

Animal Models and Medical Countermeasures Development for Radiation-Induced Lung Damage: Report from an NIAID Workshop

Authors: Williams, Jacqueline P., Jackson, Isabel L., Shah, Jui R., Czarniecki, Christine W., Maidment, Bert W., et al.

Source: Radiation Research, 177(5)

Published By: Radiation Research Society

URL: https://doi.org/10.1667/RROL04.1

The BioOne Digital Library (<u>https://bioone.org/</u>) provides worldwide distribution for more than 580 journals and eBooks from BioOne's community of over 150 nonprofit societies, research institutions, and university presses in the biological, ecological, and environmental sciences. The BioOne Digital Library encompasses the flagship aggregation BioOne Complete (<u>https://bioone.org/subscribe</u>), the BioOne Complete Archive (<u>https://bioone.org/archive</u>), and the BioOne eBooks program offerings ESA eBook Collection (<u>https://bioone.org/esa-ebooks</u>) and CSIRO Publishing BioSelect Collection (<u>https://bioone.org/csiro-ebooks</u>).

Your use of this PDF, the BioOne Digital Library, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at <u>www.bioone.org/terms-of-use</u>.

Usage of BioOne Digital Library content is strictly limited to personal, educational, and non-commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne is an innovative nonprofit that sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

WORKSHOP REPORT

Animal Models and Medical Countermeasures Development for Radiation-Induced Lung Damage: Report from an NIAID Workshop

Jacqueline P. Williams,^{*a*} Isabel L. Jackson,^{*b*} Jui R. Shah,^{*c*} Christine W. Czarniecki,^{*c*} Bert W. Maidment^{*c*} and Andrea L. DiCarlo^{*c*,1}

^aUniversity of Rochester Medical Center, Rochester, New York; ^bDuke University Medical Center, Durham, North Carolina; and ^cDivision of Allergy, Immunology and Transplantation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland

Williams, J. P., Jackson, I. L., Shah, J. R., Czarniecki, C. W., Maidment, B. W. and DiCarlo, A. L. Animal Models and Medical Countermeasures Development for Radiation-Induced Lung Damage: Report from an NIAID Workshop. *Radiat. Res.* 177, e0025–e0039 (2012).

Since 9/11, there have been concerns that terrorists may detonate a radiological or nuclear device in an American city. Aside from several decorporation and blocking agents for use against internal radionuclide contamination, there are currently no medications within the Strategic National Stockpile that are approved to treat the immediate or delayed complications resulting from accidental exposure to radiation. Although the majority of research attention has focused on developing countermeasures that target the bone marrow and gastrointestinal tract, since they represent the most acutely radiosensitive organs, individuals who survive early radiation syndromes will likely suffer late effects in the months that follow. Of particular concern are the delayed effects seen in the lung that play a major role in late mortality seen in radiation-exposed patients and accident victims. To address these concerns, the National Institute of Allergy and Infectious Diseases convened a workshop to discuss pulmonary model development, mechanisms of radiation-induced lung injury, targets for medical countermeasures development, and end points to evaluate treatment efficacy. Other topics covered included guidance on the challenges of developing and licensing drugs and treatments specific to a radiation lung damage indication. This report reviews the data presented, as well as key points from the ensuing discussion. © 2012 by Radiation Research Society

¹Address for correspondence: DAIT, NIAID, NIH, 6610 Rockledge Drive, Room 5301, Bethesda, MD 20892; e-mail: cohena@niaid.nih. gov.

INTRODUCTION

The White House Office of Science and Technology Policy's Radiological/Nuclear Threat Countermeasures Working Group has rated understanding the mechanism(s) of action of radiation-induced late effects, including pulmonary complications, as a top priority research area (1). To address this and other research needs in the area of radiation-induced damage and health effects, the Department of Health and Human Services (DHHS) has tasked the National Institutes of Health (NIH) and, specifically, the National Institute of Allergy and Infectious Diseases (NIAID) with the responsibility of identifying and developing new medical countermeasures (MCMs) for use in the event of radiological or nuclear attacks.

As part of most of the envisioned radiation scenarios, injury to the hematopoietic system and/or gastrointestinal (GI) tract appears to be the main determinant of early mortality. However, those individuals who either survive the acute radiation syndromes (ARS) or are exposed to sublethal, yet nonetheless significant, doses of radiation will be susceptible to normal tissue late effects. Often referred to as the delayed effects of acute radiation exposure (DEARE), it appears that those outcomes expressed in the lungs following total or partial-body irradiation may play a critical role in reducing long-term survival in individuals who have been exposed to a high dose of radiation (e.g., from a radiation exposure incident) (2). Thus, in concert with ongoing efforts to develop radiation countermeasures targeted at acute responding organs, additional efforts are being supported to develop specific MCMs against late lethality and morbidities, including radiation-induced pulmonary damage. Importantly, the availability and government stockpiling of products that can mitigate and/or treat DEARE, such as pulmonary injury, will increase the medical management options available to treat the potentially large numbers of casualties presenting after a radiation exposure incident.

A diverse portfolio of grants, cooperative agreements, and contracts has been awarded by the NIAID since the inception of the Radiation/Nuclear Program including many in the area of pulmonary-related DEARE. The primary goal of the program is the development of medical countermeasure products that will mitigate and/or treat radiation-induced pulmonary damage, i.e. acute (e.g., pneumonitis) and/or chronic lung sequelae (e.g., fibrosis). The agents under investigation include novel compounds, biologics such as growth factors and cytokines, nutraceuticals, cellular therapies, free radical scavengers, antiinflammatory agents, chemokine inhibitors, mucosal surface modifiers, anti-fibrotics, and other small molecules. Proposed modes of action include prevention of cellular depletion, inhibition of tissue breakdown (e.g., through preservation of the alveolar epithelial basement membrane), inhibition of the inflammatory and/or fibrotic process, and/or stimulation of tissue repair, with the ultimate goal of restoring normal pulmonary function and increasing survival. Mechanism of action studies are supported since these data are required for licensure by the U.S. Food and Drug Administration (FDA) under current regulations: 21 CFR Part 314 Subpart I, (Approval of New Drugs When Human Efficacy Studies Are Not Ethical or Feasible) and 21CFR Part 601, Subpart H, (Approval of Biological Products When Human Efficacy Studies Are Not Ethical or Feasible) (3). These regulations are commonly referred to as the FDA's Animal Rule. Additional areas of research supported by the NIAID include investigations to elucidate the mechanisms of radiation-induced lung injury, development of appropriate animal models, and identification of lung-specific biomarkers of damage.

To build on existing studies of countermeasures for other injuries and to expand on previous NIH-funded workshops (4), the Division of Allergy, Immunology and Transplantation, NIAID, NIH, held a workshop on April 19-20, 2010. The workshop brought together multiple investigators involved in lung research, including those with 2007 NIAID-funded lung awards, as well as researchers from the Centers for Medical Countermeasures against Radiation (CMCRs), awardees from the radiation combined injury portfolio, and industry scientists associated with NIAID's advanced product development efforts. Presentations were made by representatives from U.S. Government agencies and contractors involved in funding research and development of drugs for a radiation indication, as well as agencies responsible for the licensure and procurement of drugs for stockpiling. More than 60 participants were in attendance and all presenters participated in a final open panel discussion at the end of the meeting.

This report summarizes the presented data as well as key points from the discussion session. It is not intended to be an exhaustive review of all of the approaches that have been considered for mitigation/treatment of radiation damage to the lungs, but only those addressed within the NIAID grants and contract portfolio.

BACKGROUND

The Human Experience

The lung has long been recognized clinically as being highly radiosensitive, resulting in its consideration as a dose-limiting organ in many radiation therapy protocols. Its associated radiation-induced effects present as a broad spectrum of pneumonopathies (e.g. dyspnea, pneumonitis and fibrosis) and are outcomes of concern in normal lung tissue following both fractionated localized therapy (5, 6)and whole body irradiation (6, 7). As a result of this sensitivity, radiation-induced lung injury is of significant medical concern, not only with respect to partial-body therapy-related exposures involving the thorax, but also for its potential role following radiation accident scenarios, such as exposure due a nuclear detonation or the detonation of a radiological dispersion device (or "dirty bomb") resulting in individuals inhaling soluble or nonsoluble radioactive particulates. Under the latter circumstances, the irradiation might result in lung injury without significant direct damage to other organ systems yet still have the potential to cause life-threatening outcomes.

In recent decades, as supportive care protocols have improved, a number of individuals who have survived injuries associated with the acute radiation syndromes (ARS) have ultimately succumbed to a multiple organ dysfunction syndrome (MODS) or failure (MOF), with radiation-induced pneumonitis and/or pulmonary fibrosis playing a prominent role. For example, within the first 14-23 days post-exposure following the Chernobyl incident, seven patients were diagnosed with pneumonitis and, of those, two died. Of the six patients who died between 24 and 48 days post-exposure, all six had some degree of respiratory disorder and, in two, death was attributed to the lung injury (8). More recently, accidentally exposed workers at a Japanese nuclear fuel manufacturing plant (Tokaimura) who had survived doses that resulted in ARS nonetheless died from a radiation-induced MODS that involved radiation pneumonitis and other respiratory dysfunctions, such as pulmonary edema (2). Although neither of the two Tokaimura workers who exhibited MODS developed lung fibrosis (9), in another recent incident, autopsy findings from a patient in Shanghai, China, who was accidentally exposed to approximately 11 Gy of radiation and survived to day 90 post-irradiation, demonstrated both prominent interstitial pneumonia and diffuse lung fibrosis (10). These findings confirm both pneumonitis and fibrosis as end points of radiation-induced lung damage; however, it is currently uncertain if these two outcome events are pathologically or mechanistically related.

Although significant efforts are made in the clinic to limit the volume of normal lung tissue exposed to radiation due to its radiosensitivity, cases of accidental radiation exposures have been described, including patients receiving unintended thoracic irradiation during treatment of breast, lung and other cancers; the subsequent lung complications have, in some instances, led to patient deaths (11). Such outcomes underscore the critical role played by the lung in both early and late radiation lethality (4, 12, 13). Thus, there is a growing realization that, in addition to countermeasures for the classically recognized components of ARS, such as neutropenia and thrombocytopenia, agents also are needed that are specifically targeted at the pulmonary response, particularly in the context of total body irradiation (TBI) such as might be anticipated as part of a radiation incident (14).

