A Trans-Agency Workshop on the Pathophysiology of Radiation-Induced Lung Injury

Authors: Cassatt, David R., Gorovets, Alex, Karimi-Shah, Banu, Roberts, Rosemary, Price, Paul W., et al.

Source: Radiation Research, 197(4) : 415-433

Published By: Radiation Research Society

URL: https://doi.org/10.1667/RADE-21-00127.1

RADIATION RESEARCH **197**, 415–433 (2022)
0033-7587/22 $15.00

DOI: 10.1667/RADE-21-00127.1

MEETING REPORT

A Trans-Agency Workshop on the Pathophysiology of Radiation-Induced Lung Injury

David R. Cassatt,[a,1] Alex Gorovets,[c] Banu Karimi-Shah,[c] Rosemary Roberts,[c] Paul W. Price,[b] Merriline M. Satyamitra,[a] Nushin Todd,[c] Sue-Jane Wang[c] and Libero Marzella[c]

[a] *Radiation and Nuclear Countermeasures Program (RNCP) and* [b] *Office of Regulatory Affairs, Division of Allergy, Immunology and Transplantation (DAIT), National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), Rockville, Maryland; and* [c] *Center for Drug Evaluation and Research (CDER), Food and Drug Administration (FDA), Silver Spring, Maryland*

Cassatt, D. R., Gorovets, A., Karimi-Shah, B., Roberts, R., Price, P. W., Satyamitra, M. M., Todd, N., Wang, S-J. and Marzella, L. Meeting Report: A Trans-Agency Workshop on the Pathophysiology of Radiation-Induced Lung Injury. *Radiat Res*. 197, 415–433 (2022).

Research and development of medical countermeasures (MCMs) for radiation-induced lung injury relies on the availability of animal models with well-characterized pathophysiology, allowing effective bridging to humans. To develop useful animal models, it is important to understand the clinical condition, advantages and limitations of individual models, and how to properly apply these models to demonstrate MCM efficacy. On March 20, 2019, a meeting sponsored by the Radiation and Nuclear Countermeasures Program (RNCP) within the National Institute of Allergy and Infectious Diseases (NIAID) brought together medical, scientific and regulatory communities, including academic and industry subject matter experts, and government stakeholders from the Food and Drug Administration (FDA) and the Biomedical Advanced Research and Development Authority (BARDA), to identify critical research gaps, discuss current clinical practices for various forms of pulmonary damage, and consider available animal models for radiation-induced lung injury.

INTRODUCTION

Exposure to ionizing radiation induces damage to vulnerable tissues throughout the body, in particular, tissues in which cells are rapidly dividing, such as bone marrow and the small intestine. Other organs can also sustain damage, either through direct cellular damage, or through delayed effects resulting from long-term injuries and processes like inflammation. Among the tissues sensitive to ionizing radiation is the lung, in which the inflammatory response can lead to pneumonitis and fibrosis (*1–3*). In humans, pneumonitis is observed at approximately 4–12 weeks postirradiation (*4*) and is characterized by lymphoid and myeloid cell infiltration and a subsequent inflammatory response that often presents as cough, chest pain, dyspnea and fever (*5, 6*). Lung fibrosis can occur months later, especially after localized high-dose radiation exposure that would induce lethal pneumonitis if delivered to the whole thorax. Fibrosis is caused by the remodeling of the extracellular matrix, through the activation of fibroblasts and buildup of collagen, resulting in reduced pulmonary function (*7*). Pneumonitis can resolve in time; however, fibrosis is considered to be chronic (*1, 8*).

Pulmonary radiation effects have been studied primarily in cancer patients undergoing thoracic radiation therapy, but worsening radiation-induced lung injury (RILI) has also been reported in individuals exposed to ionizing radiation after industrial accidents (*9, 10*). One important difference between the clinical and accidental exposure scenarios is the degree of exposure outside the thorax. Most cancer patients receive radiation directed to thoracic tumors, sparing as much heart and lung tissue as possible, whereas unintended exposures due to radiation incidents may result in the exposure of the whole volume of the lung and heart, as well as other sensitive tissues, such as the bone marrow, kidney and gastrointestinal (GI) tract. Damage to these tissues could have a profound effect on the radiation response of the lung, such as increased risk of pneumonitis from systemic inflammatory cell mobilization.

Because of the potential for long-term RILI after radiotherapy or exposure during a radiological or nuclear public health emergency, researchers have sought to develop treatments for RILI. The U.S. Government recognizes the threat of a large-scale exposure to ionizing radiation, through detonation of a nuclear device or

[1] Address for correspondence: DAIT, NIAID, NIH, 5601 Fishers Lane, Room 7A68, Bethesda, MD 20892; email: cassattd@niaid.nih.gov.

accidental release of ionizing radiation, and therefore has sought to develop treatments to counter the damaging effects of ionizing radiation on various bodily compartments. The scope of medical countermeasure (MCM) development includes biodosimetry (determination of absorbed radiation dose), animal model development, research to understand mechanisms of injury, and the development of efficacious mitigators and therapeutics (*11*).

