Review Paper

MEDICAL COUNTERMEASURES FOR RADIATION EXPOSURE AND RELATED INJURIES: CHARACTERIZATION OF MEDICINES, FDA-APPROVAL STATUS AND INCLUSION INTO THE STRATEGIC NATIONAL STOCKPILE

Vijay K. Singh,*† Patricia L.P. Romaine,* and Thomas M. Seed‡

Abstract—World events over the past decade have highlighted the threat of nuclear terrorism as well as an urgent need to develop radiation countermeasures for acute radiation exposures and subsequent bodily injuries. An increased probability of radiological or nuclear incidents due to detonation of nuclear weapons by terrorists, sabotage of nuclear facilities, dispersal and exposure to radioactive materials, and accidents provides the basis for such enhanced radiation exposure risks for civilian populations. Although the search for suitable radiation countermeasures for radiation-associated injuries was initiated more than half a century ago, no safe and effective radiation countermeasure for the most severe of these injuries, namely acute radiation syndrome (ARS), has been approved by the United States Food and Drug Administration (FDA). The dearth of FDA-approved radiation countermeasures has prompted intensified research for a new generation of radiation countermeasures. In this communication, the authors have listed and reviewed the status of radiation countermeasures that are currently available for use, or those that might be used for exceptional nuclear/radiological contingencies, plus a limited few medicines that show early promise but still remain experimental in nature and unauthorized for human use by the FDA.
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INTRODUCTION

RADIATION ACCIDENTS such as those that occurred in Fukushima, Japan (2011), Tokaimura, Japan (1999), Goiânia, Brazil (1988), Chernobyl, Russia (1988), and Three Mile Island nuclear power station, United States (1979), all serve as warning signs of the potential hazards associated with catastrophic nuclear/radiological events (Fushiki 2013; Hu and Slaysman 1984; Koenig et al. 2005; Need et al. 2006; Ohnishi 2012; Williams and McBride 2011). In addition, threats from exposure to high doses of radiation due to terrorist attacks have become more problematic in recent years (Andersson et al. 2008; Hagby et al. 2009). These threats are exacerbated by the lack of available radiation countermeasures; in particular, those medical countermeasures for protecting against and mitigating exposure-related consequent morbidity and/or mortality responses. In such nuclear/radiological exposure scenarios, the number of victims with potential life-threatening complications could reach into the tens of thousands (Biological Effects of Ionizing Radiation VII 2005). Under such circumstances, one should expect not only a limited ability to assess precise levels of exposure to individuals but also substantial delays in delivering medical care to the affected population. This suggests the need for new types of lifesaving treatments that not only are safe and effective but also have extended time windows of effectiveness relative to exposure from the event, as critical medicines may not reach the site of the catastrophic event within days following exposure.

There are additional unmet radiation countermeasure requirements for select groups, such as military personnel and first responders, who may have received prior information about possible radiation exposure but need appropriate protective agents to be administered prophylactically prior to entry into hazardous areas for search-and-rescue

purposes. Although efforts to identify and develop radiation countermeasures were initiated decades ago, only an extremely limited number of safe and effective medical countermeasures have been fully approved by the United States Food and Drug Administration (FDA) for unwanted overexposures to radiation. None of these drugs have been designed, however, to counter specifically "acute radiation syndrome" (ARS). This situation has prompted intensified research to identify a new generation of countermeasures.

High-dose ionizing radiation exposures to the whole or substantial parts of the body often result in life-threatening injuries, primarily to those radiosensitive, self-renewing tissues, but most markedly to the hematopoietic and gastrointestinal (GI) systems. The reproductive system is also highly radiosensitive and self-renewing by nature, but acute suppression of this organ system is not life threatening per se. There are, however, a number of other critical but less radiosensitive tissues, including the respiratory, cardiovascular, and cutaneous systems, along with the liver and kidney (Baker et al. 2011; Dawson et al. 2010; Hall and Giaccia 2012; Meineke and Fliedner 2005). ARS is characterized by the differential response of the body's vital organ systems to various intensities of radiation exposure. There are at least three distinct subsyndromes—hematopoietic, GI, and neurovascular—that are dependent on the total exposure dose, the exposure dose rate, the quality of radiation, and the time and extent of bodily exposure (Cerveny et al. 1989; Dainiak 2002; Gusev et al. 2001). Cutaneous radiation injury is often linked with ARS but not considered as a fourth subsyndrome, as one can have full-blown cutaneous radiation syndrome without having ARS following either localized radiation exposure or non-deeply penetrating radiation. Each subsyndrome follows a similar clinical pattern that is divided into three phases: an initial prodromal phase occurring during the first few hours following exposure, a latent phase that shortens with increasing dose, and a manifest phase. Radiosensitivity of the various tissues does not correspond necessarily to the timing of the onset of the initial manifest phase of radiation injury. Damages are manifested in acute responding tissues within a short time interval of a few days, such as in the case of the GI tract, epidermis, and the bone marrow, but they could be delayed by many months in the case of late-responding organs such as the lungs (Oya et al. 2006). At doses that are frequently fatal within ~1–3 wk (i.e., ~4–10 Gy) but lower than the critical doses that cause GI failure, the bone marrow syndrome is expected to be the major contributor to mortality (Hall and Giaccia 2012). Moreover, even at subcritical doses for lethal GI subsyndrome (4–10 Gy), the leakage of bacteria and toxins from the damaged GI tract into the peripheral circulation has been reported to challenge the immune system with possible aggravation of the hematopoietic syndrome (Guinan et al. 2011).

Medical countermeasures for radiation fall into three broad classes. Radioprotectors or radioprotectants are prophylactic agents that are administered before exposure to prevent radiation-induced cellular and molecular damage (Stone et al. 2004). Radiation mitigators are drugs administered shortly after irradiation that accelerate recovery or repair of radiation injury. Radiation therapeutics or treatments are agents given after overt symptoms appear to stimulate repair or regeneration. Numerous candidate radiation countermeasures (specifically radioprotectants and radiomitigators) have been identified and either have been or are currently being developed for FDA approval (Dumont et al. 2010; Koenig et al. 2005; Singh et al. 2013, 2012c). Radionuclide eliminators are drugs that discorporate or block absorption of internalized radionuclides. Several candidate radiation countermeasures under this category have been identified and/or approved by FDA (Fig. 1, Table 1). Radionuclide eliminators currently licensed or under investigation include potassium iodide (KI), Prussian blue (PB), and zinc/calcium diethylenetriamine pentaacetate (Ca- and Zn-DTPA). This limited list of potentially useful drugs is alarming, especially in terms of the sheer lack of options and the limited scope of amelioration of which each drug is capable.

Though there are large numbers of radiation countermeasures for ARS under various stages of development, it is not possible to include all agents under development in this limited review. This paper is restricted to only those agents under development for ARS that are at the advanced stages such as: (a) seven promising new drugs are highlighted (Fig. 1) having FDA investigational new drug (IND) status [5-Androstenediol (5-AED), BIO 300, CBLB502/Entolimod™, Ex-RAD®, granulocyte colony-stimulating factor (G-CSF), HemaMax™, OrbeShield™]; (b) G-CSF/Neupogen® is already in the strategic national stockpile (SNS); (c) gamma-tocotrienol and AEOL 10150 have advanced to trials in nonhuman primates (NHPs); (d) amifostine is FDA-approved for limited clinical use; and (e) myeloid progenitors are under clinical trial currently for related indications. The authors have also discussed other forms of generally accepted treatments such as supportive care and radionuclides.

Gap in the knowledge

Despite advances for nearly 60 y, a fieldable radiation countermeasure agent for ARS is not yet available for human use. Although significant progress has been made, mechanisms of radiation injury are not fully understood, and so there is no clear-cut strategy for protecting tissues from such injuries. Intellectual property rights and exclusive marketing ownership encourage innovation in the area of drug development, and such incentives attract intellectuals

for facilitating and promoting the development of new products and technologies. Large pharmaceutical companies with research and development capabilities for new drug molecules do not show much interest in agents that have limited potential for revenue generation due to the relatively small size of the target treatment populations and hence relatively small demand. The major funding opportunities for the development of such agents are largely dependent on government resources. To promote such activities in the area of radiation countermeasures, the President of the United States signed into law Project BioShield on 21 July 2004, which provides new tools to improve medical

