IL-11 plus StemTPO plus enrofloxacin (*38*), captopril (ACE inhibitor) plus gentamicin plus levofloxacin (*58*), metformin in combination with sulfhydryl-containing drugs (*74*), GM-CSF plus cytokines (*79*), pegylated G-CSF plus stem cell factor plus romiplostim (*79*), 16, 16-dimethyl prostaglandin E2 plus lisinopril (ACE inhibitor) (*92*) or plus amifostine (*93*), amifostine plus glucan (*94*), a multiple antioxidant dietary supplement containing L-selenomethionine, sodium ascorbate, N-acetyl cysteine, alpha-lipoic acid, alpha-tocopherol succinate and co-enzyme Q10 (*43*), and gamma tocotrienol plus pentoxifylline (*41*). A recent meeting discussed new developments in polypharmacy for radiation injury (*95*, *96*).

COMBINED RADIATION INJURY (CI): RADIATION INJURY (RI) WITH ANOTHER TRAUMA

Often, victims exposed to radiation also present after blast with additional traumas such as hemorrhage, blast burns, wounds or infections. Combined radiation injuries (CI) were documented at Hiroshima and Nagasaki, Japan, where 60–70% of radiation victims simultaneously received thermal burns and ten percent of the 237 victims at the Chernobyl reactor meltdown received radiation exposure and thermal burns. Either radiation injury or combined injury induce body weight loss and mortality in a radiation dose-dependent manner. However, in mouse models, combined injury exhibits an earlier onset of body weight loss, earlier mortality and delayed wound healing if wound trauma occurred (*12*, *97*).

In the 1970s, the Armed Forces Radiobiology Research Institute (AFRRI) began combined injury investigations. In a murine model, radiation exposure followed by burns or wounds further reduced survival compared to burns alone, wounds alone or radiation exposure alone, and radiation exposure delayed wound healing time. In addition to penetrating skin wounds (*98*) and burn, animal models for combined injury with hemorrhage and infection were established at AFRRI. Besides worsened survival, body weight loss and delayed wound healing, combined injury with radiation plus wound or plus hemorrhage appeared to amplify and prolong skeletal tissue loss. Survivability following combined injury with radiation and wounding is related to the size of the wound (*99*), although some reports indicated that subcutaneous wounding after irradiation increased survival of irradiated mice (*100*, *101*). Injuries before or at the time of irradiation tend to decrease survivability (*97*).

As shown in Table 2, the U.S. FDA has approved a few treatments as mitigators of H-ARS (*84*, *102*). Some of these drugs are less useful for treating combined injury (*103*). The criteria for determining whether a drug/agent is considered as a combined injury medical countermeasure must include improved 30-day survival, reduced body weight loss, and improved wound healing time (for radiation and wound combinations), when the animal is exposed to radiation at $LD_{50/30}$. Several drugs/agents have been tested, but many of them failed to improve survival, mitigate body weight loss or reduce the delay in wound healing time. Table 4 lists mitigators that have shown efficacy toward CI.

MEDICAL COUNTERMEASURES FOR COMBINED RADIATION INJURY (CI)

In the case of radiation combined with wounds, natural choices for treatments included anti-inflammatory agents, antibiotics and agents that encouraged natural wound healing processes that were disrupted by radiation such as laminin deposition. In 2009, we reported that COX-2 inhibitors, celecoxib, and meloxicam, did not improve 30-day survival in B6D2F1 female mice that were exposed to 9.75 Gy of gamma radiation followed by wound trauma (*104*). In 2011, matrix metalloproteinase 2 (MMP2) inhibitor 1,10-phenanthronine, was found to inhibit MMP2 expression and then increase laminin 332 deposition in the skin in rats that were exposure to X rays (10–40 Gy) followed by wounding (*105*). In 2014, Zawaski et al. (*99*) found that Betadine or triple antibiotic ointment increased wound healing in rats exposed to 6 or 7.5 Gy and subjected to a skin wound, but did not improve 30-day survival after CI. Interestingly, Alpha-difluoromethylornithine prevented