MCM development under the U.S. Food and Drug Administration (FDA) Animal Rule (*12*) is built on the foundation of animal models that can simulate expected clinical outcomes, including the understanding of the pathophysiology and progression of injury and recovery mechanisms. Animal models have been shown to approximate human radiation injury progression, such as development of pneumonitis and fibrosis in the lung and have led to a better understanding of the mechanisms behind RILI. Because of advances made in understanding radiation injury medical management, and the licensure of leukocyte growth factors and a platelet-promoting drug that accelerate hematopoietic system recovery, victims of a radiological incident, who might have perished from the acute effects of radiation exposure, may now survive radiation exposures; however, as with most survivable health conditions, delayed effects are possible, and lung injury can present years after radiation exposure.

One limitation of the current animal models is that radiation exposure experienced in the clinic (thoracic or localized) may not simulate a large-scale, inhomogeneous body exposure following a radiological or nuclear incident. Researchers have attempted to adapt local exposure models to the needs of MCM development, moving from a whole-thorax lung irradiation (WTLI) model (*11, 13*) to a ''top-up'' exposure protocol, in which an immunosuppressive total-body irradiation (TBI) is coupled with an additional WTLI (*11*). More recently, investigators have begun investigating partial body irradiation (PBI) models in which 2.5 to 5% of the bone marrow is shielded (*14–18*), allowing for an even closer approximation to the expected human situation. These considerations are important in identifying appropriate models for study of MCMs to treat RILI.

With these issues in mind, the National Institute of Allergy and Infectious Disease (NIAID), in collaboration with the FDA and the Biomedical Advanced Research and Development Authority (BARDA) convened a two-day workshop entitled ''A Trans-Agency Workshop on the Pathophysiology of Radiation-Induced Lung Injury'' on March 20–21, 2019. Conference participants explored several aspects of RILI, including pathophysiology, mechanism of action, current treatments, animal models, biomarkers of lung injury, and the regulatory landscape for MCMs. This workshop, which was a follow-up to an earlier NIAID-sponsored workshop held in 2010 ''Animal Models and Medical Countermeasures Development for Radiation-Induced Lung Damage (*19*),'' brought together researchers, medical practitioners, program officials, and regulators to discuss facets of drug development, lung-specific biomarkers, and the path to licensure for products developed to mitigate radiation-induced injuries to the lungs. The wide-ranging participant background and experience allowed for fruitful discussions and a diversity of opinions. These discussions are meant as a guide; any definitive product pathway should be discussed with the FDA.

In Session I, Clinical Perspectives of Radiation-Induced Lung Injury, clinicians presented background on disease progression and the ways in which patients are evaluated and treated. In Session II, Regulatory Considerations for Drug Development for Radiation-Induced Lung Injury, FDA reviewers presented the agency's current thinking regarding laboratory animal models and requirements for approval of MCMs for RILI under the FDA Animal Rule. In Session III, Animal Models of Lung Injury, researchers presented their work on relevant animal models essential for understanding RILI and testing potential lung MCMs. In Session IV, Experiences from Current Drug Development for Radiation-Induced Lung Injury, representatives from various companies shared their experiences and challenges in the development of MCMs for RILI. Finally, in Session V, Biomarkers of Lung Injury, researchers presented ways in which lung injury and recovery can be tracked without relying on invasive or terminal procedures. The list of speakers is presented in Table 1. The following is a summary of the presentations and discussion.

SESSION I: CLINICAL PERSPECTIVES OF RADIATION-INDUCED LUNG INJURY

Standard-of-Care Considerations

The clinical perspective on radiation-induced lung injury and fibrosis provides insight into the pathophysiology of the disease and facilitates development of recapitulatory animal models for MCM testing. To that end, David Lederer presented an overview of acute and chronic lung injury induced by radiation therapy and discussed standards of care for patients. Radiation pneumonitis (RP) typically manifests 4 to 12 weeks after completion of radiotherapy as general respiratory symptoms, including dry cough, shortness of breath and fever (*20, 21*). The risk of a patient developing RP is heightened by concomitant chemotherapy, prior lung disease (e.g., chronic obstructive pulmonary disease), age and genetic predisposition. Other risk factors include the intensity and duration of radiation, as well as the modality by which it is delivered. The current standard of care for RP primarily involves analgesia and prednisone-induced immunosuppression. A significant proportion of patients with RP do not recover with treatment, and patients may develop chronic pulmonary fibrosis (PF) 6 to 12 months postirradiation (*22*). Pulmonary fibrosis progresses steadily for 3 to 7 years, and though the course of disease may be slowed by supplemental oxygen, pulmonary