countermeasures against a chemical, biological, radiological, or nuclear attack. After enactment of this legislation, the U.S. government initiated programs that are intended to support the development of medical radiation countermeasures and provide financial support through the Radiation and Nuclear Countermeasure Program of the National Institute of Allergy and Infectious Diseases, the National Institutes of Health, and the Biomedical Advanced Research Development Authority. Specifically, the Biomedical Advanced Research Development Authority provides significant financial support to start-up companies and other institutions for product development.
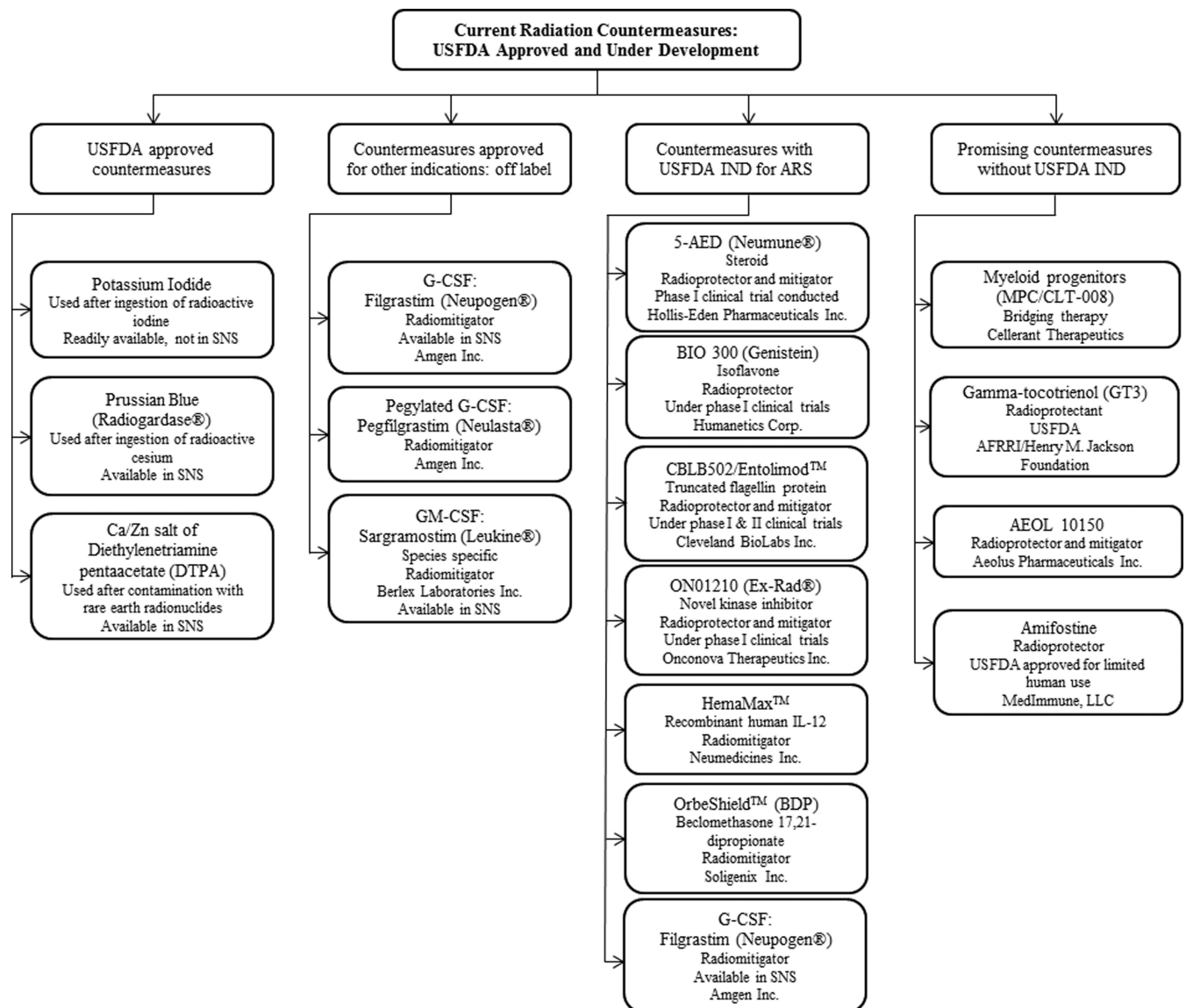
Fig. 1. Promising radiation countermeasures under development. Currently there are seven radiation countermeasures having FDA-IND status: Entolimod/CBLB502, Ex-RAD, BIO 300, OrbeShield, HemaMax, 5-Androstenediol (5-AED), and Neupogen. G-CSF and GM-CSF are available in the SNS. Other countermeasures are under different stages of development. Among the promising countermeasures being developed are myeloid progenitors (CLT-008), GT3, AEOL 10150, and amifostine. There are three countermeasures against internally deposited radionuclides: potassium iodide, Prussian blue, and Ca-/Zn-DTPA.

Table 1. Listing and characteristics of radiation countermeasures with granted FDA status and/or within SNS

Generic species	Generic name(s) (chemical names and pharmaceutical labels)	Specific use (approved use and/or off-label use)	Major advantage(s)	Major Disadvantage(s)	FDA status	SNS status	References
Chemical protectants-phosphorothioates	Amifostine/WR2721 (2-(3-aminopropyl) aminoethylphosphorothioate; Ethyol™)	Cytoprotectant— oral injury (off-label use — survival protection; systemic protection of organ systems, but specifically marrow and gut)	Highly protective with high dosing regimens; systemically active; DMFs ~1.2–3.0 (depending on tissue)	Significant toxic side-effects with clinical doses; short-time window of effectiveness	Approved — specific clinical indications (used off-label)	No	Culy et al. 2001; Glover et al. 1988; Rasey et al. 1988; Weiss 1997
Nutraceuticals-Isoflavonoids	Genistein (4'5,7 trihydroxy-isoflavonoid; Bio 300™)	Not specifically approved, experimental IND status; survival and organ system protection (i.e., marrow, gut, and lung)	Oral administration; DRF ~1.16 in rodents; minimal/no toxic side-effects	Limited time-window of drug effectiveness (~24 h)	IND	No	Day et al. 2008; Landauer et al., 2003, 2009; Zhou et al. 2005
Nutraceuticals-Vitamin E isoform	Gamma-tocotrienol (GT3)	Not specifically approved, without FDA status; promotes survival and protects both bone marrow and gut; mobilizes bone marrow stem cells and progenitors	Injury mitigative; DRF ~1.29; effective with single doses	Injectable only; time-widened but limited effectiveness window (~24–12 h pre-radiation)	None	No	Ghosh et al. 2009a; Kulkarni et al. 2010, 2012; Suman et al. 2013
Immunomodulators-TLR-5 agonist	CBLB502 (bioengineered truncated Salmonella sp. flagellin; Entolimod™)	Not specifically approved, experimental IND status; enhances survival and minimizes marrow and gut damage in select animal models (mice and NHPs)	Single/multiple dose prophylaxis possible; DRF ~1.6	Injectable only; limited effectiveness window (~24 h); flu-like side-effects	IND	No	Burdelya et al. 2008; Krivokrysenko et al. 2010, 2012
Adrenal cortical steroid metabolite 5-androstenediol	5-AED (androst-5-ene-3β, 17β-diol; Neumune®)	Not specifically approved, experimental IND status; enhances survival and minimizes marrow damage	Effective single dosing pre- and post-exposure; DRF ~1.26	Injectable only; oral dose ineffective; limited effectiveness window (~24 – 48 h pre, 2 h post-exposure); local skin (site-of-injections) irritation	IND	No	Stickney et al. 2006, 2007; Whitnall et al. 2000, 2002, 2005
Ex-RAD®	ON01210, chlorobenzylsulfone derivative	Not specifically approved, experimental IND status	Kinase inhibitor; DRF ~1.16; protector and mitigator; orally and parenterally effective	Short half-life; short administration window, effective against ~LD_{50} dose of radiation	IND	No	Ghosh et al. 2009b, 2012; Suman et al. 2012a, b.
Anti-inflammatory-corticosteroid	BDP/SGX201 (corticosteroid-beclomethasone 17,21-dipropionate; OrbeShield™)	Not specifically approved, experimental IND status; designed to mitigate radiation enteritis and to promote survival via a combined short- and long-acting drug formulation	Orally effective; significantly extends survival times in experimental canines with GI subsyndrome; minimally toxic, well-tolerated	Limited drug effectiveness; limited effectiveness window (2–24 h post irradiation); dose-dependent emesis	IND	No	Soligenix 2014

Table 1. (*Continued*)

Generic species	Generic name(s) (chemical names and pharmaceutical labels)	Specific use (approved use and/or off-label use)	Major advantage(s)	Major Disadvantage(s)	FDA status	SNS status	References
Free-radical quencher- Meso-prophyrin mimetic	AEOL 10150 (Mn prophyrin SOD mimic)	Not specifically approved, experimental IND status for another indication; designed to mitigate lung-ARS, upregulates antioxidant system	Mitigates acute radiation-induced lung injury	Limited effectiveness window (~24 h post-irradiation)	IND for another indication	No	Garofalo et al. 2014; Orrell 2006
Recombinant growth factor	rhu G-CSF/filgrastim (Neupogen®)	Approved for clinical/hemopathological indication, not approved as an ARS countermeasure; designed to stimulate neutrophil production by bone marrow in severely neutropenic patients; minimizes risk of life-threatening infections	Effective in alleviating neutropenia in immunocompromised patients; low effective dose; well-tolerated, minimally toxic; mitigates marrow-ARS	Multiple treatments required; injected only; rare but significant side-effects; monitoring of effects required	IND, used off-label	Yes	Farese et al. 2013; Gourmelon et al. 2010
Pegylated growth factor	rhu G-CSFpeg/pegfilgrastim (Neulasta®)	Approved for specific hemopathological indication; designed for sustained stimulation of neutrophil production by bone marrow; minimizes risk of life-threatening infections	Highly effective in alleviating severe neutropenia; single injection sufficient; well-tolerated, minimally toxic	Injectable only; rare, but significant side-effects noted	Used off-label	No	Farese et al. 2012
Recombinant growth factor	rhu GM-CSF (Leukine®)	Approved for specific hemopathological indication; designed to stimulate granulopoiesis by marrow in severely granulocytopenic patients; minimizes risk of life-threatening infections	Effective in alleviating severe granulocytopenia; well tolerated/minimally toxic	Multiple treatments required; injected only	Used off-label	Yes	Amgen Inc., 2013; Sanofi-Aventis, 2013
Recombinant cytokines	rhu IL-12 (HemaMax™)	Not specifically approved, experimental IND status; enhances survival and minimizes marrow and gut damage	Single/multiple dose mitigation possible	Effective in murine and NHP models	IND	No	Basile et al. 2012; Gluzman-Poltorak et al. 2014a, b; Gokhale et al. 2014; Xiong et al. 2013
Cell transfer/transplantation	CLT008 (Human), mMPC (mice)	Not specifically approved; designed for partial marrow reconstitution	Significant mitigation of ARS; effective against high doses of radiation	IV infusions only; long term toxicity is ill-defined	None	No	Singh et al. 2012b, e
Chelator	Zn/Ca DTPA	Chelating/binding/decorporating agent for transuranics (plutonium)	Oral administration; significantly reduces total body contaminants of transuranics	Requires administration and monitoring by medical personnel; bone toxicity with extended use	Approved for use as indicated	Yes	Kazzi et al. 2012

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Table 1. (Continued)

Generic species	Generic name(s) (chemical names and pharmaceutical labels)	Specific use (approved use and/or off-label use)	Major advantage(s)	Major Disadvantage(s)	FDA status	SNS status	References
Binding agents	Prussian Blue/Radiogardase®	Chelating/binding/decorporating agent for Cesium-137	Oral administration	Requires administration and monitoring by medical personnel	Approved for use as indicated	Yes	Hussar 2005
Blocking agent	KI	Thyroid uptake/blocking agent for radio-iodides (Iodine-131, Iodine-125)	Oral administration		Approved for use as indicated	No	Jarrett 1999
5-hydroxytryptamine antagonist	Granisetron/Kytril®	Blocking/minimizing emetic effects of acute radiation exposure	Oral administration		Approved for use as indicated	No	Kehlet et al, 1996
Quinolones		Broad-spectrum infective agent (e.g., 4-fluoroquinolones/ciprofloxacin)			Approved for use as indicated	Yes	Dainiak et al 2003; Flynn et al 2006; Gorin et al 2006; Waselenko et al 2004
Fluids, analgesics, anesthetics, red blood cells/platelets transfusions		Rehydrating, pain relieving, blood cell replacement agents			None	Yes	Kaplan 1985

FDA's drug evaluation and licensing processes

Animal efficacy rule for the development of radiation countermeasures. Efficacy studies of radiation countermeasures in humans cannot be conducted because it would be unethical to deliberately expose healthy human volunteers to lethal or permanently damaging biological, chemical, radiological, or nuclear substances. In 2002, the FDA issued what has become known as the "Animal Efficacy Rule" (21 CFR Parts 314), intended to expedite the development of new drugs and biologic products as medical countermeasures to chemical, biological, radiological, and nuclear threats. The Animal Rule applies only to new drug or biologic products for which definitive human efficacy studies cannot be conducted (Gronvall et al. 2007; Nightengale et al. 2002; U.S. Food and Drug Administration 2014).