Current Status of Countermeasures for Late Radiation-Induced Lung Disease

The principal radiation-induced normal tissue events in the lung are an acute-phase-alveolitis/pneumonitis and a late chronic pulmonary fibrosis (15, 16), with multiple lungrelated symptoms being expressed as part of injury progression (5, 7). Our current understanding of the underlying pathophysiologic processes leading to these end points is that, following the initiating radiation injury, the progression to late lung disease involves a complex network of cellular processes and interacting signals (17, 18). During the acute phase, inflammation is the predominant histological and physiologic feature, taking the form of macrophage infiltration into the air spaces and focal accumulations of mononuclear cells (15, 19); these events are accompanied by a decline in pulmonary function (20). Concurrent with these changes, many investigators have demonstrated immediate and subsequent cyclical alterations in cytokine and growth factor expression levels, seen both locally and systemically, that are differentially persistent throughout the developmental periods that culminate in the pneumonitic and/or fibrotic phases (17, 21, 22). Some attempts have been made to target those cytokines that appear to be critical in this progression in order to mitigate the subsequent radiation lung damage, with some success (23); however, it is unclear if the role(s) that any of the identified cytokines play in the progression to pulmonary late effects may be affected in the context of systemic (whole body) injury.

Classically, prevention of normal tissue late effects has focused on pre-treatment with radioprotective agents, notably using free radical scavengers such as amifostine. With respect to the lung, interest in this specific drug has waxed and waned over the years since the 1970s, when Yuhas first suggested that amifostine may be protective (24). Since that time, groups such as Vujaskovic *et al.* and others have continued to demonstrate at the pre-clinical level that pre-treatment with amifostine can reduce pulmonary late effects (25-27). Although some studies demonstrated a reduction in the severity of pulmonary toxicity from amifostine administration prior to concurrent cisplatin chemotherapy and radiation (28), this approach has failed to gain significant headway in the clinic. Furthermore, the need to administer the drug in advance of the exposure precludes its use in a radiation incident other than for the protection of emergency responders, and even then, its narrow temporal window of efficacy limits its application.

Another widely accepted target for mitigating strategies for the lung has been inflammation, hypothesized by many to play a significant role both in the progression of radiation-induced pulmonary effects and also in radiationinduced MODS. Certainly, clinical evidence suggests that limiting the inflammatory reaction is a reasonable approach, given the beneficial effects seen following both glucocorticosteroid and nonsteroidal anti-inflammatory drug administration targeted at the signs and symptoms of pneumonitis (29, 30). However, there is little evidence that even an intensive anti-inflammatory treatment has any effect on radiation fibrosis (31). Nonetheless, a number of pre-clinical studies have shown benefit from nonspecific, anti-inflammatory approaches (32, 33), supporting continued investigations of such an approach.

Recently, some investigators have suggested that normal tissue late effects, such as pneumonitis and fibrosis in the lung, are the result of an interaction between inflammation and oxidative damage, leading to a chronic induction of radical oxygen species (34). This hypothesis has led researchers to explore the use of antioxidants, for example, using dietary supplements such as the soy isoflavone, genistein (35), or more specific approaches, such as targeting cells' inherent antioxidant apparatus, e.g., by increasing the levels of superoxide dismutase (SOD) (36). Justification for such an approach is provided by earlier work that showed that the intra-tracheal introduction of a SOD plasmid/liposome complex reduced the induction of pulmonary late effects (37) and that over-expression of SOD appears to be protective (38). In addition, significant levels of vascular injury and remodeling have been reported in lungs after radiation (39-42). For example, pneumonitis is accompanied by pulmonary vascular endothelial dysfunction (39), loss of pulmonary vascular reactivity (41), decrease in pulmonary arterial density (42), increase in pulmonary arterial pressure (43) and right ventricular hypertrophy (42). Angiotensin converting enzyme (ACE) has been described to play a role in vascular remodeling (44-46), making it a possible target for mitigation of radiation-induced lung injury by ACE inhibitors (39).

Unfortunately, despite the promise suggested by the approaches described above, there are no currently approved countermeasures that mitigate pulmonary late effects. Furthermore, in the aftermath of a radiological or nuclear incident, additional factors may affect pulmonary end points, which need to be considered in the context of developing treatment strategies; examples of such factors include the possible changes in temporal expression and/or magnitude of response that may occur as a consequence of systemic injury as a result of TBI, internal contamination following inhalation of radionuclides, age (particularly the effect of development in children's lungs), or combined or sequential injuries.

PROGRAM OVERVIEW²

During the one and one-half day NIAID meeting, invited talks were divided into several sessions. These included "Setting the Stage for MCM Development", "NIAID's Grants and Cooperative Agreements Portfolio – Research Update", and "Industry Approaches". The possibility and mitigation of late lung cancers from radiation exposures was not addressed.

Animal Model Development for Radiation-Induced Lung Complications

It is generally accepted that the FDA Animal Rule licensure pathway will require proof of efficacy in at least two animal species (47). For this reason, animal model development within the NIAID lung program is being pursued in several species, including mice, rats, canines and nonhuman primates (NHPs).

Although a number of different animal models have been studied as a means of deciphering the pulmonary response to radiation exposure, mice are the most widely-used model, both for radiation exposure effects in general and also specifically to investigate radiation-induced lung damage (Fig. 1). However, at least one group of investigators suggested that some strains of mouse may be better models of the human lung response than other strains (I. Jackson). For example, they noted that, despite its wide-scale use, the pro-fibrotic C57BL/6J strain may be a relatively poor model of lung injury due to the observed development of pleural effusions. This pathology, although not widely observed in humans, was noted in patients who received accidental radiotherapy overexposures to the thorax (48). The group at Duke University finds that C57L, C3H, and CBA strains appear to more closely resemble the human lung response to radiation (Fig. 1) and believe that these strains may be more appropriate models. They state that in many of the rodent strains, irradiated animals actually die from pleural effusions, rather than from pneumonitis or fibrosis, and that in humans and NHPs, pleural effusions can develop, but they are normally resolved with steroid administration and do not ultimately contribute to death (I. Jackson). Specifically, they proposed that the C57L/J strain is a



FIG. 1. Radiation dose-responses for severe pneumonitis among humans receiving either single doses of UHBI (\bullet) (*117*) or single dose-equivalents of TBI (\bigcirc), most with cyclophosphamide chemotherapy prior to allogeneic bone marrow transplantation (*118*). These are compared with lethal pneumonitis in three different mouse strains receiving WTI. C57L mice are the most sensitive to radiation and the most comparable to the incidence in humans. CBA mice appear to be more resistant towards radiation pneumonitis. C57BL/6 mice were the most removed from the human data in showing no lethal incidence of pneumonitis over 6 months after lung irradiation.

potentially good model for investigating both pneumonitis (Fig. 1) and fibrosis (49), and that in any strain selected, the presence or absence of pleural effusions should be noted in any resulting publications. Excellent overviews on strain differences in lung effects (50) and potential animal model limitations to mimic human pneumonitis, pleural effusion and fibrosis are available (51).

Another important aspect of the development of MCMs for delivery to a civilian population is the identification of agents for use in special populations, e.g., children; this specific subpopulation has demonstrated a differential response compared to adults with respect to radiation injury and to many pharmaceutical agents. The development of mouse models of pediatric lung damage is being evaluated at the University of Rochester (J. Finkelstein), where studies on neonates (4-21 days of age) are ongoing (52). This group has shown that the age of the immature animal at the time of irradiation plays an important role in the outcome of the exposure; of note, age 7-10 days is critical in the neonatal animal for lung maturation (J. Finkelstein). The investigators noted that the irradiation of neonatal mice is challenging since there is a steep dose response, and total body irradiation can lead to a dose-dependent stunting in growth and, at higher doses, a failure to wean; however, total body doses ≤ 5 Gy were generally well-tolerated by the animals (J. Finkelstein). In addition, due to the likely involvement of trauma as part of a nuclear or radiological event, other models under consideration included mice subjected to radiation combined injury, specifically radiation combined with burn, detailed below (E. Kovacs).

Notwithstanding the broad database available with respect to murine models of lung injury, other workshop attendees

² Where pre-publication data are discussed, the investigator's name is provided in parentheses.

described the advantages of using rats for lung injury studies since their larger size allows for greater ease of manipulation, including achieving clearer computed tomography (CT) scanning. Interestingly, investigators from the Medical College of Wisconsin described a gender effect in the pulmonary response of rats, observing that male rats appeared to have a differential radiation sensitivity from females, including the development of different late effects (e.g., males did not develop pneumonitis in some studies, at doses with which females did) (M. Medhora). It is worth noting that a recent report suggests a similar finding of differential gender sensitivity is also seen following human radiation exposures (53); however, no such gender differences were noted in the C57L/J radiation response (I. Jackson). In light of these data, it was determined that careful selection of an end point (e.g., pneumonitis or fibrosis) should be made in the context of the chosen rodent model.

In addition to rodent models of radiation exposure, larger animals, such as canines and monkeys, are being studied for their potential use as models of the human pulmonary response. For example, researchers at Fred Hutchinson Cancer Research Center have been actively developing external irradiation canine models since the 1980s, primarily for use in the area of transplantation research, although the group has recently focused its attention on the use of this animal model for investigating lung complications following irradiation alone (R. Nash). This model is important because canine lung architecture is more similar to humans than rodents (54); and there exists a wealth of data on the use of this animal model for internal contamination as well as late lung effects.

With respect to the NHP, despite often being considered as the closest animal model to humans for many diseases and exhibiting responses to acute, high-dose radiation exposure that are representative of those expected in humans, the limited use of NHPs as models for radiationinduced lung damage provides little background information or supportive data for this model. However, there is a specific need for such a model in order to study the efficacy of drugs in treating radiation-induced lung damage. Therefore, NHP model development studies are currently underway (T. MacVittie, I. Jackson)³. Although it has been suggested by some that pig models might also be representative of radiation-induced damage to the human lung due to the structural similarity of pig lungs to those of humans (4, 55), and a number of studies have explored the use of pigs as surrogates for human responses (55-59), these animals are not currently under model development as part of the NIAID program.