TABLE 1
Workshop Speakers[a]

Name	Affiliation
Nicholas Dainiak, MD	Yale University, New Haven, CT
Joe GN ''Skip'' Garcia, MD	University of Arizona College of Medicine, Tucson, AZ
Barry Hart, PhD	Innovation Pathways, Palo Alto, CA
Isabel Lauren Jackson, PhD	University of Maryland School of Medicine, Baltimore, MD
Maureen Kane, PhD	University of Maryland School of Pharmacy, Baltimore, MD
Michael Kaytor, PhD	Humanetics Pharmaceuticals, Edina, MN
Daniel Krainak, PhD	FDA, Silver Spring, MD
Adebayo Laniyonu, PhD	FDA, Silver Spring, MD
David Lederer, MD	Columbia University, New York City, NY
Thomas MacVittie, PhD	University of Maryland School of Medicine, Baltimore, MD
John McManus	Currently Partner Therapeutics, Lexington, MA (Formerly Aeolus Pharmaceuticals, Mission Viejo, CA)
Meetha Medhora, PhD	Medical College of Wisconsin, Milwaukee, WI
Naresh Menon, PhD	ChromoLogic LLC, Monrovia, CA
Khalid Puthawala, MD	FDA, Silver Spring, MD
Stanley Stern, PhD	FDA, Silver Spring, MD
Karla Thrall, PhD	AltaSciences Preclinical Seattle, Everett, WA
Sue-Jane Wang, PhD	FDA, Silver Spring, MD
Kunyi Wu, PharmD	FDA, Silver Spring, MD

[a] Presenters and moderators were given an opportunity to review the report prior to submission.

rehabilitation, or tyrosine kinase inhibitors, these interventions are not curative (*23, 24*). The lack of effective treatments highlights the need for improved preclinical animal models for developing novel countermeasures.

Development of Therapeutic Strategies

The development of animal models for RILI is hampered by inconsistencies in animal and lung responses to radiation according to strain, sex and age of the animal, as well as the organ of interest, and the site and mode of irradiation (*25, 26*). Joe (Skip) Garcia described his group's experience with preclinical models of RILI, which they use to evaluate the efficacy of candidate therapeutic agents and identify potential molecular targets. Garcia and colleagues have shown that exposing young C5BL/6J mice to WTLI leads to increased levels of cytokines in the blood and bronchioalveolar lavage (BAL) fluid, as well as elevated amounts of protein and increased inflammatory cell presence in the BAL fluid. While exploring drugs that could be used to treat RILI, they found that simvastatin protected mice against acute lung injury and altered these phenotypic changes (*27*). Furthermore, stimulation of sphingosine 1-phosphate (S1P) receptor 1 by an S1P analog, TySIPonate, reduced vascular leakage by bolstering the vascular endothelial barrier (*28*). Additionally, studies in mice have identified a marked change in gene expression in response to WTLI.

Genomic analysis of preclinical models of RILI has yielded two molecular targets with therapeutic potential. The protein coded by *GADD45*α regulates Akt signaling by acting on the deubiquitinase UCHL1 and is implicated in the pathogenesis of acute respiratory distress syndrome (ARDS) (*29–32*). *NAMPT* encodes a B-cell maturation factor present in pulmonary endothelial cells whose expression is induced by stretching, trauma, radiation, and mechanical ventilation. Importantly, plasma levels are inversely correlated with survival in patients with ARDS. The extracellular form of NAMPT (eNAMPT) binds TLR4, triggering an inflammatory response and so acting as a damage-induced molecular marker. Additionally, single-nucleotide polymorphisms in *NAMPT* are linked to both the risk and mortality rate of ARDS. Antibodies directed against eNAMPT attenuated the severity of ARDS in a preclinical model (*33*). Moreover, in a model of RP such antibodies decreased BAL levels of inflammatory cells and proteins, as well as circulating levels of eNAMPT (*34*). *NAMPT*$^{+/-}$ mice with RP presented with lower levels of inflammatory cells and protein in BAL fluid compared to wild-type mice. Similar results were obtained in mice subjected to simulated blast-induced trauma, suggesting the therapeutic intervention potential of targeting NAMPT for lung injury of a variety of causes, including ionizing radiation.

Diagnosis and Medical Management of Lung Injury after Accidental Exposure to Radiation

Nicholas Dainiak presented on the diagnosis and medical management of lung injury after accidental exposure to radiation. The focus was on radioactive actinide isotopes, which are taken up within 2 h of exposure and incorporated into bone during its remodeling. Radioisotopes that are ingested are mostly coughed up or swallowed, but when taken up systemically, they may be transported by macrophages to the hilar lymph nodes. Approximately 5% of the <5 μm radionuclide particles measured in a nasal swab, which is the standard dose-estimation method, reach the alveoli where they can persist for ≥ 100 days. The level of exposure is compared to established limits to evaluate the clinical impact. The lung (''equivalent'') dose is measured in millisieverts and can be converted to the whole body

(''effective'') dose by multiplying by 0.12, allowing patient monitoring through mathematical modeling.