The FDA may grant marketing approval for new drug products for which safety has been established and for which the requirements of CFR Parts 314.600 are met based on adequate and well controlled animal studies to establish that the drug product is likely to produce clinical benefit in humans. The criteria of the FDA's Animal Efficacy Rule relevant to animal model development are stated below. The FDA will rely on data from animal model studies to provide substantial evidence of the effectiveness of these products only when:

1. there is a reasonably well understood pathophysiological mechanism of the toxicity of the substance and its prevention or substantial reduction by the product;
2. the effect is demonstrated in more than one animal species expected to react with a response predictive for humans, unless the effect is demonstrated in a single animal species that represents a sufficiently well-characterized animal model for predicting the response in humans;
3. the animal study's endpoint is related clearly to the desired benefit in humans, generally the enhancement of survival or prevention of major morbidity; and
4. the data or information on the kinetics and pharmacodynamics of the product or other relevant data or information, in animals and humans, allows selection of an effective dose in humans.

The Animal Efficacy Rule does not entirely eliminate the requirement of testing of the drug in humans. Clinical trials still are required to evaluate the safety of the countermeasure and to help determine the appropriate dose of the drug for efficacy in humans. Substituting animals for humans in efficacy tests was not intended to make it easier to obtain FDA approval for novel countermeasures. In fact, more information is required about the animal model itself, the mechanism and course of disease and the mechanism of action of the countermeasure, than when efficacy studies can be performed in humans. Without convincing human efficacy data, it is more difficult to understand how a countermeasure works, why it works, and to generate data

providing confidence that the countermeasure will be effective in humans when used. Animal models rarely reflect the human disease precisely. Animal-efficacy data will never be as convincing as the human-efficacy data used for other drugs or vaccines; thus a different mindset and a consistent strategy are required for the approval of countermeasures that will be needed during a chemical, biological, or radiation/nuclear defense emergency. The FDA also requires post-marketing human efficacy studies if the countermeasure is used in humans as a response to a radiation event. Therefore, as part of the FDA license application, pharmaceutical companies need to develop plans to execute such post-marketing clinical trials for drug efficacy—a testing process that no doubt would be extremely difficult while in the midst of a national public health crisis.

In sum, these challenges of the Animal Efficacy Rule requirements, compared with the traditional licensure pathway, may be one reason it has rarely been used to approve new drugs. Since 2001, only five drugs were approved, despite massive investment by the federal government to promote development of medical countermeasures to potential threats (Aebersold 2012). The first countermeasure approved was pyridostigmine bromide in 2003 for use after exposure to Soman, a nerve agent (Albuquerque et al. 2006). The second drug, Cyanokit (a lyophilized formulation of hydroxocobalamin), was approved by the FDA in December 2006 for use as an antidote in treating patients with cyanide poisoning. The third drug, Levaquin (levofloxacin), was approved in April 2012 to treat patients with the plague after exposure to *Yersinia peptis* (U.S. Food and Drug Administration 2012a). The fourth FDA-approved (December 2012) drug is Raxibacumab, a monoclonal antibody and the first biologic product to be approved under this rule, for the treatment of inhalational anthrax due to *Bacillus anthracis* in combination with appropriate antibacterial drugs and also for prophylaxis of inhalational anthrax when alternative therapies are not available or are not appropriate (U.S. Food and Drug Administration 2012b). The latest and fifth drug approved (March 2013) under this rule is the Botulism antitoxin to treat patients showing signs of botulism following exposure to botulism neurotoxin. This product is a mixture of antibody fragments that neutralizes all of the seven Botulinum nerve toxin serotypes known to cause botulism (U.S. Food and Drug Administration 2013c).

The additional drugs listed that have indeed been fully approved and licensed by the FDA as countermeasures for (non-clinical) ionizing radiation exposures that stem from internalized radionuclides (e.g., KI, PB, and Zn/Ca DTPA) have done so without implementing FDA's new "Animal Efficacy Rule."

In brief, the Animal Rule was developed to provide a basis for approving certain drugs or licensing certain biologic products as medical countermeasures to biological, chemical, radiological, and nuclear threats without efficacy data in humans, and considering the dismal record of this approval process, medical authorities and physicians who are/would be attending to the radiation-injured following a catastrophic exposure incidence will be required to consider less than optimal preventive/mitigative treatment options. These options might include using "off-labeled" medicines that are indeed FDA-approved but are not specifically indicated for use as a radiation countermeasure, or perhaps alternatively using drugs that carry an IND status but would require informed consent from human subjects prior to use. The intent of this brief review is to discuss such drugs, their characteristics, and their status in regard to FDA approval.

FDA's orphan drug designation program. Radiation countermeasures being developed for ARS and other exposure-related injuries are assigned FDA orphan drug status. The FDA's orphan drug designation program provides orphan status to drugs and biologics defined as those intended for the safe and effective treatment, diagnosis or prevention of rare diseases/disorders that affect fewer than 200,000 people in the U.S. (or less than 5 per 10,000 people in a community) or that affect more than 200,000 persons but are not expected to recover the costs of developing and marketing a treatment drug. Assignment of an orphan drug designation status to a given drug provides its manufacturer/pharmaceutical company tax reductions and the exclusive rights to the cure for a specific condition for a period of 7 y post-approval. It encourages corporations to enter a market where the high costs of drug development are less likely to be recouped quickly, due to the smaller pool of individuals needing the cure.

FDA's "fast track" drug approval process(es). Usually, the FDA considers medical countermeasures for radiation-related injuries in general, and for ARS specifically, under "fast track" approval process. The FDA's fast track programs are designed to facilitate the development and expedite the review and approval of new drugs that are intended to treat serious or life-threatening conditions and that demonstrate the potential to address unmet medical needs. The purpose is to get important new drugs to patients earlier. Filling an unmet medical need is defined as providing a therapy where none exists or providing a therapy that potentially may be superior to currently available therapy. Most countermeasures at an advanced stage of development have received FDA fast-track status.

Strategic national stockpile (SNS)

In 1998, Congress appropriated funds for the Centers for Disease Control and Prevention to acquire a pharmaceutical and vaccine stockpile to counter biological, chemical and other threats from widespread diseases that could

affect large numbers of civilians. The program was originally called the National Pharmaceutical Stockpile program, but it has since been extended to involve much more than just drugs. On 1 March 2003, the National Pharmaceutical Stockpile became the SNS program managed jointly by the Departments of Homeland Security and Health and Human Services (Centers for Disease Control and Prevention 2014). With the signing of the BioShield legislation, the SNS program was returned to Health and Human Services for oversight and guidance. In brief, the SNS is a federally owned and managed national repository of antibiotics, antivirals, chemical antidotes, antitoxins, life support pharmaceuticals, vaccines, intravenous (iv) administration supplies, airway maintenance supplies, masks, pandemic countermeasures, and medical/surgical items to supplement and resupply state and local agencies with these critical medical items in the event of an incident anywhere and at any time within the United States or its territories following incidents involving either the use of weapons of mass destruction (chemical, biological, radiological or explosive) or a major natural or technological disaster. The mission of the SNS is to provide the above items in a rapid and safe manner (Esbitt 2003; Stewart and Cordell 2007; Waselenko et al. 2004).

The SNS program is committed to having 12-Hour Push Packs delivered anywhere in the United States or its territories within 12 h of a federal decision to deploy. The 12-Hour Push Packs have been configured to be immediately loaded onto either trucks or commercial cargo aircraft for the most rapid transportation. At the same time assets from the SNS are deployed, the SNS program will deploy its Technical Advisory Response Unit to coordinate with state and local officials so the SNS assets can be efficiently received and distributed on arrival at the site.

Approaches and strategies for the prevention, mitigation, and treatment of radiation injuries

Over many decades, attempts to explain the multi-organ involvement and the generalized collapse in function of those organs after exposure to extremely high doses of ionizing radiation have led to the suggestion that the process(es) are multifactorial and not at all simple by nature. These include directly or indirectly induced cell senescence and cell death within vital tissues of the body, changes in the cellular microenvironment, modulation of the immune response, and changes in post-radiation expression of cytokines and chemokines (Dumont et al. 2010; McBride et al. 2004; Singh et al. 2012c, 2012f, 2011; Williams et al. 2010). On the molecular level, recent mechanistic studies suggest key processes involving a prolonged repair (e.g., 24–48 h) of potentially lethal double-strand breaks within DNA (deoxyribose nucleic acid) as manifested by circadian regulation of p53 expression and the onset of double-strand

break repair (Batchelor et al. 2011; Lahav 2008). This may allow a much longer time window for intervening with repair process(es) in order to achieve a more complete repair of damaged DNA than the previously proposed timeframe of only a few hours (Elkind et al. 1984; Elkind and Kamper 1970). Regardless, there is no clear understanding as to how these findings might be practically used in terms of mitigating acute radiation injury at the organ system level. One of the more critical life-threatening complications following exposure to high doses of whole-body ionizing radiation is ARS that arises from the marked suppression of regenerative hematopoiesis, driven largely by an ionizing radiation-induced death of hematopoietic stem cells (HSCs) and early progenitors (Donnelly et al. 2010; Koenig et al. 2005; Williams et al. 2010; Williams and McBride 2011). Transplanting HSC may be the remedy of choice for repairing ionizing radiation-induced injury to bone marrow. Nevertheless, because of problems associated with technical and biological aspects of the transplant procedure, stem cell transplant may not be practicable in these nuclear/radiological exposure contingencies, especially "large-scale" events involving many casualties. Moreover, there would be, undoubtedly, major difficulties in not only finding adequate matches for all individuals within the affected population but also in having available suitable medical facilities for such transplantations under various radiation exposure scenarios.