Total- and Partial-Body Irradiation Models

Any discussion of animal models for radiation damage to any organ system must include the volume of the tissue/ organ/organism that will be exposed to radiation. With lung exposures, there are a number of different radiation protocols that might be considered, so it is critical to understand how each represents the expected human response to both the radiation exposure and the MCM. For example, as part of a large-scale nuclear or radiological event, it is likely that the majority of those exposed will experience a total body, albeit heterogeneous, exposure. However, in the absence of supportive care, external doses of TBI delivered at levels currently considered necessary to cause late lung damage would be acutely lethal, so irradiation protocols involving TBI are often not selected in studies of late pulmonary effects. Nonetheless, to more effectively model the accident scenario, some investigators have elected to use high-dose TBI exposures, and rely on bone marrow transplantation to reconstitute the animal's bone marrow compartment and minimize mortality resulting from hematopoietic complications (M. Medhora). Other researchers have adopted high-dose exposure protocols, in which only a part of the animal is irradiated (partial-body irradiation, PBI). PBI models include those in which only the thorax is irradiated (whole-thorax lung irradiation, WTLI) (Z. Vujaskovic), and variations on this WTLI exposure in which only one side of the lung is irradiated (with the other lung serving as an unirradiated control) (R. Nash). In several of these models, the heart is shielded in order to eliminate the complicating factor of radiationinduced cardiovascular damage.

However, a drawback with some of the models discussed above is, indeed, the absence of hematopoietic myelosuppression and/or gastrointestinal (GI) damage, since one would anticipate these playing a role in a real-life, human exposure scenario and possibly affecting the progression of lung damage. To address this issue, a NHP-PBI model is being developed in which only the tibia and feet of the animals are shielded, allowing for approximately 5% active marrow to be retained (T. MacVittie, I. Jackson)⁴. As may be anticipated, depending on the dose, some of the animals do not survive the resultant GI and/or hematopoietic damage, but among those that do survive the acute syndromes, all have experienced bone marrow myelosuppression and recovery; however, significant downstream damage to the GI has been observed. In fact, many of these animals go on to develop a late "GI syndrome," which manifests as a failure to reconstitute normal villus structure and an inability to absorb nutrients (thus leading to weight

³ Z. Vujaskovic *et al.*, Variations in mast cell hyperplasia in the irradiated lungs of different mouse strains, rats and nonhuman primates. Presented at the Fifty-sixth Annual Meeting of the Radiation Research Society, 2010.

⁴ T. Shea-Donohue *et al.*, An acute radiation syndrome (ARS) nonhuman primate (NHP) research platform: prolonged gastrointestinal (GI) dysfunction observed in NHPs surviving the acute heme and GI syndromes. Presented at the Fifty-Fifth Annual Meeting of the Radiation Research Society, 2009.

loss that can necessitate their removal from the study)⁴. Nonetheless, these animals also begin to demonstrate late lung complications within months of exposure, as diagnosed by computed tomography (CT) scans (T. MacVittie, J. MacManus, I. Jackson). It was proposed that this model is a good representative of what might be seen clinically in personnel following accidental TBI.

This PBI model, along with a WTLI model, is being developed by NIAID through a product development contract. However, the radiation damage resulting from these kinds of PBI exposures provide significant challenges and require early and intense medical management of the animals in order to ensure that they survive the ARS stages of illness. An alternative protocol, proposed as part of a CMCR animal models workshop (60), is to expose the animals to a sub-lethal TBI radiation exposure, but with an additional localized "top-up," high-dose radiation exposure to the thorax, thus retaining the systemic injury associated with TBI without risking acute lethality. In this model, the anticipated pulmonary end points are retained, with pneumonitis being observed at 8-12 weeks and fibrosis starting at around 26 weeks post-irradiation (J. Williams).

Radiation plus Other Stressors (Two-Hit Models)

In the wake of the detonation of an improvised nuclear device, it is expected that irradiation will be combined with other traumatic injuries in 65-70% of all casualties, with 40% of those involving skin burns (61). Therefore, in addition to mouse models for radiation alone, investigators are developing a radiation combined injury model in C57BL/6 mice (62), in which radiation exposure is delivered concomitantly with skin burn; the effects of this double-hit injury are being studied for their effect on lung function and overall mortality. Burn in the presence of 2-9 Gy of total body irradiation has been modeled, and preliminary data from animals given a combined insult demonstrated decreased survival at early time points, prolonged pulmonary neutrophil infiltration, and increased expression levels of pulmonary chemokines and cytokines (E. Kovacs).

In a second dual-injury model, irradiated mice were subsequently infected (9–12 months post-radiation) with influenza or exposed to aerosolized lipopolysaccharide (LPS) in order to assess the long-term acquired or inherent immune functionality of the irradiated lung. As compared to age-matched unirradiated animals, LPS exposure led to a delayed, but ultimately increased, recruitment of inflammatory cells into the lungs of the previously irradiated animals, and was followed by a slower recovery (*63*). Following influenza exposure, differential levels of immune cell recruitment were seen between irradiated and age-matched animals, although viral titers were equivalent between the treatment arms (J. Finkelstein).

Early Expression of Biomarkers that are Predictive of Late Lung Effects

The totality of mechanisms involved in radiation-induced lung injury are not well understood, and many pathways have been studied in attempts to elucidate how radiation exposure leads to late complications (64). Some of these pathways involve the extracellular expression of critical factors, such as transforming growth factor (TGF)-\beta1 (65, 66), interleukin (IL)-6 (67), and nitric oxide (68), and a number of reviews have described the impact that these and other potential mechanisms may have on the progression to late normal tissue injury (34, 69-73). However, many investigators have suggested that the systemic (circulatory) expression of some of these cytokines and growth factors has the potential for use as biomarkers of injury since there are both immediate and late changes in expression levels associated with the various phases of progression. Although it is likely that the majority of the acutely expressed factors may serve only as surrogates of injury, it is hoped that at least some might prove to be predictive in nature, with their altered expression levels indicating which patients are susceptible to the development of late complications. Data were presented supporting the analysis of a panel of acutephase cytokine levels measured in peripheral blood that may serve as surrogate markers of pulmonary injury; the panel included IL-1, IL-6, and CXCL1 (KC in the mouse) (52). Interestingly, increased expression levels of both IL-6 and KC were demonstrated again at late time points (≥ 9 months post-radiation) (63), suggesting that these factors may indeed act as biomarkers of late injury and also may function as potential targets for mitigation (J. Finkelstein).

These findings were consistent with other evaluations of inflammatory cytokines in the post-irradiated lung, which have suggested that cytokine and growth factor expression levels are bi- or multi-phasic, with some responses occurring within hours of irradiation, and other responses manifesting weeks to months later (17, 74). It has previously been noted that biochemical and structural indicators could also be predictive of late changes (75); however, these data are more difficult to assess clinically.

Approaches to Treating Radiation-Induced Pulmonary Damage

Anti-oxidant and anti-inflammatory drugs. Synthetic SOD mimetics are antioxidant agents that have shown promise in the treatment of ongoing radiation damage, presumably by reducing chronic oxidative stress. A number of such compounds are being developed as lung radiation damage mitigators and include MnTE-2-PyP(5+) (76), MnTnHex-2-PyP(5+) (77) and AEOL10150 (78). AEOL10150 is a catalytic, metalloporphyrin antioxidant that reduces radiation damage to the lung, even when delivered up to 72 h after WTLI (79) (80–82). This agent, when given during and after radiation exposure, has also been shown to increase survival in mice (79, 83). The

compound scavenges peroxynitrite, inhibits lipid peroxidation, and has SOD activity. Metalloporphyrins as a class of compounds have been shown to attenuate O₂-, ONOO- and H₂O₂-mediated injury. AEOL10150 is also an effective rescue treatment for Cl(2)-induced airway hyper-responsiveness, airway inflammation, injury-induced airway epithelial cell regeneration, and oxidative stress (84). Another series of compounds that falls under this category are SODcatalase mimetics, two different classes of Mn-ligand complexes known collectively as "EUK compounds." EUK-207 and EUK-189 are salen Mn complexes that scavenge multiple ROS and RNS species and show protective efficacy in a variety of in vivo disease models, including being among the most effective mitochondrialprotective antioxidant agents known (85) and capable of prolonging survival in SOD2^{-/-} mice, a model for severe mitochondrial oxidative injury (86). The mitochondrialprotective properties of salen Mn complexes have been confirmed in ischemia (87) and radiation-induced mitochondrial injury models (88). EUK-189 was previously shown to mitigate radiation injury in hematopoietic (89) and lung models (90), and to inhibit radiation-induced oral mucositis (88). EUK-207, a newer salen Mn complex designed for greater stability, has a longer plasma half-life than EUK-189 and mitigates radiation injury in the kidney, skin, and CNS, as well as the lung (S. Doctrow, J. Williams) (88). In a rat thoracic irradiation model (91), EUK-207 mitigated pneumonitis and fibrosis-related end points, as well as reducing lung 80HdG levels, an indicator of oxidative injury. Another group of SOD/catalase mimetics (the EUK-400 series) are, unlike EUK-207 and EUK-189 and several other Mn-containing antioxidant agents, orally bioavailable. These compounds show anti-apoptotic activity but are Mn porphyrins, and showed greater cytotoxicity than the salen Mn complexes (92). Both the salen Mn and Mn porphyrin classes of "EUK" compounds have been found to inhibit radiation-induced endothelial cell apoptosis in cell culture studies (93).

Proprietary formulations of oxidized glutathione variants made by Novelos Therapeutics, Inc. (Nov002 and Nov205), are also undergoing testing for their ability to reduce radiation-induced lung damage (K. Held). The Nov002 compound, composed of oxidized glutathione plus cisplatin in a 1,000:1 molar ratio (94), has been shown to decrease chemotherapy-induced hematopoietic toxicity (95). In contrast, Nov205 is oxidized glutathione plus inosine in a 1:1 molar ratio and has been studied in clinical trials for patients with chronic hepatitis C who did not respond to standard therapies. Both of these drugs are believed to act as immuno-modulators and have anti-inflammatory properties. They are both stable with a long shelf life, and have shown minimal toxicity in humans. In addition, both forms of the drug are being evaluated clinically under Investigational New Drug (IND) applications filed with the FDA. Their potential efficacy is being evaluated in a mouse model, with end points such as breathing rate, body and lung weight, survival, and histopathology planned⁵.