After first medically stabilizing the patient, high-activity insoluble radionuclides can be removed from the lung by BAL; however, BAL may be improved using surfactants. To explore the effectiveness of surfactant use, large-animal efficacy studies are needed. Radionuclide-specific decorporation therapy, using an ion exchange resin and extracorporeal dialysis or chelator (e.g., diethylenetriaminepentaacetic acid, DTPA), requires continued use for several years to be effective at removing radionuclides from bone. Radiation pneumonitis leads to heavy, edematous, bleeding lungs with septal thickening, interalveolar fibroblast proliferation and lymphocytic infiltration (*35, 36*). The resulting Th1 response leads to vasodilation, hypotension, and maldistribution of blood flow, resulting in systemic inflammatory response syndrome and ultimately multiple organ failure (*37, 38*). New therapies that restore the immune balance by promoting a compensatory Th2 response and/or correct the maldistribution of blood flow are needed.

The criticality accident that occurred at a uranium reprocessing facility at Tokaimura, Japan in 1999 is a real-world example of the health effects of radiation exposure (*10*). Addition of excess uranyl acetate solution to a tank resulted in a criticality event and exposure of three workers to intense neutron and gamma-ray radiation. The two workers closest to the tank immediately experienced pain, nausea, and difficulty breathing, and died after several months of treatment in the hospital. They were estimated to have received doses of 13.9 and 7.4 Gy; autopsies showed pulmonary edema, hemorrhage, congestion and pneumonia. The third worker, who was behind a desk approximately 4 m from the tank, survived. The severity and complexity of the pulmonary injuries caused by this and other accidents (*39*) highlights the need for novel lung-targeted therapeutics.

Quantitation of Lung Injury Using Imaging

To establish the efficacy of new therapeutic interventions in animal models, it is crucial to be able to quantitatively evaluate changes in the severity of RILI over time. Daniel Krainak provided an overview of imaging modalities for this purpose. The ideal imaging modality for RILI would yield quantitative data and enable simultaneous assessment of the dose distribution and would not affect the dose distribution of the injurious radionuclide. Such a modality would additionally be predictive of human pathophysiology and therapeutic efficacy.

Among the available quantitative imaging modalities, e.g., two-dimensional X-ray, ultrasound, and magnetic resonance-based techniques, computed tomography (CT) has high resolution, can provide four-dimensional data, and is the most widely available (*40*). Data generated by these imaging modalities can be analyzed densitometrically [monitoring of the density of a region of interest (ROI)] or volumetrically (monitoring of the volume of a ROI; the data can be compared to spirometry findings). The ROI may be the entirety of one or both lungs (global analysis), or a portion thereof (regional analysis); the latter allows assessment of local effects. The aim is to measure the change objectively and quantitatively over time in a parameter relevant to the pathogenesis of RILI. Densitometric analysis is based on signal attenuation by tissue; air shows low attenuation and bone exhibits high attenuation (*41–43*). Thus, densitometry may be used to assess the severity of pulmonary fibrosis; the greater the density on imaging, the more severe the fibrosis. The density data may additionally be correlated with measures of lung function such as the forced expiratory volume in 1 s (*44, 45*).

For a quantitative imaging modality to be useful, the magnitude of the effect must be sufficiently greater than any uncertainty in the measurement likely to be encountered in clinical practice. The reliability of any quantitative imaging modality is influenced by a host of factors related to the patient (e.g., variation in physiology and anatomy), the means of acquisition (e.g., protocol, slice thickness, reconstruction), and the measurement method (e.g., degree of automation, algorithm, software version). The reliability of imaging data is evaluated in three steps: Establishment of analytical performance, determination of acceptability, and assessment of clinical relevance. Validation should focus on the measurement actually used among the many generated by imaging modalities.

SESSION II: REGULATORY CONSIDERATIONS FOR DRUG DEVELOPMENT FOR LUNG RADIATION INJURY

Like other investigational drugs, products developed for the treatment of RILI must go through the FDA's approval process. However, due to the nature of the condition, these products cannot be tested for efficacy in standard clinical trials, as it would be unethical and unfeasible to carry them out. Instead, sponsors are encouraged to pursue FDA approval under the Animal Rule, which allows product development to proceed in the absence of human efficacy trials, so long as adequate and well-controlled animal efficacy studies have been conducted in an appropriate animal model to establish that the drug is reasonably likely to benefit humans affected by the injury (*12*).