TABLE 4
Countermeasure treatment for CI

Agent	Activity/limitations	Ref.(s)
Cox-2 inhibitors: Celecoxib and meloxicam	Failed to improve 30-day survival after CI	(*104*)
MMP2 inhibitor:1,10-phenanthronine	Increased laminin 332 deposit to the skin and accelerate skin wound healing after CI	(*105*)
Betadine	Increased wound healing but did not improve 30-day survival after CI	(*99*)
Triple antibiotic ointment	Increased wound healing but did not improve 30-day survival after CI	(*99*)
Timolol beta-adrenergic receptor antagonist	Increased wound healing after CI	(*109*)
Alpha-difluoromethylornithine	Prevented hippocampus-dependent cognitive impairment after CI	(*106*)
Ciprofloxacin	Increased IL-3 in blood, 30-day survival, and ATP production after CI	(*91*)
Captopril+ gentamicin+ Levofloxacin	Increased 30-day survival after RI but not CI	(*58*)
Neulasta + Alxn4100TPO	Increased 30-day survival after RI and CI	(*88*)
Neulasta + L-Citrulline	No significantly increased survival after CI	(*89*)
Mouse Ghrelin	Increased survival, mitigated H-ARS, GI-ARS, and brain hemorrhage after CI	(*107*)

hippocampus-dependent cognitive impairment in male C57BL/6 mice exposed to 4 Gy of gamma radiation after combined injury (*106*).

Ciprofloxacin is a U.S. FDA-approved fluoroquinolone antibiotic that has been included in the Strategic National Stockpile for dispensing during a national emergency to control bacterial infection. Besides the antimicrobial activity, several groups reported immunomodulatory effects of ciprofloxacin in rodent models and human clinical trials improving a wide spectrum of conditions. In 2015, the authors found that oral administration of ciprofloxacin improved 30-day survival, mitigated body weight loss, accelerated skin-wound healing, upregulated IL-3, and preserved ATP production in B6D2F1 female mice exposed to 9.5 Gy ^{60}Co gamma rays followed by wound trauma (*91*).

We also investigated combined treatments (aka polypharmacy) to maximize countermeasure development to concurrently treat both radiation injury and CI. When B6D2F1 female mice were exposed to 9.5 Gy of ^{60}Co gamma radiation alone or followed by wound trauma, captopril in drinking water in combination with topical gentamicin and oral levofloxacin failed to improve 30-day survival after CI, but was effective at increasing survival after radiation injury (*58*). In 2017, we reported that combined treatment with Neulasta® and Alxn4100TPO increased 30-day survival of B6D2F1 female mice exposed to 9.5 Gy of ^{60}Co gamma rays alone (RI) or followed by wound trauma (CI). Alxn4100TPO is a TPO receptor agonist that significantly increased megakaryocytes in bone marrow and platelets in circulation (*88*). In 2021, using the same animal model, the authors reported that treatment with Neulasta® and L-Citrulline administered 24 h after combined injury improved survival, although this did not reach statistical significance (*89*). The authors also studied mouse ghrelin administered to mice after radiation injury or CI. Mouse ghrelin significantly increased 30-day survival, mitigated H-ARS, GI-ARS, and decreased brain hemorrhage after combined injury (*107*).

Agents that have been successful in alleviating combined radiation exposure with burn injury include verapamil, chitosan-wrapped human defensin 5, glucagon-like peptide 2, or cervical sympathetic ganglia block (*108*). The beta 2 adrenergic receptor agonist timolol improved epidermal burn wound closure after radiation exposure combined with burn injuries (*109*). Finally, the burn/wound contact dressing Silverlon has received U.S. FDA approval for use in treating wounds/burns and dermatitis after irradiation (*10*).

Additional drugs/agents/procedures efficacious at improving 30-day survival for combined injury have been published elsewhere. These include Neupogen®, Neulasta®, Alxn4100TPO, mouse mesenchymal stem cells (MSCs), gentamicin, Silvadene, WR-151327, amifostine, bone marrow transplantation, Neulasta® and ghrelin, Neulasta® and ciprofloxacin, S-TDCM and gentamicin (*12*, *18*, *97*).