ACE inhibitors. Suppression of the renin angiotensin system was described more than two decades ago to ameliorate radiation pneumonitis and fibrosis in the lungs (39). Recently, ACE inhibitors were found to be more effective than an angiotensin receptor blocker (33). Several different types of ACE inhibitors have been tested, either alone or in combination with other drugs, for their ability to minimize radiation-induced lung damage; these inhibitors include fosinopril, enalapril and captopril. In studies performed in rats, either TBI was given with bone marrow transplant⁶ or WTLI was administered⁷. The three drugs mitigated morbidity after TBI when started up to 4 h after radiation. Enalapril was effective even when started one week after TBI. All three drugs were shown to attenuate onset of fibrosis even when administration was delayed one week post-irradiation in the WTLI model (M. Medhora). However, captopril and enalapril, but not fosinopril mitigated morbidity through pneumonitis from 42-70 days after WTLI. Captopril has a short half-life, so enalapril was the preferred choice. These results suggest the mechanism of action of ACE inhibitors may be a class effect of the drugs rather than mitigation by structural side groups, which differ in the three drugs tested. In independent drug combination studies, addition of captopril to a protocol of SOD/catalase mimetic, EUK-207, led to improved survival (86% vs. 47%) compared to EUK alone⁸, and when statins were added to the captopril and EUK-207 combination, 100% survival was observed at 6 months post-radiation (J. Williams).

Nutraceutical approaches. As noted earlier, genistein, a soy isoflavone with antioxidant properties, has shown promise in treating radiation lung injury (35, 91). Genistein, known to block NF κ B activation, delays lethality in radiation-exposed rats and decreases micronuclei formation in irradiated lung fibroblasts (R. Hill). It has also been shown to mitigate pneumonitis and fibrosis even when initiated 1 week after irradiation (96). Another promising approach to limiting radiation damage to the lung is the use of a Chinese herb, triptolide (TPL), which is a potent, biologically-active compound isolated from the medicinal 'Thunder God Vine', *Tripterygium wilfordii*. TPL is a small

⁵ L. Gerweck *et al.*, Novel agents for mitigation of radiationinduced pulmonary injury. Presented at the Fifty-Sixth Annual Meeting of the Radiation Research Society, 2010.

⁶ M. Medhora *et al.*, Efficacy of structurally different angiotensin converting enzyme (ACE) inhibitors for mitigation of radiation pneumonitis. Presented at the Fifty-Sixth Annual Meeting of the Radiation Research Society, 2010.

⁷ E. R. Jacobs *et al.*, Angiotensin converting enzyme (ACE) inhibitors mitigate pulmonary fibrosis in rats. Presented at the Fifty-Sixth Annual Meeting of the Radiation Research Society, 2010.

⁸ F. Gao *et al.*, Angiotensin converting enzyme (ACE) inhibitors in combination with EUK-207 for mitigation of radiation pneumonitis. Presented at the Fifty-Sixth Annual Meeting of the Radiation Research Society, 2010.



FIG. 2. Statins increase survival in irradiated mice. Statins were administered either immediately after irradiation (15 Gy) or 8 weeks post exposure. *Statistically significant increases in survival at greater than 25 weeks post-irradiation as compared to untreated controls were noted for both treatment arms. Modified from Williams *et al.*, 2004 (*32*).

molecule that has been shown to alter mitochondrial function (97) and enhance the anti-tumor effect of radiation (98). TPL has also been in clinical trials for polycystic kidney disease, having already shown efficacy in animal models of the disease (99). When administered to mice after irradiation, TPL improved survival, breathing rate and lung compliance, indicators of lung fibrosis (S. Yang). Radiation-induced lung inflammation was also reduced by post-irradiation treatment with TPL, and its mechanism of action appears to be mediated by inhibition of immune cells and pro-inflammatory molecules⁹.

Cell therapies. Mesynchymal stem cells (MSCs) are multi-potent stem cells that can differentiate into a variety of different cell types. These cells have been shown to reduce radiation-induced damage in a number of different nonhematopoietic organ systems, including the GI tract (100, 101), skin (102), and the salivary gland (103, 104), and are now undergoing testing for their ability to treat radiation damage to the lungs (R. Nash). In a canine model, in which only one lung was irradiated (with the other serving as an unirradiated control), no pneumonitis was seen at the dose levels used, although small areas of inflammation were noted at the periphery and fibrosis developed by week 26 (R. Nash). Post-exposure infusion of MSCs showed promise in limiting damage, as assessed by bronchoalveolar lavage (BAL) at 36 weeks post-irradiation and pulmonary function tests (R. Nash).

Statins and growth factors. A number of statin drugs, both alone and in combination with other agents, have been studied in several mouse strains (J. Williams). Administration of simvastatin alone showed no survival benefit; however, when combined with the SOD/catalase mimetic, EUK-207, the combination of drugs reduced macrophage infiltrate numbers, down-regulated pro-inflammatory cytokines, and led to an increase in survival¹⁰. Other statins also increased survival when administered immediately after or at 8 weeks post-radiation exposure (Fig. 2) (J. Williams). Keratinocyte growth factor (KGF) studies are also being planned in a canine model, in order to assess the potential efficacy of the compound in ameliorating radiation-induced lung damage (R. Nash).

Other novel approaches. The threat of radiation exposure to the lung is not always from a prompt gamma exposure, but can also be due to prolonged exposure from internalization (inhalation) of radioactive particles. Studies in a canine (beagle) model have demonstrated that the use of bronchoalveolar lavage (BAL) will facilitate the removal of inhaled ¹⁴⁴Ce, resulting in greatly increased survival times in treated animals when compared to animals not subjected to BAL (*105*). BAL also removed about 50% of inhaled ²⁴¹Am particles and similarly improved survival in dogs (*106*). Currently, therefore, BAL is the only treatment available to clear nonsoluble radioactive particle in the lung, and has been used following incidents of internal contamination (*107*); however, it is an invasive procedure.

¹⁰ J. P Williams *et al.*, A combined therapeutic approach to pulmonary mitigation following a radiological event. Presented at the Fifty-Sixth Annual Meeting of the Radiation Research Society, 2010.

⁹ C. Chen *et al.*, Triptolide reduces radiation-induced cytokines in lung inflammation model. Presented at the Fifty-Sixth Annual Meeting of the Radiation Research Society, 2010.

The lead drug under investigation for this indication, P-552, is a small molecule, sodium-channel blocker based on the structure of amiloride, a potassium-sparing diuretic used clinically for over 20 years. This molecule is also in clinical development for the treatment of cystic fibrosis (*108*) and xerostomia (*109*). When delivered post-exposure by aerosol inhalation with saline, P-552 induces a synergistic increase in airway surface hydration (W. Thelin). It has been suggested that the P-552 molecule, when combined with small amounts of saline, offers the possibility of accelerating clearance of inhaled radioactive particles with little risk and in a minimally-invasive manner by thinning the fluids in the lung and encouraging clearance of the radionuclide by the cilia (W. Thelin).

The final novel compound under study through the NIAID program is Homspera – an orally-available, Substance P (SP) analog that is a NK1 receptor agonist and modulator of inflammation. SP has been shown to enhance bone marrow recovery after radiation exposure (*110*). Radiation depresses levels of SP; however, if levels are increased, inflammation and fibrosis are reduced (J. Finkelstein). Homspera also appears to affect the GI and hematopoietic compartments. This drug is being evaluated for its efficacy in reducing pulmonary fibrosis as a modulator of neurogenic inflammation, and its ability to reduce bone marrow toxicity effects on stem-cell populations.

DISCUSSION

Following the presentations, an open discussion allowed meeting participants to interact with a panel of government officials and contractors involved in the advanced development and licensure of drugs for a radiation/lung damage indication. The panel included representatives from the NIAID, the Biomedical Advanced Research and Development Authority (BARDA), HHS, the Office of Counterterrorism and Emergency Coordination (OCTEC) and the Division of Medical Imaging Products, Center for Drugs Evaluation and Research (CDER), FDA, and the University of Maryland School of Medicine. A listing of all panelists can be found in the Acknowledgments section. An overview of the discussions is provided below.

Expected Concept of Operations (CONOPS) following a Radiological or Nuclear Incident

Expectations were expressed that, following a large-scale radiological or nuclear event, individuals exposed to heterogeneous or partial-body (shielded) radiation exposures could subsequently develop late lung complications. There are currently no medical countermeasures (MCMs) in the strategic national stockpile (SNS) – coordinated by the Centers for Disease Control (111) – to treat radiation damage to the lungs. Therefore, there is a critical need to carry out studies within the NIAID-funded portfolio to

provide a pipeline for advanced development of such drugs. However, for an agent to be considered for such emergency usage, additional conditions must be met not always considered as part of drug licensure:

- Given that biodosimetry methods and devices, which can rapidly and accurately estimate after-the-fact dose are not yet available, it is possible that any therapies targeting radiation-induced lung injury, provided after a radiological or nuclear event, may be incorrectly administered to someone who has not received a dose of radiation sufficient to warrant its use. For this reason, successful Phase I safety trials (if data are not already available) will be an imperative as part of the development process, and the number of patients required might be larger than what would be needed for a classical, drug licensure pathway. It may be possible to reference available, clinical efficacy data for a drug licensed for another lung indication, although consultation with the appropriate FDA division would be warranted, especially if this pathway is being considered.
- Since a drug for a radiation-induced lung indication would likely be used alongside other treatments, drug interactions will need to be assessed. In addition, since the drug would be used in a civilian population, its effects in special populations (e.g., elderly, pediatric or immuno-compromised patients) would need to be determined.
- Any toxicology and/or PK/PD studies for the drug would need to be carried out in both irradiated and nonirradiated animals since these variables can be different in an irradiated, and thus, myelo-suppressed, animal.

Another driving force behind the CONOPS is the guidance from the government that delivery of a MCM may not be possible within the immediate hours or even days of an incident and, therefore, administration schedules beginning at times >24 h to 72 h post-exposure represent appropriate testing windows for drugs to treat acute radiation effects. This is similarly true regarding the development of drugs for a radiation-induced late effect, and investigators were strongly encouraged to consider testing using delayed administration scheduling.