Regulatory Review Issues in Dosimetry Associated with Radiation-Induced Lung Injury

Under the FDA Animal Rule, careful consideration must be given to ensure that the selected radiation injury model is robust and closely translatable to the human condition. Stanley Stern discussed the challenges and nuances associated with modeling radiation injury in animals. For the purposes of designing a valid injury model, an important distinction should be made between radiation ''dosimetry''

and ''dosing''. While ''dosimetry'' refers to the measurement of radiation absorbed within a subject, ''dosing'' refers simply to the act of exposing the subject to a known amount of radiation. This distinction becomes critical when comparing radiation injury between animals and humans, as the same degree of exposure may not necessarily result in comparable damage among different species. To ensure that the selected exposure model is reproducible and representative of a mass casualty scenario, several factors should be controlled, including particle type, energy spectra, and dosimetry curves between species. Additionally, researchers should ensure that their animal exposure model generates a lung injury that is qualitatively similar to that observed in diseased humans (i.e., biological similarity).

In the event of a public health emergency from a nuclear detonation, the full extent of injury to an individual could go well beyond radiation, to include a concert of environmental insults that are likely to interact, thus making real-world lung injuries difficult to model. These factors could include injuries from the initial blast (e.g., skin wounds and overpressure to the lungs and eardrums), blunt force trauma, as well as thermal radiation burns to the exterior of the body, all of which require more study (*46*). Due to the complexity of the issue, it would be more prudent to first focus drug development on radiation alone rather than attempting to model radiation plus blast injuries.

To give insight into current models, the work of Karla Thrall and colleagues, in which rhesus macaques were exposed to WTLI using a linear accelerator (LINAC) device commonly used for radiation therapy in cancer patients, was discussed (*13, 47*). The LINAC delivered ~10 Gy at a rate of 1.0 Gy/min, resulting in a lethal dose (LD) in approximately one half of the animals (LD_{50}). It should be noted that the dose rate delivered by LINAC devices depends on the filter applied (*48*). In another study, Marks and colleagues (*49*) studied the probability of RP developing in cancer patients treated with partial-lung irradiation. The early manifestation of RP includes difficulty breathing, cough and fever, as well as inflammation of the lungs, while more advanced manifestations include fibrosis and infection (*50*). Surprisingly, their results showed no clear relationship between radiation dose and RP development, exemplifying the need to better understand causes of the disease. Furthermore, physicians have yet to agree upon a standardized grading system for assessing the severity of RP. In closing, use of LINAC devices to model high-dose irradiation in animals should be done, when possible. Drug developers are also strongly encouraged to factor dosimetry (i.e., absorbed dose) into their proposed models.

Clinical Pharmacology Considerations for Products Developed for Radiation-Induced Lung Injury under the Animal Rule

In the absence of human efficacy trials, the correct dosage for a novel lung injury drug must be formulated based on a combination of animal research and pre-existing knowledge (*12, 51*). Kunyi Wu presented an overview of pharmacological methods used to select an effective dose for treating humans with lung radiation injury. For drugs that are administered systemically, two main approaches are typically used for dose selection: pharmacokinetics (PK) and pharmacodynamics (PD). A PK analysis is included in most drug applications. The PK approach is applicable when: 1. the dose-response relationship has been established in animals; 2. dose response has not yet been established in humans; and 3. based on the drug's mechanism of action (MOA), it is reasonable to assume that dose response will be comparable among species. Using the PK approach, a dose determination is made based on animal testing (drug absorption, metabolism, etc.) in both healthy and diseased animals, as well as prior knowledge (MOA, safety profile). When using a PK approach to select a dose for humans, the selected dose should ideally be higher than the effective dose used in animals, to minimize the risk of delivering a sub-therapeutic dosage.

In contrast to the PK approach, PD hinges on the identification of a biological marker that corresponds to a desired clinical outcome. The biological marker (PD marker) is then used as a proxy to estimate the dose required to reach the desired clinical outcome. This way, a human dose can be estimated based on the dose that results in similar PD marker levels to those achieved in successfully treated animals. To apply the PD approach, the PD marker must be directly related to the drug's MOA and must be closely related to the desired clinical outcome. An example is the supplemental application for pegfilgrastim (Neulasta®), a drug normally indicated for patients with neutropenia (low neutrophil levels) associated with bone marrow suppressing chemotherapy. For the pegfilgrastim hematopoietic acute radiation syndrome (ARS) indication, a PD approach was possible because the drug's MOA is known to directly impact neutrophils, and animal studies showed that shortening the duration of neutropenia (the PD marker) raises the probability of survival (the desired clinical outcome). Thus, the final dose regimen of pegfilgrastim for ARS in irradiated humans was selected for its ability to shorten the duration of neutropenia most significantly.