Different approaches have been suggested to mitigate high-dose radiation injury and decrease radiation-induced mortality due to the hematopoietic syndrome. These include anti-Gram-negative bacteria antibiotics treatment (Brook et al. 2004), complemented by antibiotics of broad coverage (e.g., 4 fluoroquinolone-ciprofloxacin) and judicious use of select antimycotics, antivirals, and fluids (Dainiak et al. 2003). A continuous post-irradiation iv treatment with a combination of antibiotics and recombinant gut permeability-increasing-protein BP21 also has shown promising results in mitigating radiation-induced bone marrow aplasia (Guinan et al. 2011). By contrast, another drug, amifostine, is an exceedingly well studied, well recognized radioprotectant that is clearly efficacious in terms of its radioprotective properties; unfortunately, the drug is quite toxic (hypotensive and emetic by nature) when administered at doses that are significantly radioprotective (Wasserman and Brizel 2001). Nevertheless, the FDA has approved amifostine for limited use and for specific indications (for preventing radiation injury in the salivary glands and reducing head and neck cancer in patients receiving radiotherapy to reduce xerostomia) (Eisbruch 2011; Kouvaris et al. 2007; Singh et al. 2012c). Amifostine has been used intraperitoneally (ip), intramuscularly (im), and iv in various studies. The effect of such radiation-protective agents should be theoretically maximal only when given a short time before or after radiation exposure (Dumont et al. 2010; Landauer et al.

1992; Singh et al. 2012c; Weiss and Landauer 2009). Several radiation countermeasures are under investigation based on different mechanisms, such as antioxidants, anti-apoptotic agents, cytokines and growth factors, and anti-inflammatory agents (Dumont et al. 2010; Seed 2005; Singh et al. 2012c; Weiss et al. 2009). As stated above, in this review, the authors discuss selected agents that are at an advanced stage of development or already approved by the FDA.

Efficacy comparison for various radiation countermeasures

The extent of survival protection against acute irradiation injury is generally expressed as a dose modification factor (DMF) or dose reduction factor (DRF). DRF and DMF values are calculated by dividing the radiation LD_{50} (lethal dose of 50% mortality) for drug-treated, irradiated animals by the LD_{50} for irradiated animals treated only with the vehicle used to administer the drug. For example, the DRF for a 30-d survival in the mouse model quantifies protection of the hematopoietic system and is, no doubt, one of the more useful experimental measures for comparing a drug's efficacy against ARS (Brown et al. 1988; Yuhas and Storer 1969). Because the probit regression lines are not always parallel, one should perform the complete DRF analysis. Even when using DRF, comparison of different agents may be difficult because the DRF can vary as a function of species, strain, age, gender, vehicle, route of drug administration, and the time of drug administration in relation to irradiation. In comparing the DRFs of different drugs, another important issue is to consider relative toxicity of the drug doses being used for deriving DRF. The therapeutics index of a drug is defined as the ratio of the drug LD_{50} to the effective dose of the drug. In general, the larger the therapeutic index, the safer the drug will be for the individual experimental animal or human alike (Weiss et al. 2009).

Countermeasures against external ionizing radiation: injury preventing and mitigating pharmacologics

Amifostine/ethyol. Amifostine (WR2721), or more specifically 2-(3-aminopropyl) aminoethylphosphorothioate, is currently the only systemically effective radioprotective agent that has been fully approved (June 1999) for human use by the FDA (Glover et al. 1988; Rasey et al. 1988; Weiss 1997). The drug was initially developed by US BioScience Inc., and is currently produced and marketed by MedImmune Inc. (Gaithersburg, MD) (MedImmune 2013). Despite this FDA "approval for use" status, the drug has been authorized for use for only a very narrowly defined medical indication, namely the reduction of xerostomia that results from post-irradiation injury of salivary glands in patients undergoing radiotherapy for the treatment of head and neck cancers (Culy and Spencer 2001). This primary indication for amifostine's use (and approval) is closely tied to a secondary indication; namely, for the cytoprotection of irradiated oral epithelium and the prevention of oral mucositis. Despite this very limited drug indication, amifostine is well recognized as a potent cytoprotectant for many of the body's major organ systems when administered at sufficiently large doses (i.e., 300–500 mg/kg). The latter drug dosing levels are well within the range of currently prescribed doses for the cytoprotection of the cancer patient's salivary glands and the minimization of radiation-induced xerostomia. Under standard dosing regimens, amifostine is generally well tolerated by the majority of patients; serious side effects are rare, but minor toxic responses occur frequently and include nausea and vomiting. Adverse cutaneous reactions are commonly noted following subcutaneous (sc) injection. The slow administration of amifostine is designed to limit the frequency and intensity of these relatively mildly toxic side effects. Nevertheless, these side effects are performance decrementing. Accordingly, amifostine has not been approved for general use (non-clinical) in radiation protection of high-risk personnel or the population at large. In part, this lack of approval stems from the recognition that amifostine is moderately toxic and induces nausea and vomiting in a substantial fraction of treated individuals when delivered at dose levels that are protective against ARS. However, alternative indications have been proposed and include: a) global cytoprotection with significant survival benefit when administered at high drug doses; b) selective protection of specific progenitorial tissue compartments at low drug doses; and c) protection against late-arising, radiation-induced cancers with low drug doses (Seed 2005). Recently, radioprotective doses for amifostine have been demonstrated that appear to lie between 25 and 50 mg kg^{-1}. Mature, lineage-restricted progenitors appear to be more responsive to the protective effects of low doses of amifostine than the more primitive, multipotential progenitors (Seed et al. 2014). Amifostine is a remarkably effective, potent, and systemically active drug that has the capacity to provide not only substantial cytoprotection to various vital tissues but also to promote survival in otherwise fatal nuclear/radiological exposure situations.

In addition to the toxic side-effects, there are other limitations in using this drug for non-clinical purposes: first, amifostine is currently administered by iv infusion; other routes of drug delivery have been explored but remain to be fully authorized/approved by the FDA. Secondly, amifostine has an extremely short, pre-exposure time window of radioprotectiveness (i.e., generally less than 1 h).

A sizable effort has been made to research and develop new formulations and delivery systems for amifostine in order to better manage drug toxicity and to enhance overall drug efficacy. This effort is essential if amifostine is ever to be authorized for use during nuclear/radiological contingencies.

In this regard, an effort has been made to reduce the toxicity of amifostine by one of several methods: a) a simple dose-reduction alone or coupled with supplementation with non-toxic drug adjuvants (Seed et al. 1999); b) use of anti-emetics; and c) slow-release delivery of drugs (Srinivasan et al. 2002). In general, all of these approaches have proven to be effective in reducing drug toxicity but not entirely eliminating it.

It has been demonstrated that low doses of amifostine ($\sim$25–75 mg kg^{-1}) delivered shortly prior ($\sim$30 min) to acute, whole-body γ-radiation exposure can provide significant levels of protection to experimental mice and their vital progenitorial compartments of bone marrow and that this added protection most likely translates into increased rates of survival (Seed et al. 1999). Assuming an uncertainty in the quantitative relationships between drug dosing in different species, these amifostine dosing levels roughly equate to $\sim$2–6 mg kg^{-1} (or $\sim$74–222 mg m^{-2}) in humans (Freireich et al. 1966). Prior clinical tests have shown that comparably low amifostine dosing levels ($\sim$267 mg m^{-2} delivered by sc injection) in a small cohort of young, normal male volunteers was well tolerated with no significant side effects reported and little to no nausea experienced (Shaw et al. 1988).

Upper and lower GI toxicity associated with high dose (cytoprotective doses) of amifostine can be ameliorated with the application of 5-hydroxytryptamine receptors (5-HT-receptor) inhibitor-based anti-emetics, such as Kytril®. Kytril administrations (80 mg kg^{-1}) delivered shortly before amifostine injections reduced both the frequency of the emetic responses and the fraction of emetic animals. At the lowest dose tested, 12.5 mg kg^{-1} of amifostine, 90% of the animals tolerated this dosing exceedingly well and showed no signs of emesis (T.M. Seed, unpublished observations[§]).

An attempt to develop amifostine for non-clinical purposes has been made through sustained drug delivery. For this effort, a "slow releasing" implantable pellet has been designed and tested in the laboratory using a small rodent model (Srinivasan et al. 2002). The implanted amifostine-embedded pellet provides not only an extended time-window of radioprotection ($\sim$4–6 h) but also reduces the severity and delays the onset of performance decrementing effects of the drug.

Despite the modest advancements in attempting to maximize amifostine's usefulness as a radioprotectant, none of the systems (as described above) to date entirely eliminates amifostine's toxicity. Although the work on amifostine and related aminothiols has been promising in terms of developing and fielding a safe and effective radioprotector, additional research is clearly needed in order to improve current drug design and delivery strategies.

[§]T.M. Seed, unpublished observations.

CBLB502/Entolimod. Currently being clinically developed as a radiation countermeasure, CBLB502 is a potent and stable agent derived from the flagellin protein of *Salmonella* bacteria (*Salmonella enterica serovar Dublin*). Its pharmacologic action is based on binding to toll-like receptor 5 (TLR5) of targeted cells and activating NF-κB signaling. Biologically, purified flagellin protects mice from lethal doses of γ-total-body irradiation (TBI) (Vijay-Kumar et al. 2008). Cleveland BioLabs, Inc. (Buffalo, NY, USA) identified CBLB502 (now known as Entolimod) as a TLR5 ligand that significantly improved the radioprotective efficacy of flagellin while having reduced toxicity and immunogenicity (Burdelya et al. 2008). As a truncated derivative of the *Salmonella* flagellin protein, CBLB502 acts by triggering TLR5 signaling to activate NF-κB. The FDA has granted IND status to CBLB502 as a radiation countermeasure for ARS, and it is currently in clinical development (Cleveland BioLabs, Inc. 2014). Data from a human safety study indicates that CBLB502 was well tolerated systemically, and biomarker results corresponded to previously demonstrated biomarkers in animal models for ARS (Krivokrysenko et al. 2012). Like other radiation countermeasures for ARS, CBLB502 has been granted fast-track status by the FDA.