Finally, only FDA-licensed drugs (licensed in the U.S. for any indication) can be stockpiled, and released from the stockpile for use following a radiological or nuclear incident can only be done if the specific drug is FDA-approved for the radiation indication, or through issue of an emergency use authorization (EUA) for that drug (*112*). In either of these pathways, it is important that data be obtained in appropriate, Good Laboratory Practice (GLP)-compliant animal models, as per FDA's Animal Rule (*3*).

MCM Development for Radiation Injury to the Lung

Because the ultimate goal of NIAID's program is licensure of a drug to treat late lung complications following

radiation exposure from a radiological or nuclear incident, efforts have focused on developing animal models that would be acceptable to the FDA for licensure via FDA's Animal Rule pathway. Unlike licensure via the traditional drug development pathway, this alternative pathway includes the following requirements for licensure:

- establishment of a reasonably well-understood pathophysiological mechanism of radiation damage;
- that effects of radiation exposure and MCM mitigation are demonstrated in at least one animal species expected to react with a response predictive of humans;
- the animal study outcomes are clearly related to the desired benefit in humans;
- data on MCM pharmacokinetics (PK) and pharmacodynamics (PD) are available to allow selection of an effective dose in humans.

It is critical to link together data obtained in each animal model (including mechanism of action, radiation and drug dose, PK and PD), and then show that the animal models are appropriate representations of the damage observed in humans, and that amelioration of damage by the drug at a given dose in the animal models is predictive of the response that will be observed in humans.

Working with the FDA toward MCM licensure

Some explanations and guidance were offered to the workshop participants by regulatory officials present on the best way to move forward with their prospective agents. For example, for protein or small molecule approaches, FDA jurisdiction over MCMs for radiation damage to the lungs following a radiological or nuclear incident is in the Division of Medical Imaging Products, Center for Drug Evaluation and Research (CDER). Cell therapy approaches would involve divisions within the Center for Biologics Evaluation and Research (CBER). FDA communications for these types of drug development programs are iterative, with initial contact preferably handled by liaison groups within each FDA center, which can provide companies with guidance on licensure pathways for drugs for this indication. Initial contact within CDER for medical countermeasures for radiation exposure should be with the FDA Office of Counter-Terrorism and Emergency Coordination (OCTEC). Applicants pursuing cell therapies and certain biologics should contact the Senior Advisor for Counterterrorism/ Medical Countermeasures, Office of the Director, CBER. It is extremely important that any potential sponsor of a drug for a radiation lung indication engage with the appropriate branch within the CDER or CBER, to determine which models and end points are acceptable to the FDA before designing pivotal studies to ascertain drug efficacy. Additional information on important considerations for investigators considering pursuing FDA approval for a radiation indication can be found elsewhere (113).

Safety. As with FDA licensure of any drug, safety studies will be critical. Since a drug for a radiation-induced lung indication would likely be used concomitantly with other treatments, drug interactions must be assessed. In addition, since the drug would be used in a civilian population, its effects in special populations (e.g., elderly, pediatric or immuno-compromised patients) would need to be determined. Any toxicology and/or PK/PD studies for the drug would need to be carried out in both irradiated and nonirradiated animals, since these variables can be different in an irradiated, and thus, myelo-suppressed animal. Even with the FDA Animal Rule licensure pathway, Phase 1 studies in healthy volunteers would still be required, and due to the intended use, the number of patients required might be larger than what would be needed for a classical drug licensure pathway. It may be possible to reference available, clinical efficacy data for a drug licensed for another lung indication, such as idiopathic pulmonary fibrosis, or for treatments in cancer patients that have received either TBI or thorax irradiations, although consultation with the appropriate FDA division would be warranted, especially if this pathway is being considered.

Challenges for Development of Lung Therapies

As with any organ-specific therapy for radiation damage, there are challenges in developing both an irradiated animal model for the indication as well as drugs to treat the variety of syndromes that may result from radiation exposure to the lungs. This is further complicated by the fact that lung effects can take many months to evolve, therefore, animal studies take longer to yield results.

Development of animal models and supportive care protocols. Participants and panelists discussed the possibility of performing studies in mouse versus rat models. Obviously, each model has its advantages; however, the consensus was that it was probably best at this time to continue concurrent development of models in both rodent species. Use of knockout and/or transgenic mice may be advisable in order to discern mechanism of action of the drug, but at least one or two other animal models should be selected for efficacy studies.

It was agreed that whatever rodent is selected for small animal studies, it is critical to fully standardize the model and validate it for its ability to detect efficacy of a MCM. Validation should include referencing "gold standards" and comparing proposed new treatments to standard of care. For example, the synthetic steroid, dexamethasone, is used clinically to treat lung injury (114), and in the case of radiation-induced damage, would likely be used by a physician. Dexamethasone has also been shown to minimize fibrosis in animal models of lung damage (115); therefore, researchers developing an MCM should consider the use of this treatment in the models that they propose, and obtain guidance from the FDA before initiating such studies.

WORKSHOP REPORT

Name	Affiliation	Expertise
David Cassatt, Ph.D.	DAIT, NIAID	Drug development, immunology, radiation biology
Susan Doctrow, Ph.D.	Boston University	Oxidative stress & disease, synthetic catalytic ROS scavengers, small molecule drug development
Jacob Finkelstein, Ph.D.	University of Rochester	Alveolar epithelium in injury responses, adult and pediatric lung animal models
Leo Gerweck, Ph.D.	Massachusetts General Hospital	Tumor cell radiation sensitivity
Marcy Grace, Ph.D.	BARDA	Radiation biodosimetry, biomarkers of radiation damage
Kathryn Held, Ph.D.	Massachusetts General Hospital	Radiation biology, radiation modifiers, oxidative stress, DNA damage and cell death mechanisms
Richard Hill, Ph.D.	University of Toronto	Tumour hypoxia, radiation effects on wound healing, lung responses to irradiation
I. Lauren Jackson, B.S.	Duke University	Radiation biology/pathology, molecular genetics, and rodent lung model development
Elizabeth Kovacs, Ph.D.	Loyola University Medical Center	Burn injury, innate immunity, pulmonary inflammation, cutaneous wound healing
Thomas MacVittie, Ph.D.	University of Maryland School of Medicine	Radiation biology, nonhuman primate models or radiation damage for MCM testing
Ronald Manning, Ph.D.	BARDA	Radiochemistry, analytical chemistry, small molecule drug development
John McManus, B.S.	Aeolus Pharmaceuticals	Drug development, project management and finance
Meetha Medhora, Ph.D.	Medical College of Wisconsin	Radiation pneumopathy, molecular biology, vascular biology, ACE inhibitors
Richard Nash, M.D.	Fred Hutchinson Cancer Research Center	Canine lung irradiation model, stem cell transplantation, oncology
Mark Poznansky, M.D., Ph.D.	Massachusetts General Hospital	Mechanisms of immunity, vaccines and immunotherapies for cancer, infectious diseases
Jui Shah, Ph.D.	DAIT, NIAID	Regulatory affairs, pharmacology, toxicology
William Thelin, Ph.D.	Parion Sciences	Mucociliary clearance of particles from the lung, cystic fibrosis
Jacqueline Williams, Ph.D.	University of Rochester	Normal tissue radiation responses, mechanism of radiation lung damage, animal model development
Shanmin Yang, M.D.	University of Florida	Rodent lung irradiation models, small molecule synthesis and drug development
Lurong Zhang, M.D., Ph.D.	University of Florida	Radiation biology, bioactive peptide design and mechanisms, drug pharmacokinetics

 TABLE 1

 Invited Workshop Speakers and Areas of Expertise^a

"Invited speakers were given the opportunity to comment on the meeting report before its submission.

Standard supportive care in many animal research facilities includes administration of fluids, antibiotics, analgesics for pain, and antipyretics for fever. Intensive supportive care, such as would be expected for human patients following a radiological or nuclear incident, is typically provided only to large animals due to the infeasibility of administering such support to small animals. There is some debate as to whether MCMs should be tested with or without supportive care measures, with those advocating against the delivery of supportive care arguing that the medical infrastructures may be compromised after a radiological or nuclear incident, making it challenging. Data suggest that supportive care is an effective MCM on its own, improving survival of animals when compared to nonsupported animals and vielding a radiation dose modification factor of ~ 1.3 (116). The question of whether to provide supportive care to an irradiated animal - and to what extent – is especially important for certain TBI models of lung damage (discussed below), since animals must survive both the GI and hematopoietic syndromes before manifesting lung injury; however, only discussions with the FDA can delineate the animal care protocol that should be employed for development of a particular MCM.

Defining radiation exposure parameters. Because there are different types of radiation exposure (e.g., radionuclides, X rays, pure gamma exposures from a source), and all can have different dose rates, this can be a confounding factor in an in vivo efficacy study. In addition, other factors, such as time of day of irradiation, anesthesia, use of acidified water, and food composition (e.g., high levels of soy), can affect radiation responses. Therefore, care must be taken to consider these effects when planning a study. Because lung damage can result from internal contamination (through radionuclide inhalation in a fallout scenario) and/or external exposure (from a prompt burst of radiation), it is important to consider if both routes of exposure lead to similar late lung effects. With regards to complications from internal radionuclide contamination in the lung, pulmonary effects are generally mild and are mostly hematopoietic (J. Finkelstein).

As discussed above, several radiation exposure protocols are currently in use within research laboratories. These include partial-body [shielded so only the lung (one or both sides) or thorax is exposed] as well as TBI (both with and without bone marrow transplant). In addition, some investigators are coupling a low-lethal TBI exposure with a localized, higher dose to the lungs in an attempt to more closely model expected radiation exposure scenarios following a radiological or nuclear incident. It is not yet clear which model will be considered to be the most appropriate by the FDA; however, draft guidance provided by the agency in 2009 provides important information to potential drug sponsors on what must be submitted for licensure of an MCM for this and other radiation indications (47).