An important caveat for PK and PD is that while both methods can be used to evaluate the effectiveness of systemic drugs, efficacy of locally acting drugs cannot be determined using PK, which requires systemic testing. In addition, due to the effects of radiation on the GI tract, oral dosages should not be assumed to be equivalent between healthy and radiation-exposed subjects. To this point, matters may be complicated by the fact that radiation-induced damage to the GI tract changes over the course of the injury; therefore, this represents an important consideration for PK studies that can be properly addressed with multi-dose testing.

Nonclinical Considerations for Product Development Under the Animal Rule

Adebayo Laniyonu provided a framework for lung injury product development under the FDA Animal Rule and discussed regulatory and research considerations for drug approval. Approval under the Animal Rule can proceed if certain milestones are met during animal testing. Some of the more crucial conditions are as follows: Toxicology for the drug must be well established, efficacy must be demonstrated in more than one animal species, the animal study end point must be clearly related to human benefit, and PK/PD analysis must allow selection of an effective dose in humans. As always, studies should be conducted in accordance with the U.S. Animal Welfare Act and the Public Health Safety (PHS) policy on the humane care and use of laboratory animals.[2] It is also important to note that Animal Rule applications are eligible for various forms of expedited FDA review such as ''fast track'' and ''priority review.''

On the research front, while human efficacy data collected under Good Clinical Practice (GCP) standards are not required for applications under the Animal Rule, data collected from animal studies will be held to the same standard of quality. Therefore, experiments should be well designed (i.e., controlled, randomized, blinded), should model the human condition as closely as possible, and data quality controls and assurances must be in place. For example, it would be wise to mimic the supportive care (e.g., inclusion of antimicrobials, leukocyte growth factors, etc.) that humans exposed to radiation would be expected to receive. Furthermore, animal studies of RILI should be designed to encompass the entire natural course of the disease, as it has yet to be fully characterized. Future research should also approach lung radiation injury as a complex multi-organ disease, rather than a disease of the lungs alone. PBI models (with some degree of bone marrow sparing) are a good first step towards inducing lung injury, as well as allowing the full evolution of the multi-organ disease (e.g., hematopoietic (H)- and GI-ARS) to unfold in animal models.

There are challenges in the development of models and in the conduct of nonhuman primate (NHP) survival studies for lung MCMs under the Animal Rule; therefore, alternatives such as more extensive survival studies in small animals, and exploration of efficacy end points such as pulmonary function could be considered by the FDA. In any case, sponsors should discuss specific animal models and primary and secondary end points with the FDA.

SESSION III: ANIMAL MODELS OF LUNG INJURY

A key component of MCM testing is choosing appropriate animal models. In practice, researchers have chosen rodents and large animals such as NHPs and a variety of radiation exposure methods. There are several challenges in developing animal models of lung injury: 1. radiation exposures that induce lung injury are well above levels that cause fatal bone marrow injury; 2. the length of time between radiation exposure and mortality (the accepted end point for studies under the FDA Animal Rule) may be many months; and 3. the animal injury and its medical management may not mimic the clinical experience.

Researchers have developed animal models to dampen the lethal effects of H-ARS by shielding various amounts of bone marrow, through use of WTLI (*52, 53*), ''top-up'' exposure protocol (TBI at a sublethal exposure level followed by WTLI) (*11*) or shielding of between 2.5% and 5% of the bone marrow (*14–18*), thereby sparing enough bone marrow to provide adequate protection against lethality from infection and hemorrhage. Researchers are also examining secondary end points to determine the extent of lung injury and recovery, well before mortality is expected. Examination of these secondary end points allows researchers to determine how well the animal model acts as a surrogate for the human lung injury. The speakers in this session addressed these aspects of their selected large and small animal models, as well as the gaps that exist in current development.

Mouse Models of Radiation Pneumonitis/Fibrosis for Medical Countermeasure Development in the Context of the FDA Animal Rule

Isabel (Lauren) Jackson presented data from her group's development of a mouse WTLI model, using an X-ray source. Mouse studies have shown that the two major pathologies often observed after radiation exposure (pneumonitis and fibrosis) are not necessarily linked, and studies have demonstrated that different mouse strains have varied susceptibilities to pneumonitis and fibrosis (*54–56*). The C57BL/6J strain, commonly used for murine radiation studies, tended to develop fibrotic lungs, with late pleural effusions; CBA/J mice also tended to develop pneumonitis and pleural effusions; and in contrast, C57L/J mice developed early pneumonitis and late fibrosis, which is a progression more commonly observed in humans (*26, 57*). Mortality tended to take approximately 180 days to reach equilibrium in this C57L/J strain (*58*). The radiation exposures that induced mortality in this strain tended to be close to exposures that caused similar mortality in humans, as well as in NHP WTLI models.