To avoid apoptosis, tumors employ genetic mechanisms that allow them to survive. The radioprotection strategy using TLR ligands (specifically CBLB502) is based on such properties of tumors; it involves mimicking the tumor's ability to avoid apoptosis using pharmacological activators of NF-κB and p53 (protein 53, a tumor-suppressor protein). A single injection of CBLB502, either before lethal TBI (24 h prior) or up to 48 h following irradiation, protected mice from both GI and hematopoietic subsyndromes with significantly improved survival (Krivokrysenko et al. 2010). CBLB502 also demonstrated radioprotective and radiomitigative potential in lethally irradiated NHPs (Burdelya et al. 2008). A single im injection of CBLB502 significantly increased the survival of rhesus NHPs exposed to 6.5 Gy TBI and promoted the regeneration of their small intestine, spleen, thymus, and bone marrow when administered from 1 to 48 h after irradiation (Krivokrysenko et al. 2010). The severity and duration of irradiation-induced thrombocytopenia and neutropenia decreased significantly with CBLB502 treatment.

Recently, two cytokines, G-CSF and interleukin-6 (IL-6), were identified as candidate biomarkers for the radioprotective and radiomitigative efficacy of CBLB502. Induction of both G-CSF and IL-6 by CBLB502 is TLR5-dependent and dose-dependent. Also it is critically important for CBLB502's aid in increasing survival of irradiated animals (Krivokrysenko et al. 2012). Administration of either G-CSF or IL-6 neutralizing antibody abrogated the radiomitigation by CBLB502. These biomarkers are likely to be useful for the accurate prediction of CBLB502 dose, providing radioprotection or radiomitigation in humans. Cleveland BioLabs, Inc.

is preparing pre-EUA application for submission to FDA (Cleveland BioLabs, Inc. 2014).

5-Androstenediol (5-AED)/Neumune. 5-AED (androst-5-ene-3beta,17beta-diol) has been advanced as a possible countermeasure for treating the hematological component of ARS. It has been used in animal models to stimulate both innate and adaptive immunity and treat infection and radiation-induced immune suppression. One of five radiation countermeasures shown to enhance survival in irradiated NHPs is 5-AED (the other four are Neupogen/G-CSF, HemaMax, AEOL 10150 and Entolimod/CBLB502). The 5-AED has been investigated as a radioprotector as well as a radiomitigator (Stickney et al. 2007, 2006; Whitnall et al. 2002). A single sc injection of 5-AED 24 to 48 h prior to or 2 h after a lethal dose of TBI enhanced survival in mice (Whitnall et al. 2000, 2002), although injection 2 h after irradiation had lower efficacy than a pre-irradiation injection (i.e., 12% vs. 78%, respectively) (Whitnall et al. 2005). Later, the radioprotective efficacy of 5-AED was confirmed in separate studies in irradiated mice and NHPs (Loria et al. 2000; Stickney et al. 2006). Administration of 5-AED elevated granulocyte-macrophage colony-forming cell numbers in bone marrow and increased neutrophils, monocytes, and natural killer cells in blood of irradiated animals (Stickney et al. 2007, 2006; Whitnall et al. 2001). The 5-AED also improved radiation-induced thrombocytopenia (Stickney et al. 2007, 2006; Whitnall et al. 2001, 2002). However, 5-AED did not protect mice receiving high doses of radiation causing GI subsyndrome, and there was no effect on crypt counts in irradiated mice **. Administration of 5-AED enhanced survival of irradiated primary human hematopoietic progenitor (CD34^{+} cells) in vitro (Xiao et al. 2007).

To elucidate other mechanisms that may be responsible for the survival-enhancing effects of 5-AED in irradiated animals, studies were extended to investigate the cytokine profile induced by 5-AED in mice. The 5-AED significantly increased levels of both G-CSF and IL-6 in peripheral blood of mice (Singh et al. 2005). An antibody neutralization study suggested that endogenous G-CSF was involved in survival enhancement by 5-AED. Also 5-AED modulated apoptotic and cell cycle pathway proteins in irradiated mice; 5-AED administration appeared to limit DNA strand breakage in splenocytes from irradiated mice (Grace et al. 2012).

Recently conducted clinical trials suggest that parenteral administration of Neumune in aqueous suspension may be a safe and effective means to stimulate innate immunity and alleviate neutropenia and thrombocytopenia associated with ARS (Stickney et al. 2010). Neumune administration significantly increased circulating neutrophils and platelets in the peripheral blood of adult and elderly subjects.

ON01210/Ex-RAD®. ON01210 (a chlorobenzylsulfone derivative known as Ex-RAD) is a novel, small-molecule kinase inhibitor under development as a radiation countermeasure. Ex-RAD provided significant protection against [60]Co γ-TBI when administered sc (500 mg kg^{-1}) to C3H/HeN mice 24 h and 15 min before irradiation. Ex-RAD's estimated DRF is 1.16 (Ghosh et al. 2009b). In another study, Ex-RAD showed a significant survival benefit after prophylactic oral administration of the drug (Suman et al. 2012a). In addition to its protective efficacy, Ex-RAD's mitigative properties have been explored to some extent. Ex-RAD protected 90% of C3H/HeN mice compared to 50% survival in a control group when administered 24 and 36 h after radiation exposure (7.5 Gy ^{137}Cs) (Kumar 2010).

Not only does Ex-RAD increase survival rates, it also has been shown to aid in the recovery of the hematopoietic system. This drug accelerated the recovery of peripheral blood elements in irradiated mice when administered either sc or orally (Ghosh et al. 2012; Suman et al. 2012a). In addition, Ex-RAD-treated mice (either through oral or sc route) contained higher numbers of granulocyte macrophage-colony forming units than in vehicle-treated mice. Bone marrow obtained from irradiated mice indicated that Ex-RAD protected cells from radiation-induced apoptosis after exposure to [60]Co γ-irradiation (Ghosh et al. 2012). Ex-RAD also assists in the recovery of the GI system, with a higher number of surviving intestinal crypts after acute radiation exposure in Ex-RAD-treated mice than irradiated controls (Ghosh et al. 2012). These effects may be due in part to signaling pathways that are affected by Ex-RAD (Suman et al. 2012b). Recently, Kang et al. (2013) demonstrated that Ex-RAD manifests its protective effects through the up-regulation of PI3-kinase/AKT pathways in cells exposed to radiation.

Ex-RAD (and also OrbeShield—discussed below) has been granted FDA IND status. Both drugs have demonstrated oral efficacy. Oral administration holds better clinical promise as an effective countermeasure for first responder use as well as for at-risk civilian populations in a nuclear accident. Onconova Therapeutics Inc. (Newtown, PA), the pharmaceutical drug developer, has completed two phase-I clinical studies using Ex-RAD in healthy volunteers and has reported no evidence of systemic side effects (Onconova Therapeutics Inc. 2014). Currently, this countermeasure is being tested for efficacy in NHP models.

BDP/OrbeShield™. BDP (beclomethasone 17,21-dipropionate, a highly potent, topically active corticosteroid) has a local effect on inflamed tissue (Phillipps 1990). OrbeShield demonstrated a statistically significant survival advantage in a canine model of the GI subsyndrome of ARS (Soligenix 2014). Sixteen canines were irradiated with 12 Gy TBI at 0.7 Gy min^{-1}. All animals received

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** V.K. Singh, unpublished observations.

autologous bone marrow infusion 4 h after TBI to reduce the duration and impact of the hematopoietic ARS. Supportive care included iv fluids, electrolytes, broad-spectrum iv antibiotics, anti-emetics, and irradiated whole blood transfusion support. Four control canines did not receive BDP, six canines received BDP 2 h after irradiation, and six received BDP starting 24 h after irradiation. The BDP treatment was 2 mg orally every 6 h for 14 d, then 2 mg orally twice daily until day 100. The median survival of three treatment groups was: 8 d control, 100 d for 2 h BDP, and 87 d for 24 h BDP ($p = 0.04$ for both BDP groups compared to control) (Georges et al. 2012). These findings suggest that BDP has the potential to rescue inflammation in the radiation damaged GI mucosa and improve survival when therapy is initiated as late as 24 h after high dose irradiation. Recently, the FDA has granted IND status to OrbeShield (Soligenix 2014). OrbeShield has been formulated for oral administration in patients with GI subsyndrome as a single product consisting of two tablets; one tablet is intended to release BDP in the proximal portions of the GI tract, and the other tablet is intended to release BDP in the distal portions of the GI tract. This agent has also been granted orphan drug and fast-track status by the FDA.

AEOL 10150. AEOL 10150 is a new radiation countermeasure currently being researched and developed by Aeolus Pharmaceuticals, Inc. (Mission Viejo, CA). AEOL 10150 is a novel, well tolerated, 'broad-spectrum catalytic antioxidant' (meso-porphyrin mimetic; $C_{48}H_{56}C_{15}MnN_{12}$) with significant protective and mitigative activities relative to ARS, in particular acute pulmonary injury (Batinic-Haberle et al. 2014; Gridley et al. 2007; Pearlstein et al. 2010; Zhang et al. 2012). Extended survival and minimization of acute pathology has been demonstrated using sustained, drug dosing regimens (e.g., daily treatments for 28 d) using both mouse and NHP models (Orrell 2006). Recently, Garofalo et al. (2014) investigated whether administration of AEOL 10150 after 11.5 Gy exposure to whole thorax lung ($LD_{100/180}$) could reduce radiation-induced lung injury and improve overall survival in a NHP model. AEOL 10150 administration (beginning at 24 h post-irradiation daily, sc injections at a concentration of 5 mg kg^{-1} for a total of 4 wk) demonstrated efficacy as a mitigator against fatal radiation-induced lung injury. Treatment with the drug resulted in 28.6% survival following exposure to a radiation dose that was 100% fatal in the control cohort. Computed tomography scans demonstrated less quantitative radiographic injury (pneumonitis, fibrosis, effusions) in the AEOL 10150-treated cohort at day 60 post-irradiation, and AEOL 10150-treated animals required less dexamethasone support. Analysis of serial plasma samples suggested that AEOL 10150 treatment led to lower relative transforming growth factor-beta-1 levels when compared with the control animals. The results of this study demonstrate that treatment with AEOL 10150 results in reduced clinical, radiographic, anatomic, and molecular evidence of radiation-induced lung injury. This agent is also being investigated as a countermeasure for GI subsyndrome (Aeolus Pharmaceuticals 2013).

Injury preventing and mitigating nutraceuticals

BIO 300 (Genistein). Isoflavone derived from soya (*Glycine max*) is known as genistein (4′,5,7-trihydroxyisoflavone) and is one of the flavonoid-family members that is currently being investigated specifically as a radiation countermeasure. Genistein is a well known phytoestrogen, antioxidant and protein tyrosine kinase inhibitor that modulates signal transduction pathways (Valachovicova et al. 2004). Genistein is being developed as a radiation countermeasure by Humanetics Pharmaceuticals (Minneapolis, MN) under the name BIO 300 (Dumont et al. 2010; Zenk 2007). The FDA has granted orphan drug designation and IND status to BIO 300 for the prevention of ARS. Humanetics already has conducted two phase-I trials and reported that BIO 300 is safe and well tolerated when administered orally for 14 d in healthy volunteers (Humanetics Pharmaceuticals 2014).