Identifying appropriate end points (primary and secondary) to assess efficacy of lung MCMs. FDA requires use of end points that indicate clinical benefit, which for many indications is a mortality end point. For an MCM proposed to treat radiation injury to the lung, reduction of major morbidity by the therapeutic may represent another possible end point. An improvement in survival of 25-30% as well as a DMF of greater than 1.1 is desirable in a drug for which licensure for this indication is being sought. However, determination of major morbidity in the lung is a difficult clinical end point to assess, and improvements in "quality of life" of affected patients are difficult to quantify and are even more so in animal models. For example, it is unclear if a finding of reduced lung function, which is ameliorated by a candidate drug, would indicate an appropriate major morbidity, and if enhanced lung function in an animal model following treatment would represent an acceptable end point under the Animal Rule.

Therefore, it is important to be able to prove clinical relevance of a treatment if a survival endpoint is not considered. For example, human lung damage is often assessed by CT scan, so it would therefore be important to attempt CT scans of irradiated animals to relate those findings to a clinically-relevant outcome. Such scans work well in larger animals, but are logistically challenging in rodents such as mice. Lung compliance and/or respiratory rate could also represent surrogate end points; however, many variables can affect the latter. A more thorough consideration of potential end points to be considered can be found elsewhere (60).

Research Gaps

This meeting highlighted several important gaps in the research that is currently being funded by the NIAID and other U.S. Government agencies. Further studies are still needed to better understand the impact of several factors on lung sensitivity to radiation exposure, as well as responses to therapeutics. These factors include age, gender and immune competency. There is also still a need to further validate existing models for radiation damage to the lungs, and to better understand the mechanisms responsible for the radiation damage and cellular and organ responses to the injury.

ACKNOWLEDGMENTS

The authors thank conference participants for contributions to the discussion; especially the investigators whose presentations served to stimulate discussion on topics important to this research area (see Table 1), and those who assisted with the preparation of this report. Special thanks to Dr. Marcy Grace (BARDA), Dr. Brad Leissa (OCTEC, CDER, FDA),

Dr. Thomas MacVittie (University of Maryland School of Medicine), Dr. Bert Maidment (NIAID), Dr. Rafel (Dwaine) Rieves (Division of Medical Imaging, CDER, FDA), and Dr. Jui Shah (Office of Regulatory Affairs, NIAID) for serving on the panel and for their valuable contributions to the discussion. The authors also thank NIAID staff (Dr. David Cassatt and Dr. Erika Davies) for their careful review of this manuscript.

REFERENCES

- Pellmar TC, Rockwell S. Priority list of research areas for radiological nuclear threat countermeasures. Radiat Res. 2005;163(1):115–23.
- Endo A, Yamaguchi Y. Analysis of dose distribution for heavily exposed workers in the first criticality accident in Japan. Radiat Res. 2003; 159(4):535–42.
- Food and Drug Administration. New drug and biological drug products: evidence needed to demonstrate effectiveness of new drugs when human efficacy studies are not ethical or feasible. Fed Regist. 2002; 67:37988–98.
- 4. Stone HB, Moulder JE, Coleman CN, Ang KK, Anscher MS, Barcellos-Hoff MH, et al. Models for evaluating agents intended for the prophylaxis, mitigation and treatment of radiation injuries. Report of an NCI Workshop, December 3–4, 2003. Radiat Res. 2004; 162(6):711–28.
- 5. Movsas B, Raffin TA, Epstein AH, Link CJ, Jr. Pulmonary radiation injury. Chest. 1997; 111(4):1061–76.
- Oya N, Sasai K, Tachiiri S, Sakamoto T, Nagata Y, Okada T, et al. Influence of radiation dose rate and lung dose on interstitial pneumonitis after fractionated total body irradiation: acute parotitis may predict interstitial pneumonitis. Int J Hematol. 2006; 83(1):86–91.
- Schneider RA, Schultze J, Jensen JM, Hebbinghaus D, Galalae RM. Long-term outcome after static intensity-modulated total body radiotherapy using compensators stratified by pediatric and adult cohorts. Int J Radiat Oncol Biol Phys. 2008; 70(1):194–202.
- United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR). Report to the General Assembly, Annex J: Exposure and Effects of the Chernobyl Accident. Sources, Effects and Risks of Ionizing Radiation. 2000.
- Uozaki H, Fukayama M, Nakagawa K, Ishikawa T, Misawa S, Doi M, et al. The pathology of multi-organ involvement: two autopsy cases from the Tokai-mura criticality accident. BJR Suppl. 2005; 27:13–6.
- Changlin Y, Genyao Y. Multi-organ failure in a radiation accident: the Chinese experience of 1990. BJR Suppl. 2005; 27:47–54.
- Mettler FA, Ortiz-Lopez P. Accidents in radiation therapy. Gusev IA, Guskova AK, Mettler FA, editors. Washington, DC: CRC Press; 2001.
- 12. Vlasov PA, Kvacheva Iu E. [The pathomorphology of the pulmonary infectious complications in acute radiation sickness (based on the autopsy data from persons who died as a result of the accident at the Chernobyl Atomic Electric Power Station]. Ter Arkh. 1996; 68(3):23–6. Patomorfologiia legochnykh infektsionnykh oslozhnenii ostroi luchevoi bolezni (po materialam autopsii lits, pogibshikh v rezul'tate avarii na Chernobyl'skoi AES.
- Baranov AE, Selidovkin GD, Butturini A, Gale RP. Hematopoietic recovery after 10-Gy acute total body radiation. Blood. 1994; 83(2):596–9.
- 14. Moulder JE, Cohen EP. Future strategies for mitigation and treatment of chronic radiation-induced normal tissue injury. Semin Radiat Oncol. 2007; 17(2):141–8.
- Travis EL, Down JD, Holmes SJ, Hobson B. Radiation pneumonitis and fibrosis in mouse lung assayed by respiratory frequency and histology. Radiat Res. 1980; 84(1):133–43.
- 16. Rubin P, Finkelstein JN, Siemann DW, Shapiro DL, Van Houtte

P, Penney DP. Predictive biochemical assays for late radiation effects. Int J Radiat Oncol Biol Phys. 1986; 12(4):469–76.

- Rubin P, Johnston CJ, Williams JP, McDonald S, Finkelstein JN. A perpetual cascade of cytokines postirradiation leads to pulmonary fibrosis. Int J Radiat Oncol Biol Phys. 1995; 33(1): 99–109.
- Fleckenstein K, Zgonjanin L, Chen L, Rabbani Z, Jackson IL, Thrasher B, et al. Temporal onset of hypoxia and oxidative stress after pulmonary irradiation. Int J Radiat Oncol Biol Phys. 2007; 68(1):196–204.
- 19. Szabo S, Ghosh SN, Fish BL, Bodiga S, Tomic R, Kumar G, et al. Cellular inflammatory infiltrate in pneumonitis induced by a single moderate dose of thoracic x radiation in rats. Radiat Res. 2010; 173(4):545–56.
- 20. Thomas O, Mahe M, Campion L, Bourdin S, Milpied N, Brunet G, et al. Long-term complications of total body irradiation in adults. Int J Radiat Oncol Biol Phys. 2001; 49(1):125–31.
- 21. Franko AJ, Sharplin J, Ghahary A, Barcellos-Hoff MH. Immunohistochemical localization of transforming growth factor beta and tumor necrosis factor alpha in the lungs of fibrosis-prone and "nonfibrosing" mice during the latent period and early phase after irradiation. Radiat Res. 1997; 147(2):245–56.
- 22. Hong JH, Chiang CS, Tsao CY, Lin PY, McBride WH, Wu CJ. Rapid induction of cytokine gene expression in the lung after single and fractionated doses of radiation. Int J Radiat Biol. 1999; 75(11):1421–7.
- 23. Anscher MS, Thrasher B, Rabbani Z, Teicher B, Vujaskovic Z. Antitransforming growth factor-beta antibody 1D11 ameliorates normal tissue damage caused by high-dose radiation. Int J Radiat Oncol Biol Phys. 2006; 65(3):876–81.
- 24. Yuhas JM. Radiotherapy of experimental lung tumors in the presence and absence of a radioprotective drug, S-2-(3-aminopropylamino)ethylphosphorothioic acid (WR-2721). J Natl Cancer Inst. 1973; 50(1):69–78.
- 25. Travis EL, Parkins CS, Holmes SJ, Down JD, Fowler JF. WR-2721 protection of pneumonitis and fibrosis in mouse lung after single doses of x rays. Int J Radiat Oncol Biol Phys. 1984; 10(2): 243–51. Epub 1984/02/01.
- Vujaskovic Z, Feng QF, Rabbani ZN, Samulski TV, Anscher MS, Brizel DM. Assessment of the protective effect of amifostine on radiation-induced pulmonary toxicity. Exp Lung Res. 2002; 28(7):577–90.
- Down JD, Laurent GJ, McAnulty RJ, Steel GG. Oxygendependent protection of radiation lung damage in mice by WR 2721. Int J Radiat Biol Relat Stud Phys Chem Med. 1984; 46(5): 597–607. Epub 1984/11/01.
- 28. Komaki R, Lee JS, Milas L, Lee HK, Fossella FV, Herbst RS, et al. Effects of amifostine on acute toxicity from concurrent chemotherapy and radiotherapy for inoperable nonsmall-cell lung cancer: report of a randomized comparative trial. Int J Radiat Oncol Biol Phys. 2004; 58(5):1369–77. Epub 2004/03/31.
- Magana E, Crowell RE. Radiation pneumonitis successfully treated with inhaled corticosteroids. South Med J. 2003; 96(5): 521–4.
- 30. Sekine I, Sumi M, Ito Y, Nokihara H, Yamamoto N, Kunitoh H, et al. Retrospective analysis of steroid therapy for radiationinduced lung injury in lung cancer patients. Radiother Oncol. 2006; 80(1):93–7.
- Molls M, Herrmann T, Steinberg F, Feldmann HJ. Radiopathology of the lung: experimental and clinical observations. Recent Results Cancer Res. 1993; 130:109–21.
- 32. Williams JP, Hernady E, Johnston CJ, Reed CM, Fenton B, Okunieff P, et al. Effect of administration of lovastatin on the development of late pulmonary effects after whole-lung irradiation in a murine model. Radiat Res. 2004; 161(5):560–7.
- 33. Ghosh SN, Zhang R, Fish BL, Semenenko VA, Li XA, Moulder JE, et al. Renin-Angiotensin system suppression mitigates

experimental radiation pneumonitis. Int J Radiat Oncol Biol Phys. 2009; 75(5):1528–36.