Other non-invasive secondary lung parameters include lung density, as measured using CT scans, and enhanced pause (Penh), which measures airway constriction and appears to be a reliable predictor of mortality (*53, 57*). Assessment of the expression of genes linked to tissue damage and repair pathways may also be a promising way

[2] https://olaw.nih.gov/policies-laws/phs-policy.htm.

of following progression and mechanisms of lung injury. Other secondary indicators of lung damage include increased lung mass, wet lung weights, and inflammation/collagen deposition as revealed histologically. Many of these parameters are measured at necropsy, limiting their usefulness in following disease progression; however, these measurements are important for model development to show similarity between tissue injuries noted in various mouse strains and what the Jackson lab describes as being observed in human RILI. For example, pleural effusions observed in CBA/J and C57BL/6J mice are described as not being seen in human radiation-induced lung injury (*57*), whereas in the C57L/J strain, progression and exposure thresholds of pneumonitis and fibrosis are more similar to what is observed clinically. These secondary end points are essential in the understanding of the pathogenesis of radiation injury and provide context for the mortality data and the effects of a given MCM on lung damage and recovery.

Whole-Thoracic Lung Irradiation in NHPs

Karla Thrall presented data from an NHP model of WTLI. To build on earlier studies conducted at the University of Maryland School of Medicine (UMSOM) using male monkeys, female rhesus macaques received WTLI at various levels via LINAC and observed for 180 days. Two important aspects of studies in larger animals are that additional blood draws can be taken, allowing for additional minimally invasive monitoring, and that Institutional Animal Use and Care Committees (IACUCs) may require additional medical management, such as administration of dexamethasone to animals in respiratory distress (which was done in this study). The institutional lethality curve for the female monkeys was similar to that of male animals from the UMSOM study, and there was no significant difference between the dose-response relationships, LD values or slopes (*13, 47*). In the Thrall study, measurements of pulmonary function were taken, which included non-sedated respiratory rate (NSRR), heart rate, blood gas and CT scans. Of these, CT scans and NSRR track disease progression. The CT scans are also useful in measuring the extent of lung damage compared to baseline and the likelihood that an animal will eventually meet euthanasia criteria. The researchers also found that courses of dexamethasone reduced respiratory distress, although this treatment was ineffective against severe tissue damage. Histopathology performed at time of euthanasia showed that non-survivors had a higher number of severe histopathological findings (*47*). In all, this study demonstrated the robustness of the NHP WTLI model and the usefulness of secondary measurements. Other measurements beyond those listed above could include echocardiogram, pulse oximetry, and more frequent heart rate monitoring.

Rat Model of Radiation-Induced Lung Injury: Relevance to the NIAID Radiation Countermeasure Program

Because of concerns that WTLI might not represent the full spectrum of the contributors to lung damage, researchers have investigated rodent PBI models in which only a portion of the bone marrow (generally 7.5% or less) is shielded. Meetha Medhora presented published data from her laboratory's rat WTLI model (*59*) as well as her efforts to replicate mortality and histological findings in the PBI model, with approximately 7.5% of the bone marrow in one of the hind limbs shielded from radiation (*14*). When these shielded WAG/RijCmcr rats received 13 Gy X-ray irradiation, there was substantial mortality in the first 21 days that could be significantly ameliorated by medical management consisting of early (days 3–7), subcutaneous injection of saline and antibiotics (enrofloxacin given days 2–28). One difference from their earlier WTLI model was mortality due to late-stage renal failure when the kidneys were in the radiation field. One aspect noted in both the WTLI and PBI/BM7.5 models is that pneumonitis is a function of both lung and heart injury (*60, 61*). Pneumonitis is similar between both models and is associated with pleural effusions. They also studied special populations (pediatric and geriatric) and found increased mortality in juveniles, while older rats were relatively resistant to pneumonitis compared to young adults (*62*). The group has also evaluated MCMs beyond supportive care and found that survival was improved with the angiotensin converting enzyme (ACE) inhibitor lisinopril. ACE inhibitors also attenuated pulmonary fibrosis after WTLI (*59*); however, since pneumonitis after doses of 13–15 Gy to the whole thorax is lethal, it is not possible to observe robust pulmonary fibrosis that occurs later in time after higher doses of radiation to partial volumes of the lung. In addition to mortality, other indications of lung and multi-organ injury were measured. These indicators include breathing interval (i.e., the inverse of breathing rate, which accounts for animal attrition due to RILI euthanasia criteria), body weight and CT scans. As expected, untreated rats showed the most dramatic decreases in breathing interval and body weight, and CT scans revealed increases in lung density due to radiation. Other non-invasive imaging could include single photon emission CT, optical imaging, and magnetic resonance imaging (MRI), which can measure the level of lung perfusion (*63*).