Genistein protects mice against the potential lethal effects of ^{60}Co γ-irradiation when administered sc (25–400 mg kg^{-1}) 24 h before whole-body irradiation (Landauer et al. 2003). The DRF was determined to be 1.16 when administered sc. Multiple doses of genistein administered orally (daily for 4 d before and after irradiation, or once daily for 7 d consecutively before γ-irradiation) significantly protected mice against irradiation (Landauer et al. 2009; Zhou and Mi 2005). Genistein also has been shown to reduce lung injury in mice when administered before irradiation (7.75 Gy) (Day et al. 2008). Genistein stimulates induction of low levels of hematopoietic cytokines (Singh et al. 2009). A single sc injection of genistein 24 h before irradiation provided significant radioprotection to the hematopoietic progenitor cell compartment within bone marrow of the exposed animal. Genistein also promoted the recovery of nucleated cells, myeloid and erythroid lineages in bone marrow of irradiated mice (Davis et al. 2007; Zhou and Mi 2005). Pretreatment with genistein appears to provide, in part, protection from acute myelotoxicity by limiting the extent of radiation-induced senescence of very primitive hematopoietic tissue repopulating progenitors (Davis et al. 2008). Progenitors from genistein-treated mice expressed fewer DNA damage-responsive and cell-cycle checkpoint genes than did progenitors from untreated or vehicle-treated mice. Recently, nanoparticle formulation of genistein was shown to increase mouse protection, increase bone marrow cellularity, and decrease radiation-induced death of hematopoietic stem and progenitor cells (Ha et al. 2013).

A combination of sc injected genistein and orally administered captopril (angiotensin converting enzyme inhibitor) increased the radioprotective efficacy of genistein in C57BL/6J mice (Day et al. 2013). Enhanced survival was supported by a reduction of radiation-induced anemia, improved recovery of nucleated bone marrow cells, splenocytes, and circulating red blood cells. The drug combination enhanced early recovery of marrow progenitors. Genistein alone and genistein plus captopril protected hematopoietic progenitor cells and suppressed the induction of radiation-induced micronuclei; by contrast, captopril alone had no effect on these endpoints. Captopril alone and genistein plus captopril, although not genistein alone, suppressed radiation-induced erythropoietin production. The above report suggests that genistein and captopril treatments serve to protect the hematopoietic system from acute radiation damage in a complementary manner by targeting different pathways of injury and repair (Day et al. 2013).

Gamma-tocotrienol (GT3). GT3 is one of the eight isomers (tocols) of vitamin E. It is a potent inhibitor of HMG-CoA (3-hydroxy-3-methylglutaryl-coenzyme A) reductase (Baliarsingh et al. 2005; Qureshi et al. 1986). In recent years, it has received a great deal of attention by researchers and appears to be one of the more promising radioprotective tocols tested to date. When administered 24 h before ^{60}Co γ-irradiation, GT3 significantly protected mice against radiation doses as high as 11.5 Gy, and its DRF as a radioprotector (24 h before irradiation, 200 mg kg^{-1} dose, sc route) was 1.29 in mice. GT3 treatment accelerated hematopoietic recovery in peripheral blood and enhanced recovery of hematopoietic progenitors in bone marrow of irradiated mice (Ghosh et al. 2009a; Kulkarni et al. 2010). GT3 treatment resulted in significant induction of G-CSF and IL-6 in mice, though the concentration of IL-6 was significantly lower than that of G-CSF (Kulkarni et al. 2012; Singh et al. 2006). Mouse survival studies with GT3 suggested the most efficacious time for administration was 24 h prior to irradiation, possibly due to the induction of key hematopoietic cytokines during that time window. Prophylactic GT3 administration demonstrated upregulation of anti-apoptotic genes and downregulation of pro-apoptotic genes (both at the transcription and the protein levels) at 4 and 24 h after irradiation (Suman et al. 2013). Jejunal crypt analysis, using TUNEL staining, showed protection of GI tissue with GT3 treatment. Similar to that mentioned above for other countermeasures, the authors have demonstrated that the administration of G-CSF antibody abrogates the radioprotective efficacy of GT3 (Kulkarni et al. 2013).

As a vitamin E isomer, GT3 has antioxidant properties common to other tocols. After irradiation, GT3 decreased vascular oxidative stress, reduced intestinal injury, and accelerated the recovery of soluble markers of endothelial function (Berbee et al. 2009). Radiation exposure decreased tetrahydrobiopterin in lungs, which can result in vasoconstriction and neurovascular dysfunction; GT3 administration reversed this effect. Both GT3 and tetrahydrobiopterin supplementation reduced post-irradiation vascular peroxynitrite production (Berbee et al. 2011a). Berbee et al. reported an additive effect of GT3 and pentoxifylline (methylxanthine derived phosphodiesterase inhibitor). This combination significantly improved survival of mice against ^{60}Co γ-irradiation compared with either drug administered alone (Berbee et al. 2011b). The GT3 and pentoxifylline combination protected all mice against TBI doses as high as 12 Gy, in addition to improving bone marrow colony-forming units, spleen colony counts, and platelet recovery compared to GT3 alone (Berbee et al. 2011b). Currently, GT3 is being investigated for its efficacy against ^{60}Co γ-irradiation using a NHP model.

Injury-mitigating therapeutic growth factors and recombinant cytokines

G-CSF (filgrastim, Neupogen), pegylated G-CSF (pegfilgrastim, Neulasta®), and GM-CSF (sargramostim, Leukine®) belong to a class of agents known as "colony stimulating factors" and are FDA approved for the treatment of chemotherapy-induced neutropenia, but none are approved for radiation-induced neutropenia. No prospective randomized trials have proven either the efficacy or long-term safety of hematopoietic growth factors in humans exposed to radiation. However, experience using these cytokines after accidental radiation exposure has been gained during incidents involving small numbers of patients, as tracked by REAC/TS (Oak Ridge, TN) and in smaller clinical studies (Dainiak et al. 2011b; Hirama et al. 2003; Ishii et al. 2001; Maekawa 2002; Meineke et al. 2003; Singh et al. 2014; The Radiation Emergency Assistance Center/Training Site 2013; Waselenko et al. 2004). G-CSF (Neupogen) and GM-CSF (Leukine) are available in the SNS (HHS 2013). Procurement and use of these drugs from the SNS would require a formal EUA. The Centers for Disease Control currently holds both IND and EUA applications with the FDA for the use of G-CSF in the event of a nuclear or radiological emergency (Centers for Disease Control and Prevention 2007). In a large mass casualty event, off-label use by individual clinicians might occur from sources outside the SNS, but FDA still recommends a EUA. Incident managers will probably provide direction on this issue during a mass casualty event.

G-CSF/filgrastim/Neupogen® and pegylated G-CSF/ pegfilgrastim/Neulasta®. The Centers for Disease Control and Prevention currently has an IND Application (with the FDA) containing a detail clinical protocol for how Neupogen (G-CSF) would be administered to exposed victims in the event of a radiological nuclear incident (U.S. Food and Drug Administration 2013a). Earlier work with G-CSF and other

growth factors as a radiation countermeasure has been reviewed recently (Singh et al. 2015). G-CSF treatments might not be self-sufficient in the effective clinical management of ARS, but it most certainly aids in that management and helps to restore granulopoietic function in acutely suppressed marrow (Gourmelon et al. 2010). There is a recent report for G-CSF (filgrastim) evaluation showing that it increased survival of NHPs (a cohort of 46 randomized animals, 24 filgrastim-treated and 22 control) exposed to an $LD_{50/60}$ dose (7.5 Gy, an approximate mid-lethal dose) of linear particle accelerator (LINAC)-derived photon radiation. Filgrastim (10 µg kg^{-1}) was administered beginning 1 d after irradiation and continued daily until the absolute neutrophil count was >1,000 µL^{-1} for 3 d consecutively. All NHPs in this study received medical management/supportive care (Farese et al. 2013). Filgrastim administered at this dose and schedule effectively mitigated the lethality of the hematopoietic subsyndrome of ARS. Filgrastim significantly reduced 60 d overall mortality (20.8%, 5/24) compared to the controls (59.1%, 13/22). Filgrastim also decreased the duration of neutropenia but did not affect the absolute neutrophil count nadir. Survival was increased significantly over controls.

In another study from the same laboratory, the efficacy of pegfilgrastim (Neulasta) was demonstrated in NHPs. Administration of pegfilgrastim at days 1 and 7 was most effective at improving neutrophil recovery compared to daily administration of filgrastim or a single injection of pegfilgrastim on day 1, after severe, radiation-induced myelosuppression in rhesus macaques (*Macaca mulatta*) (Farese et al. 2012).

Granulocyte monocyte colony stimulating factor (GM-CSF)/sargramostim/Leukine®. The role of GM-CSF to increase recovery of hematopoiesis after irradiation is similar to that of G-CSF (Singh et al. 2014). GM-CSF is currently approved for a total of five indications with the first indication approval granted in 1991. In three of the five approved indications, it is used for the mitigation of neutropenia following chemotherapy with or without radiation (U.S. Food and Drug Administration 2013b). The injury to the bone marrow caused by acute radiation exposure is qualitatively similar to that caused by chemotherapy with or without radiation. The recommended dose for GM-CSF (sargramostim) is 5–10 µg kg^{-1} d^{-1} sc or (200–400 µg m^{-2} d^{-1}) (Amgen Inc. 2013; Sanofi-Aventis 2013). Some toxicity that develops in patients treated with GM-CSF includes local erythema, hypotension, chills, and fever (Weisdorf et al. 2006). No comprehensive comparative data with that of G-CSF are available.

HemaMax™/NMIL12-1 (recombinant human interleukin-12 or rhuIL-12). Currently recombinant human IL-12 is being developed as a radiomitigator by Neumedicines Inc. (Pasadena, CA, USA). The pharmacokinetics, pharmacodynamics, and efficacy of mouse and human IL-12 in mice and rhesus NHP (*Macaca mulatta*), respectively, have been reported (Basile et al. 2012). Allometrically equivalent doses of mouse and human HemaMax preparations significantly increased mouse and NHP survival, respectively, when administered 24 h post-irradiation with similar pharmacokinetics. In the NHP study, survival benefit was accompanied by higher leukocyte, thrombocyte, and reticulocyte counts during nadir (12–14 d) and less body weight lost when compared to vehicle (Gluzman-Poltorak et al. 2014a). Another group confirmed the radiomitigation potential of rhuIL-12 in NHPs (Xiong et al. 2013).