- Zhao W, Robbins ME. Inflammation and chronic oxidative stress in radiation-induced late normal tissue injury: therapeutic implications. Curr Med Chem. 2009; 16(2):130–43.
- Para AE, Bezjak A, Yeung IW, Van Dyk J, Hill RP. Effects of genistein following fractionated lung irradiation in mice. Radiother Oncol. 2009; 92(3):500–10.
- 36. Khan MA, Van Dyk J, Yeung IW, Hill RP. Partial volume rat lung irradiation; assessment of early DNA damage in different lung regions and effect of radical scavengers. Radiother Oncol. 2003; 66(1):95–102.
- 37. Epperly MW, Travis EL, Sikora C, Greenberger JS. Manganese superoxide dismutase (MnSOD) plasmid/liposome pulmonary radioprotective gene therapy: modulation of irradiation-induced mRNA for IL-I, TNF-alpha, and TGF-beta correlates with delay of organizing alveolitis/fibrosis. Biol Blood Marrow Transplant. 1999; 5(4):204–14.
- Rabbani ZN, Anscher MS, Folz RJ, Archer E, Huang H, Chen L, et al. Overexpression of extracellular superoxide dismutase reduces acute radiation induced lung toxicity. BMC Cancer. 2005; 5:59.
- 39. Ward WF, Molteni A, Ts'ao CH. Radiation-induced endothelial dysfunction and fibrosis in rat lung: modification by the angiotensin converting enzyme inhibitor CL242817. Radiat Res. 1989; 117(2):342–50.
- 40. Molteni A, Moulder JE, Cohen EF, Ward WF, Fish BL, Taylor JM, et al. Control of radiation-induced pneumopathy and lung fibrosis by angiotensin-converting enzyme inhibitors and an angiotensin II type 1 receptor blocker. Int J Radiat Biol. 2000; 76(4):523–32.
- 41. Zhang R, Ghosh SN, Zhu D, North PE, Fish BL, Morrow NV, et al. Structural and functional alterations in the rat lung following whole thoracic irradiation with moderate doses: injury and recovery. Int J Radiat Biol. 2008; 84(6):487–97.
- 42. Ghosh SN, Wu Q, Mader M, Fish BL, Moulder JE, Jacobs ER, et al. Vascular injury after whole thoracic x-ray irradiation in the rat. Int J Radiat Oncol Biol Phys. 2009; 74(1):192–9.
- 43. Ward WF, Lin PJ, Wong PS, Behnia R, Jalali N. Radiation pneumonitis in rats and its modification by the angiotensinconverting enzyme inhibitor captopril evaluated by highresolution computed tomography. Radiat Res. 1993; 135(1):81–7.
- Heeneman S, Sluimer JC, Daemen MJ. Angiotensin-converting enzyme and vascular remodeling. Circ Res. 2007; 101(5):441–54.
- 45. Holecki M, Szewieczek J, Chudek J. Effects of angiotensinconverting enzyme inhibitors beyond lowering blood pressure– are they important for doctors? Pharmacol Rep. 2011; 63(3): 740–51.
- Galderisi M, de Divitiis O. Risk factor-induced cardiovascular remodeling and the effects of angiotensin-converting enzyme inhibitors. J Cardiovasc Pharmacol. 2008; 51(6):523–31.
- 47. Food and Drug Administration. Guidance for Industry: Animal Models — Essential Elements to Address Efficacy Under the Animal Rule. In: Center for Drug Evaluation and Research (CDER) Center for Biologics Evaluation and Research (CBER), editor.: Office of Communications, Division of Drug Information, FDA; 2009.
- 48. International Atomic Energy Agency. Accidental Overexposure of Radiotherapy Patients in Bialystok. Vienna. 2004.
- 49. Jackson IL, Vujaskovic Z, Down JD. Revisiting strain-related differences in radiation sensitivity of the mouse lung: recognizing and avoiding the confounding effects of pleural effusions. Radiat Res. 2010; 173(1):10–20.
- 50. Sharplin J, Franko AJ. A quantitative histological study of straindependent differences in the effects of irradiation on mouse lung during the early phase. Radiat Res. 1989; 119(1):1–14. Epub 1989/07/01.

- 51. Travis EL, Tucker SL. The relationship between functional assays of radiation response in the lung and target cell depletion. The British Journal of Cancer Supplement. 1986; 7:304–19. Epub 1986/01/01.
- 52. Johnston CJ, Hernady E, Reed C, Thurston SW, Finkelstein JN, Williams JP. Early alterations in cytokine expression in adult compared to developing lung in mice after radiation exposure. Radiat Res. 2010; 173(4):522–35.
- 53. Fucic A, Gamulin M. Interaction between ionizing radiation and estrogen: What we are missing? Med Hypotheses. 2011.
- 54. Chen Q, Klein JS, Gamsu G, Webb WR. High-resolution computed tomography of the mammalian lung. Am J Vet Res. 1992; 53(7):1218–24.
- Hopewell JW, Rezvani M, Moustafa HF. The pig as a model for the study of radiation effects on the lung. Int J Radiat Biol. 2000; 76(4):447–52.
- Herrmann T, Baumann M, Voigtmann L, Knorr A. Effect of irradiated volume on lung damage in pigs. Radiother Oncol. 1997; 44(1):35–40.
- Rezvani M, Heryet JC, Hopewell JW. Effects of single doses of gamma-radiation on pig lung. Radiother Oncol. 1989; 14(2): 133–42.
- Rezvani M, Hopewell JW. The response of the pig lung to fractionated doses of X rays. Br J Radiol. 1990; 63(745):41–50.
- Herrmann T, Voigtmann L, Knorr A, Lorenz J, Johanssen U. The time-dose relationship for radiation-induced lung damage in pigs. Radiother Oncol. 1986; 5(2):127–35.
- Williams JP, Brown SL, Georges GE, Hauer-Jensen M, Hill RP, Huser AK, et al. Animal models for medical countermeasures to radiation exposure. Radiat Res. 2010; 173(4):557–78.
- Kishi HS. Effects of the "special bomb": recollections of a neurosurgeon in Hiroshima, August 8-15, 1945. Neurosurgery. 2000; 47(2):441–5; discussion 5–6.
- Palmer JL, Deburghgraeve CR, Bird MD, Hauer-Jensen M, Kovacs EJ. Development of a combined radiation and burn injury model. J Burn Care Res. 2011; 32(2):317–23.
- 63. Johnston CJ, Manning C, Hernady E, Reed C, Thurston SW, Finkelstein JN, et al. Effect of total body irradiation on late lung effects: Hidden dangers. Int J Radiat Biol. 2011.
- 64. Cappuccini F, Eldh T, Bruder D, Gereke M, Jastrow H, Schulze-Osthoff K, et al. New insights into the molecular pathology of radiation-induced pneumopathy. Radiother Oncol. 2011.
- 65. Anscher MS. Targeting the TGF-beta1 pathway to prevent normal tissue injury after cancer therapy. Oncologist. 2010; 15(4):350–9.
- 66. Kim JY, Kim YS, Kim YK, Park HJ, Kim SJ, Kang JH, et al. The TGF-beta1 dynamics during radiation therapy and its correlation to symptomatic radiation pneumonitis in lung cancer patients. Radiat Oncol. 2009; 4:59.
- 67. Sakai M, Iwakawa M, Iwakura Y, Ohta T, Tsujii H, Imai T. CD44 and Bak expression in IL-6 or TNF-alpha gene knockout mice after whole lung irradiation. J Radiat Res (Tokyo). 2008; 49(4):409–16.
- Nozaki Y, Hasegawa Y, Takeuchi A, Fan ZH, Isobe KI, Nakashima I, et al. Nitric oxide as an inflammatory mediator of radiation pneumonitis in rats. Am J Physiol. 1997; 272(4 Pt 1): L651–8.
- Graves PR, Siddiqui F, Anscher MS, Movsas B. Radiation pulmonary toxicity: from mechanisms to management. Semin Radiat Oncol. 2011; 20(3):201–7.
- 70. Wilson MS, Wynn TA. Pulmonary fibrosis: pathogenesis, etiology and regulation. Mucosal Immunol. 2009; 2(2):103–21.
- Brush J, Lipnick SL, Phillips T, Sitko J, McDonald JT, McBride WH. Molecular mechanisms of late normal tissue injury. Semin Radiat Oncol. 2007; 17(2):121–30.
- 72. Tsoutsou PG, Koukourakis MI. Radiation pneumonitis and

fibrosis: mechanisms underlying its pathogenesis and implications for future research. Int J Radiat Oncol Biol Phys. 2006; 66(5):1281–93.