PERSPECTIVES ON COMBINED INJURY COUNTERMEASURES

Among all U.S. FDA-approved drugs and non-U.S. FDA-approved candidates, combined therapy with MSCs and Neulasta® should stand a great chance to significantly increase survival after combined injury as well as radiation injury, because whole bone marrow (containing progenitor stem cells and MSCs) transplantation resulted in 100% survival after lethal CI, treatment with MSCs showed survival improvement by 30% after lethal CI, and Neulasta® has been demonstrated to mobilize neutrophils from bone marrow to peripheral blood. Thus, if Neulasta® can enhance MSC capability to save more lives after CI, then lower numbers of MSCs would be needed, which would lead to a lower chance of developing lung fibrosis later. However, the obvious drawback in using MSCs is that medical assistance from appropriately trained personnel is required, which is not feasible in a mass casualty scenario. In addition, the quality control and/or safety of MSCs are not regulated yet. On the other hand, results from the combined treatment

with a TPO mimetic and Neulasta® appeared promising to concurrently treat combined injury as well as RI.

DISCUSSION

The availability of appropriate preclinical animal models was one of the limiting factors for developing radiation medical countermeasures for radiation injury and combined injury and the identification of suitable biomarkers of radiation exposure. Now the characterization of additional animal models is well under way. For instance, radiation exposure biomarker comparisons across mice, NHPs and 2 varieties of minipigs have been performed (*110*), proteomic profiles from heart, lung, and liver samples of irradiated minipigs have been analyzed (*111*) and cutaneous models of both large swine (*112*) and minipigs are under development (*113*) to complement studies using minipigs to examine GI-ARS (*114*) and H-ARS (*115*). For investigating the radiation injury and countermeasure efficacy within large animal models however, only NHP and canine have been fully characterized. The NHP is the gold standard for drug development and U.S. FDA regulatory approval under the Animal Rule. Additional suitable large animal models for H-ARS and GI-ARS need to be developed and validated to expedite the development and regulatory approval of radiation countermeasures. Although the minipig is an intriguing model, it is in a primitive state of investigation as an ARS animal model compared to the mouse, rat, canine, and NHP. Countermeasures are typically tested in rodents using 30-day survival as the major endpoint; a 60-day survival endpoint is used in canines or NHP. These time intervals have been selected to reflect the ability of the agent to provide protection or mitigation of ARS following total- or partial-body radiation exposure. DEARE is also gaining interest in the field of radiation medical countermeasure investigation.

Only a limited number of radiation countermeasures have been fully approved by the U.S. FDA for the purpose of controlling the health hazards arising from ionizing radiation exposures. The U.S. FDA has approved ten agents that mitigate irradiation-associated injuries. Four of these agents, KI (ThyroShield), Zn-DTPA, Ca-DTPA, and Prussian Blue, are agents that bind, chelate, or block internalized radionuclides so that body-burdens of radioisotopes can be minimized. Six growth factors or cytokines have been approved by the U.S. FDA since 2015 as radiomitigators for H-ARS. Two additional agents, amifostine and palifermin, have been approved by the U.S. FDA for very narrow clinical indications (*26*, *87*, *116*); though these agents are used in the clinic for radiation-related limited indications, these are not approved for H-ARS. Of the aforementioned agents, only amifostine is classified as a radioprotector. This agent has poor toxicity and safety profiles, a common limitation with many of the small molecule agents. There are a few additional cytokines and growth factors, such as erythropoietin (EPO), IL-3, and IL-11, that have been approved by the U.S. FDA for limited indications arising from radiotherapy- and chemotherapy-induced neutropenia in cancer patients. There are several agents that have dual use and are being evaluated as radiation countermeasures for H-ARS and GI-ARS.

The efficacy of radioprotectors/mitigators in the setting of exposure to particulate radiation such as protons or high-linear energy transfer (LET) irradiations such as neutrons and heavy ions is a less explored area since the majority of studies utilized low-LET γ radiation or X rays. Finally, the use of combinations of different agents has not been extensively tested. Thus, combinations of countermeasures with different mechanisms of action may be superior to single agents in the same manner that combination cancer chemotherapy is often superior to treatment with individual agents.

CONCLUSIONS

The above listing of potential agents provides a testament to the research and development effort currently being directed toward the discovery and development of safe and effective medical countermeasures for unintentional radiation exposures. However, the number of useful and fully authorized agents remains remarkably limited. *Radiation Research* has substantially contributed and will continue to contribute to the dissemination of information regarding the development of radiation medical countermeasures through the publication of peer-reviewed research and review articles.

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