Recently, Neumedicines Inc. reported a significant increase in survival when NHPs were treated with a single, low-dose of rhuIL-12. This study had additional treatment groups that received G-CSF for 18 consecutive days and another that received G-CSF for 18 consecutive days in combination with a single dose of rhuIL-12 (Gluzman-Poltorak et al. 2014b). No supportive care was provided. The combination of G-CSF plus rhuIL-12 appeared to augment the recovery of trilineal hematopoiesis to a greater extent than with just rhuIL-12; however, this did not translate to improved survival. These data demonstrate that G-CSF can be safely administered after rhuIL-12. To demonstrate the safety of HemaMax, Neumedicines, Inc., conducted a Phase-Ib study where healthy human volunteers were administered a single dose predicted to be effective in humans for treating hematopoietic syndrome based on NHP data; this trial suggests rhuIL-12 to be safe and well tolerated at this dose (Gokhale et al. 2014; Neumedicines 2014). An additional study, investigating the mechanism of action of rhuIL-12 in healthy human subjects, suggests that rhuIL-12 administration induced IL-12Rβ2$^+$, CD16$^+$CD56$^+$ natural killer cell migration from the peripheral blood into the tissue compartment, through a mechanism facilitated by interferon-γ-induced CXCL10 chemokine and its receptor CXCR3 (Neumedicines 2014). These studies suggest that rhuIL-12 has potential to be an effective radiation mitigator against radiation lethality (Gluzman-Poltorak et al. 2014a).

Injury-mitigating, therapeutic cell transplants: Cellular therapy

Myeloid progenitor cells (MPC). Cellerant Therapeutics (San Carlos, CA, USA) has developed culture conditions to produce large numbers of mouse myeloid progenitors from hematopoietic stem cells. The myeloid progenitors can improve survival against high levels of radiation. Following transplantation into an irradiated host, myeloid progenitors derived from three major histocompatibility complex-disparate mouse strains expand and differentiate in vivo, giving rise to myeloid, erythroid, and dendritic cells as well as platelets. Additional long-term

studies are required in animals receiving myeloid progenitors and exposed to different doses of ionizing radiation causing hematopoietic and GI subsyndromes to rule out graft versus host disease. Such studies are vital for success of this product in the clinic.

In collaboration with Cellerant Therapeutics, the authors studied myeloid progenitor cells for use as a bridging therapy for radiation injuries. The aim of this study was to elucidate the potential of mouse myeloid progenitor cells (mMPC) to mitigate lethal doses of ^{60}Co γ irradiation and x rays in various strains of mice. Different cell doses of pooled allogeneic mMPC generated ex vivo from AKR, C57Bl/6, and FVB mice were transfused iv into haplotype-mismatched recipient BALB/c or CD2F1 mice at various times after irradiation to assess their effect on 30-d survival. The results demonstrated that cryopreserved allogeneic mMPC significantly improves survival in both strains of mice irradiated with lethal doses of ^{60}Co γ-radiation (CD2F1, 9.2 Gy) and x-ray exposures (BALB/c, 9 Gy) that are known to cause ARS in hematopoietic tissues (Singh et al. 2012b). The survival benefit was mMPC-dose dependent and significant, even when mMPC administration was delayed up to 7 d post-irradiation. It was shown further that mMPC administration mitigates death from ARS at radiation doses up to 15 Gy (^{60}Co γ-radiation, CD2F1), which are radiation exposure levels that cause mice to succumb to multi-organ failure, and determined that the DRF of 5 X 10^6 mMPC administered 24 h post irradiation of CD2F1 mice is 1.73. Even at high doses of up to 14 Gy ^{60}Co γ radiation, mMPC administration could be delayed up to 5 d in CD2F1 mice and still provide significant benefit to 30-d survival. Additional studies are needed to monitor mMPC-transplanted mice for the long term to investigate graft vs. host disease and to evaluate histopathology of various organs of transplanted mice.

The authors also studied the GI tract structural integrity in mice receiving higher doses of radiation exposure causing GI injury and mMPC treatment (Singh et al. 2012e). Intestinal tissues were harvested at different times after irradiation and analyzed for architecture, surviving crypts, and villus height and number. The effect of infused mMPC on bacterial translocation from gut to heart, spleen, and liver was also investigated in irradiated mice by bacterial tissue cultures and estimated endotoxin in serum samples. It was observed that the infusion of mMPC significantly improved survival of mice receiving high doses of radiation, decreased the number of bacterial infections, and lowered endotoxin levels in serum. The histopathology of jejunum from irradiated and mMPC-transfused mice revealed improved gut structural integrity compared to untreated controls. In brief, the results of this study further support the authors' contention that the transfusion of mMPC acts as a bridging therapy, not only for the hematopoietic system

but also for GI system recovery following acute, potentially lethal radiation injury by improving GI structural integrity and inhibiting bacterial translocation in the GI tract of lethally irradiated mice.

Cellerant Therapeutics is developing CLT-008 as a treatment for chemotherapy-induced neutropenia, protection following exposure to acute radiation, as an adjunct to cord blood transplantation, and it currently is in a Phase-I study with patients undergoing cord blood transplants for the treatment of hematological malignancies (Cellerant Therapeutics 2013). They also have another product under development called CLT-009 for thrombocytopenia.

Clinical support measures

Supportive care can be, in its own right, an extremely effective radiation countermeasure when judiciously applied (DiCarlo et al. 2011; Farese et al. 2012; MacVittie et al. 2005). Supportive care includes the administration of cytokines, blood products, antibiotics, antiemetics, antidiarrheals, fluids and electrolytes, and topical burn creams. Supportive care in animal studies may be classified as "full support" (defined as administration of fluids, blood products, antibiotics, and in some cases parenteral nutrition) or "aggressive support" (defined as individualized care, including cytokines and hematopoietic stem cell transplant). Antibiotics also improve survival in mice after combined injury (Ledney and Elliott 2010). There are several reports demonstrating the efficacy of antibiotic use and platelet transfusions in canines exposed to radiation (Furth et al. 1953; Jackson et al. 1959; Perman et al. 1962). Supportive care has also been reported to increase survival in NHPs (Farese et al. 2012; MacVittie et al. 2005). Based on experimental data, it is thought that supportive care is capable of changing survival of LD$_{50/60}$ doses (50% of the population surviving at 60 d) from 3.5–4 Gy (without medical care) up to 5–6 Gy with supportive care such as the use of transfusions and antibiotics (Dainiak et al. 2003; Hall et al. 2012). Further, the additions of colony stimulating factors (CSF) and intensive care may raise the LD$_{50/60}$ to 6–8 Gy. The highest survival rates will come from those with less than 6 Gy dose without physical trauma. Supportive care is intended to maintain the patient until surviving stem cells can be stimulated to resume blood cell (neutrophil and platelet) production. The management of radiation-exposed individuals with severe ARS and combined injury is highly labor- and resource-intensive; single casualties of the Tokaimura criticality radiation accident required significant personnel and resources for prolonged periods of intensive medical care (Ishii et al. 2001).

Antimicrobial, antifungal, and antiviral agents. Sufficiently intense exposure to ionizing radiation suppresses immune response and damages vital organs, placing affected

individuals at risk to infections with bacteria, fungi, and viruses. Since infections are a major cause of mortality after acute, high dose radiation exposures, preventing and treating infections are extremely important in the care of exposed individuals (Dainiak et al. 2011b). Several studies indicate that antibiotics given alone or in combination were effective in reducing mortality of irradiated canines (Dainiak et al. 2003). The $LD_{50/30}$ value increased significantly when antibiotics supplemented the general supportive care regimens in canines and NHPs with ARS (Dainiak et al. 2003; Farese et al. 2013). The topical application of gentamycin and silvadene improved survival in mice receiving combined injury (varying doses of radiation and wound) (Ledney and Elliott 2010).

Exposed individuals with an absolute neutrophil count $< 0.5 \times 10^6$ cells mL^{-1} or having neutropenic fever (>38° C) are at increased risk for opportunistic and nosocomial infections and may benefit from prophylactic antimicrobial therapy (Dainiak et al. 2003; Flynn and Goans 2006; Gorin et al. 2006; Waselenko et al. 2004). Antibiotic therapy is generally specified according to microbiological diagnostics tests, but if not available, third generation cephalosporin or monotherapy is applied.

Antifungal therapy is recommended if febrile patients do not respond to antibiotics. Use of fluconazole or alternative agents is common for suppression of yeast colonization. Fluconazole (at ~400 mg oral per day) has been shown to lessen invasive fungal infections and mortality in patients undergoing allogeneic bone marrow transplantation (Dainiak et al. 2003; Goans 2002). Fluconazole prophylaxis is ineffective against Aspergillus, *Candida krusei*, and resistant Candida species.

Antiviral therapy is recommended for victims showing signs of viral infection. Prophylactic antiviral therapy with valcyclovir or acyclovir is recommended for individuals with a history of infection with herpes simplex virus or with a positive serology for type 1 or 2 herpes simplex virus (Waselenko et al. 2004). In such patients, immunosuppression confers a heightened risk of viral reactivation.

Fluids and electrolytes. Adequate supplies of iv fluids might not be readily available in all situations involving mass radiation casualties. Nevertheless, survival of some patients with milder cases of fluid and electrolyte loss may be enhanced by replacement therapy; thus judicious use of these medical supplies would be warranted. Measurement of the extent and relative volumes of diarrhea and vomiting will help guide the fluid replacement. Those with more vomiting than diarrhea will suffer the greater loss of chlorides and may develop alkalosis, while those with secretory, cholera-like diarrhea may develop hypokalemia and hyponatremia with total-body salt depletion. In the event of combined-burn injury involving more than 10% of the body surface, crystalloid infusions are just as satisfactory as colloid, but a higher volume of infusate may be necessary (Kaplan 1985).