- Anscher MS, Vujaskovic Z. Mechanisms and potential targets for prevention and treatment of normal tissue injury after radiation therapy. Semin Oncol. 2005; 32(2 Suppl 3):S86–91.
- 74. Rube CE, Wilfert F, Palm J, Konig J, Burdak-Rothkamm S, Liu L, et al. Irradiation induces a biphasic expression of proinflammatory cytokines in the lung. Strahlenther Onkol. 2004; 180(7):442–8.
- Travis EL. Early indicators of radiation injury in the lung: are they useful predictors for late changes? Int J Radiat Oncol Biol Phys. 1980; 6(9):1267–9.
- 76. Yakovlev VA, Rabender CS, Sankala H, Gauter-Fleckenstein B, Fleckenstein K, Batinic-Haberle I, et al. Proteomic analysis of radiation-induced changes in rat lung: Modulation by the superoxide dismutase mimetic MnTE-2-PyP(5+). Int J Radiat Oncol Biol Phys. 2010; 78(2):547–54.
- Gauter-Fleckenstein B, Fleckenstein K, Owzar K, Jiang C, Batinic-Haberle I, Vujaskovic Z. Comparison of two Mn porphyrin-based mimics of superoxide dismutase in pulmonary radioprotection. Free Radic Biol Med. 2008; 44(6):982–9.
- Vujaskovic Z, Batinic-Haberle I, Rabbani ZN, Feng QF, Kang SK, Spasojevic I, et al. A small molecular weight catalytic metalloporphyrin antioxidant with superoxide dismutase (SOD) mimetic properties protects lungs from radiation-induced injury. Free Radic Biol Med. 2002; 33(6):857–63.
- 79. Rabbani ZN, Batinic-Haberle I, Anscher MS, Huang J, Day BJ, Alexander E, et al. Long-term administration of a small molecular weight catalytic metalloporphyrin antioxidant, AEOL 10150, protects lungs from radiation-induced injury. Int J Radiat Oncol Biol Phys. 2007; 67(2):573–80.
- Day BJ, Shawen S, Liochev SI, Crapo JD. A metalloporphyrin superoxide dismutase mimetic protects against paraquat-induced endothelial cell injury, in vitro. J Pharmacol Exp Ther. 1995; 275(3):1227–32.
- 81. Szabo C, Day BJ, Salzman AL. Evaluation of the relative contribution of nitric oxide and peroxynitrite to the suppression of mitochondrial respiration in immunostimulated macrophages using a manganese mesoporphyrin superoxide dismutase mimetic and peroxynitrite scavenger. FEBS Lett. 1996; 381(1–2):82–6.
- Day BJ, Fridovich I, Crapo JD. Manganic porphyrins possess catalase activity and protect endothelial cells against hydrogen peroxide-mediated injury. Arch Biochem Biophys. 1997; 347(2): 256–62.
- 83. Rabbani ZN, Salahuddin FK, Yarmolenko P, Batinic-Haberle I, Thrasher BA, Gauter-Fleckenstein B, et al. Low molecular weight catalytic metalloporphyrin antioxidant AEOL 10150 protects lungs from fractionated radiation. Free Radic Res. 2007; 41(11): 1273–82.
- 84. McGovern T, Day BJ, White CW, Powell WS, Martin JG. AEOL10150: A novel therapeutic for rescue treatment after toxic gas lung injury. Free Radic Biol Med. 2010.
- 85. Melov S, Doctrow SR, Schneider JA, Haberson J, Patel M, Coskun PE, et al. Lifespan extension and rescue of spongiform encephalopathy in superoxide dismutase 2 nullizygous mice treated with superoxide dismutase-catalase mimetics. J Neurosci. 2001; 21(21):8348–53.
- 86. Doctrow S, Baudry M, Huffman K, Malfroy B, Melov S. Salen Manganese Complexes: Multifunctional Catalytic Antioxidants Protective in Models for Neurodegenerative Diseases of Aging. Medicinal Inorganic Chemistry: ACS Symposium Series: Oxford University Press; 2005.
- 87. Liesa M, Luptak I, Qin F, Hyde BB, Sahin E, Siwik DA, et al. Mitochondrial transporter ATP binding cassette mitochondrial erythroid is a novel gene required for cardiac recovery after ischemia/reperfusion. Circulation. 2011; 124(7):806–13.
- 88. Rosenthal RA, Fish B, Hill RP, Huffman KD, Lazarova Z,

Mahmood J, et al. Salen Mn complexes mitigate radiation injury in normal tissues. Anticancer Agents Med Chem. 2011; 11(4): 359–72.

- Srinivasan V, Doctrow S, Singh VK, Whitnall MH. Evaluation of EUK-189, a synthetic superoxide dismutase/catalase mimetic as a radiation countermeasure. Immunopharmacol Immunotoxicol. 2008; 30(2):271–90.
- 90. Langan AR, Khan MA, Yeung IW, Van Dyk J, Hill RP. Partial volume rat lung irradiation: the protective/mitigating effects of Eukarion-189, a superoxide dismutase-catalase mimetic. Radiother Oncol. 2006; 79(2):231–8.
- 91. Mahmood J, Jelveh S, Calveley V, Zaidi A, Doctrow SR, Hill RP. Mitigation of radiation-induced lung injury by genistein and EUK-207. Int J Radiat Biol. 2011; 87(8):889–901.
- 92. Rosenthal RA, Huffman KD, Fisette LW, Damphousse CA, Callaway WB, Malfroy B, et al. Orally available Mn porphyrins with superoxide dismutase and catalase activities. J Biol Inorg Chem. 2009; 14(6):979–91.
- *93.* Vorotnikova E, Rosenthal RA, Tries M, Doctrow SR, Braunhut SJ. Novel synthetic SOD/catalase mimetics can mitigate capillary endothelial cell apoptosis caused by ionizing radiation. Radiat Res. 2010; 173(6):748–59.
- Townsend DM, Pazoles CJ, Tew KD. NOV-002, a mimetic of glutathione disulfide. Expert Opin Investig Drugs. 2008; 17(7): 1075–83.
- 95. Jenderny S, Lin H, Garrett T, Tew KD, Townsend DM. Protective effects of a glutathione disulfide mimetic (NOV-002) against cisplatin induced kidney toxicity. Biomed Pharmacother. 2010; 64(1):73–6.
- 96. Mahmood J, Jelveh S, Calveley V, Zaidi A, Doctrow SR, Hill RP. Mitigation of Lung Injury after Accidental Exposure to Radiation. Radiat Res. 2011.
- Su Y, Yang S, Xiao Z, Wang W, Okunieff P, Zhang L. Triptolide alters mitochondrial functions. Adv Exp Med Biol. 2007; 599:139–46.
- Wang W, Yang S, Su Y, Xiao Z, Wang C, Li X, et al. Enhanced antitumor effect of combined triptolide and ionizing radiation. Clin Cancer Res. 2007; 13(16):4891–9.
- Leuenroth SJ, Bencivenga N, Igarashi P, Somlo S, Crews CM. Triptolide reduces cystogenesis in a model of ADPKD. J Am Soc Nephrol. 2008; 19(9):1659–62.
- 100. Zhang J, Gong JF, Zhang W, Zhu WM, Li JS. Effects of transplanted bone marrow mesenchymal stem cells on the irradiated intestine of mice. J Biomed Sci. 2008; 15(5):585–94.
- 101. Semont A, Francois S, Mouiseddine M, Francois A, Sache A, Frick J, et al. Mesenchymal stem cells increase self-renewal of small intestinal epithelium and accelerate structural recovery after radiation injury. Adv Exp Med Biol. 2006; 585:19–30.
- 102. Agay D, Scherthan H, Forcheron F, Grenier N, Herodin F, Meineke V, et al. Multipotent mesenchymal stem cell grafting to treat cutaneous radiation syndrome: development of a new minipig model. Exp Hematol. 2010; 38(10):945–56.
- 103. Coppes RP, Stokman MA. Stem cells and the repair of radiationinduced salivary gland damage. Oral Dis. 2011; 17(2):143–53.
- 104. Lombaert IM, Wierenga PK, Kok T, Kampinga HH, deHaan G,

Coppes RP. Mobilization of bone marrow stem cells by granulocyte colony-stimulating factor ameliorates radiation-induced damage to salivary glands. Clin Cancer Res. 2006; 12(6):1804–12.

- 105. Muggenburg BA, Boecker BB, Hahn FF, McClellan RO. Lung lavage therapy to lessen the biological effects of inhaled 144Ce in dogs. Radiat Res. 1990; 124(2):147–55.
- 106. Muggenburg BA, Mewhinney JA. Removal of inhaled 241Am oxide particles of various sizes from beagle dogs using lung lavage and chelation treatment. Health Phys. 1981; 41(1):123–33.
- 107. Voelz GL, Lawrence JN, Johnson ER. Fifty years of plutonium exposure to the Manhattan Project plutonium workers: an update. Health Phys. 1997; 73(4):611–9.
- 108. Hirsh AJ, Sabater JR, Zamurs A, Smith RT, Paradiso AM, Hopkins S, et al. Evaluation of second generation amiloride analogs as therapy for cystic fibrosis lung disease. J Pharmacol Exp Ther. 2004; 311(3):929–38.
- 109. Singh ML, Papas AS, Fox PC, Stevens AL, Boucher RC, Thelin BT. Development of P552-02 to treat xerostomia in Sjgrens Syndrome. Arthritis Rheum. 2009; 60(Suppl 10):494.
- 110. An YS, Lee E, Kang MH, Hong HS, Kim MR, Jang WS, et al. Substance P stimulates the recovery of bone marrow after the irradiation. J Cell Physiol. 2011; 226(5):1204–13.
- 111. Centers for Disease Control. Emergency Preparedness and Response: Strategic National Stockpile. 2010.
- 112. Food and Drug Administration. Draft Guidance: Emergency Use Authorization of Medical Products. In: Office of Counterterrorism Policy and Planning, editor.: Office of Counterterrorism Policy and Planning (HF-29), Office of the Commissioner, FDA; 2005.
- 113. DiCarlo AL, Poncz M, Cassatt DR, Shah JR, Czarniecki CW, Maidment BW. Medical countermeasures for platelet regeneration after radiation exposure. Report of a workshop and guided discussion sponsored by the National Institute of Allergy and Infectious Diseases, Bethesda, MD, March 22–23, 2010. Radiat Res. 2011; 176(1):e0001–15.
- 114. Azoulay E, Canet E, Raffoux E, Lengline E, Lemiale V, Vincent F, et al. Dexamethasone in patients with acute lung injury from acute monocytic leukemia. Eur Respir J. 2011.
- 115. Dik WA, McAnulty RJ, Versnel MA, Naber BA, Zimmermann LJ, Laurent GJ, et al. Short course dexamethasone treatment following injury inhibits bleomycin induced fibrosis in rats. Thorax. 2003; 58(9):765–71.
- 116. MacVittie TJ, Farese AM, Jackson W. Defining the full therapeutic potential of recombinant growth factors in the post radiation-accident environment: the effect of supportive care plus administration of G-CSF. Health Phys. 2005; 89(5):546–55.
- 117. Van Dyk J, Keane TJ, Kan S, Rider WD, Fryer CJ. Radiation pneumonitis following large single dose irradiation: a reevaluation based on absolute dose to lung. Int J Radiat Oncol Biol Phys. 1981; 7(4):461–7.
- 118. Sampath S, Schultheiss TE, Wong J. Dose response and factors related to interstitial pneumonitis after bone marrow transplant. Int J Radiat Oncol Biol Phys. 2005; 63(3):876–84. Epub 2005/10/04.