Platelets and red blood cells. The criteria for managing ARS patients during the period of bone marrow hypoplasia are quite similar to those applied for other severely neutropenic patients. Since the patients will be producing white blood cells in limited amounts, in the case of sepsis, they may not show the typical signs of infection. The most reliable sign, and often the only one, will be a fever (>38 °C). Platelet substitution prevents bleeding during the bone marrow hypoplastic period, but over-transfusion should be avoided. The rationale for avoiding surgery during the period of acute marrow suppression is to prevent infection and excessive bleeding. Platelet transfusion is recommended with thresholds as follows: 10,000 μL^{-1} if close monitoring is possible and no bleeding or other complications are present; 20,000 μL^{-1} if close monitoring is not possible and bleeding but no other complications are present; and 50,000 μL^{-1} if additional trauma, surgery, cerebral oedema, transfusions are present.

Indications for red blood cell (erythrocyte) transfusion are based on defined levels of hemoglobin as established by hospital criteria. Patients at higher risk of coronary disease or stroke may receive transfusions if hemoglobin < 10 g dL^{-1}.

Countermeasures against internally deposited radionuclides

In addition to having medical countermeasures for "external radiation exposure scenarios," there are other radiation exposure scenarios that would result in serious medical consequences if left unattended; the more important of these exposure situations involves internally-deposited radionuclides. For the latter exposures, a very limited number of mitigating agents have been approved by the FDA and are ready for field-use in cases of unintended exposures that are either accidental or terrorist-associated in nature. These countering agents include KI, PB, and Zn/Ca-DTPA and are used primarily to limit and/or reduce "body-burdens" of internalized radionuclides via blocking uptake, chelating, or a combined action.

Blocker—potassium iodide (KI). KI is an FDA-approved blocking agent used to prevent adverse health effects caused by internal contamination from radioactive iodine. KI is no longer stockpiled in SNS as it is readily available elsewhere. Iodine-131 is released most frequently after incidents involving nuclear reactors. In terms of primary use, KI is generally applied prior to or shortly following the threat of exposure to radioactive iodine in order to block uptake of isotopic iodides by the thyroid. For adults or adolescents (>12 y of age), daily oral doses of 130 mg will be sufficient to block thyroid

uptake of radioiodine. Recommended KI doses for children (3–12 y), infants (1 mo to 3 y), or neonates (birth to 1 y) are 50 mg, 25 mg, and 12.5 mg, respectively (Jarrett 1999). Guidance on KI dosing for the general public has been developed through the joint effort of the FDA Center for Drug Evaluation and Research and the U.S. Department of Health and Human Services (U.S. Food and Drug Administration 2002).

Binder—Prussian blue (PB)/Radiogardase®. Chemically, PB is insoluble ferric hexacyanoferrate and goes by the trade name of Radiogardase. PB is an FDA-approved binding agent for treatment of internal contamination with radioactive cesium-137 and radioactive (and also non-radioactive) thallium (Hussar 2005). PB is stockpiled within the SNS. PB is administered orally and is well tolerated at a recommended dosing schedule of 1–3 g, 3 times per day. After exposure, PB binds to the isotopes and safely transports them out of the body.

Chelators—Zinc/calcium diethylenetriamine penta-acetate (Zn/Ca DTPA). There are two salts of this nucleate chelating agent, namely Zn-DTPA and Ca-DTPA, and both can be used to treat individuals contaminated with transuranic- and/or rare earth radionuclides. The FDA Center for Drug Evaluation and Research approved these agents for radiation contingency events in 2003, and these chelating agents are available in the SNS (The Radiation Emergency Assistance Center/Training Site 2013). Treatments (in adults) generally involve iv infusions of 1 g in 5 mL Ringer's lactate solution (or normal saline) (Radiation Emergency Assistance Center/Training Site 2013). If the quantity of plutonium internalized is enough to cause tissue reactions, it is suggested that DTPA should be used regardless of the level of uranium present (Kazzi et al. 2012).

Countermeasures for radiation-induced emesis

Granisetron/Kytril®. Granisetron {endo-N-[9-methyl-9-azabicy(3.3.1) non-3-yl] -1-methyl-1 H-indazole-3caroxamide hydrochloride} is a highly effective anti-emetic drug with potent antagonistic effects on 5-HT. This is a commonly used clinical anti-emetic agent for reducing the toxic side effects of radio- and/or chemotherapeutic treatment modalities. The drug is not radioprotective per se but serves only to minimize the prodromal responses of acute, high dose irradiation (Kehlet et al. 1996). This drug was researched and marketed by F. Hoffmann La Roche Ltd. (Basel, Switzerland). Full FDA approval for human use was granted in 2001 for the alleviation of toxic side effects (nausea and vomiting) associated with cancer therapy.

Granisetron is most effective when given orally once a day with a 2 mg dose. The drug can be administered daily up to 14 d in order to prevent emesis. The main advantage of the drug is that it is nontoxic, well tolerated and highly efficacious. With appropriate dosing, Granisetron significantly minimizes the initial performance-decrementing effects associated with the prodromal responses of acute exposures. The major disadvantage is that the drug will mask prodromal responses that serve as useful bioindicators of acute radiation exposure. The drug is very limited in its protective actions and does not protect tissue from radiation injury.

Additional countermeasures not requiring FDA approval

For the sake of completeness, several additional countermeasures have proven to be useful in select cases of radionuclide contamination (Gusev et al. 2001; Hubner and Fry 1979; Jarrett 1999; Nesterenko et al. 2004). These include sodium bicarbonate for uranium contamination; penicillamine for stomach lavages or as a purgative following ^{60}Co contamination; aluminum hydroxide for oral or stomach lavaging; and magnesium sulfate or alginates and fruit pectins for oral administration to absorb and block gut uptake of ingested radionuclides. The World Health Organization recommendation for analgesic treatment levels I–III is as follows: Level I - non-steroid anti-inflammatory drugs except aspirin; Level II - low effect opiates; Level III - high effect opiates; and if Level III is not effective, combine with corticoids and neuroleptics.

Lessons learned as a result of major radiological accidents have served to improve the effectiveness of basic clinical management protocols, including basic dosimetric procedures and improved procedures to decontaminate individuals both externally and internally.

SUMMARY AND CONCLUSION

The success in the development of radiation countermeasures will depend on a better understanding of the damage resulting from radiation exposure. Stem cell biology will definitely help to develop a new generation of radiation countermeasures. G-CSF improves granulopoietic activity and does not prevent radiation-induced apoptosis of pluripotent hematopoietic stem and progenitor cells. Although cytokines are promising for treatment of radiation casualties, they lack unanimous endorsement by health professionals. The WHO panel of experts used the Grading of Recommendations Assessment Development and Evaluation tool to extract and analyze data from reports of cytokine administration and/or bone marrow transplantation in individuals with hematopoietic syndrome after exposure to ionizing radiation. The lack of control groups in humans restricts these analyses. Nevertheless, together with results of controlled trials in large animals and clinical trials in unirradiated humans, these analyses support the strong recommendation for G-CSF or GM-CSF administration in humans with hematopoietic syndrome following acute radiation exposure (Dainiak et al. 2011a and b).

All of the radiation countermeasures discussed in this paper hold promise. Only three agents, OrbeShield, Ex-RAD, and BIO 300, demonstrate oral efficacy for ARS. Several agents have shown potential as radiomitigators, which can be useful in a mass casualty scenario. CBLB502, HemaMax, AEOL 10150, and 5-AED have been evaluated in NHPs with encouraging results, and all these agents have been claimed as radiomitigators. CBLB502 and 5-AED have efficacy also as radioprotectors. The radioprotective efficacy of GT3 is currently being evaluated in NHPs, and initial indication is encouraging [††]. Previously evaluated chemical radioprotectors, such as amifostine and tempol, are highly efficacious agents but have significant limitations in terms of use due to side effects; it needs to be reevaluated and possibly reformulated for alternative use in select radiation-exposure contingencies. Other promising agents are myeloid progenitors that have been selectively modified, in vitro cultured (CLT008/CLT009), or pharmaceutically-induced (via tocol administrations) to be released from the bone marrow into the blood. These progenitors can be administered a few days after radiation exposure without significant compromise in their efficacy. Only time will tell whether or not these "cellular approaches" are useful or not. Here the concerns are homing patterns of the progenitors in various tissues and possible graft vs. host reactions.

Additional preclinical studies in large animals will be needed to firmly establish the mechanism of action and efficacy for the above-mentioned radiation countermeasures under development. The Animal Efficacy Rule requires greater reliance on large animal models for preclinical safety and efficacy studies, and national resources are limited in this area. Availability of adequate animal models is one of the limiting factors for developing novel radiation countermeasures, and discovering clear and unambiguous biomarkers of radiation exposure is another. G-CSF and IL-6 have been identified as surrogate efficacy biomarkers for CBLB502 (Krivokrysenko et al. 2012). Additional long-lived large animal models for ARS need to be developed and validated to facilitate advanced development of radiation countermeasures. These animal models can be used for efficacy studies and also for developing efficacy biomarkers and dose conversion of the drug from animals to humans.

Medical management of patients exposed to intentional or accidental ionizing radiation is complex and demands many resources. The outcome of supportive care is definitely promising, but resource scarcities after a nuclear detonation/mass casualty scenario would greatly reduce the number of patients who could receive comprehensive and intensive care, including sufficient fluid and extensive blood product transfusion support. The primary responsibilities for optimizing resource use and outcomes will reside with health care professionals.

Additional research is needed to identify new therapeutic approaches and to develop novel countermeasures for radiation injury. There are several additional radiation countermeasures under development such as captopril (Davis et al. 2010; Medhora et al. 2012), CBLB613 (Singh et al. 2012d), CBLB612 (Shakhov et al. 2012), delta-tocotrienol (Li et al. 2010), epidermal growth factor (Doan et al. 2013), fibroblast growth factor-2 (Zhang et al. 2010), fibroblast growth factor-peptide (Ma et al. 2013), insulin-like growth factor-1 (Zhou et al. 2013), tempol (Mitchell et al. 2012), tocopherol succinate (Singh et al. 2012a), thrombopoietin receptor agonist (ALXN4100TPO) (Satyamitra et al. 2011), palifermin (Finch et al. 2013), superoxide dismutase (Guo et al. 2003), 3,3'-diindolylmethane (Fan et al. 2013), oltipraz (Kim et al. 1998), phosphoinisitide-3 kinase inhibitor (LY294002) (Lazo et al. 2013), anticeramide antibody (Rotolo et al. 2012), phenylbutyrate (Miller et al. 2011), R-spondin1 (Bhanja et al. 2009), and somatostatin analog (SOM230) (Fu et al. 2011). Since it was not possible to discuss all agents under development in this review, the authors selected some of those agents that are at advanced stages of the development.

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