Acute Radiation-Induced Lung Injury: Linking ARS and Delayed Effects of Acute Radiation Exposure (DEARE), Concurrent Multiple Organ Injury (MOI), and MCMs using an NHP Research Platform

The rhesus macaque (*Macaca mulatta*) has been an important large animal model of radiation injury. The development of the NHP lung injury model in the laboratory of Thomas MacVittie arose from long-term DEARE studies, which included prolonged GI injury and

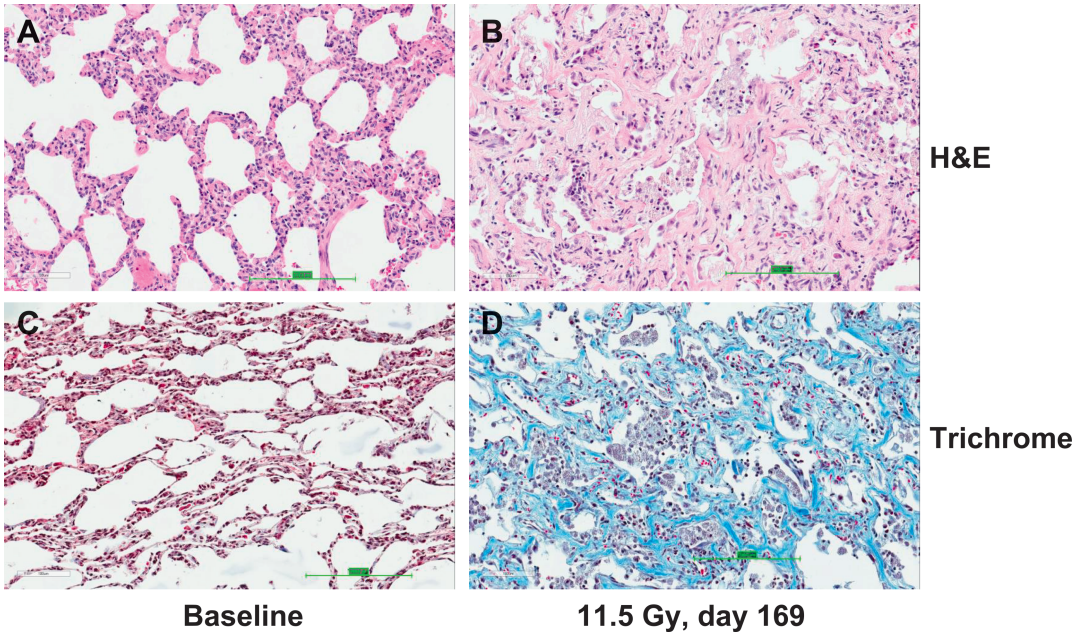

FIG. 1. The lungs of rhesus macaques collected from nonirradiated animals (panels A and C) and animals that received 11.5 Gy PBI with 5% of bone marrow sparing (panels B and D), collected 169 days postirradiation. Samples were stained with hematoxylin and eosin stain (panels A and B) or Masson's trichrome, indicating collagen deposition (panels C and D). This figure was presented at the meeting.

recovery kinetics (*18*), and acute and delayed kidney injury (*64*). This research platform allows for the examination of lung injury in the context of multi-organ injury, estimation of the radiation dose distribution to an organ volume, and characterization of the prescribed, descriptive dose to the specific dose delivered to the organ. Prado *et al*. used three-dimensional dose calculations combined with corrections for tissue heterogeneity to estimate doses to the lung, kidney and heart relative to the prescribed dose delivered to midline tissue (*65, 66*). Data presented at the workshop included the primary end point (survival) as well as secondary end points, such as clinical, cellular, radiographic, and histological indices, non-invasive NSRR, oxygen saturation (SpO_2), and CT scans, which provided evidence of pneumonitis, fibrosis, and pleural and pericardial effusions, and complete histology from animals euthanized for cause at predetermined time points (Fig. 1). The PBI/BM-sparing model with approximately 5% or 2.5% sparing (PBI/BM5 and PBI/BM2.5) was tested and was found to differentiate between combined H-/GI-ARS and acute kidney injury, which occur within the first 50 days, and DEARE, characterized by lung, chronic kidney and heart injury, which generally occurs after the 90-day latent period (*15, 17, 64, 67*). One vital component was the type of medical management provided. In the NHP, antibiotics, fluids, and nutritional support based on clinical triggers were augmented with dexamethasone in animals experiencing lung distress. Dexamethasone appeared to extend survival time and mitigate pleural effusion. They found that with PBI/BM5 shielding and under their medical management protocol, 10 or 11 Gy irradiation from LINAC provided enough animals that had survived H-/GI-ARS, yet experienced mortality from lung injury (*15*). They also demonstrated that CT scans could show ongoing pneumonitis/fibrosis and pleural effusions that could be scored on a severity scale or percentage of lung damaged (Fig. 2). Though pleural effusions were not observed in C57L/J mice, they occurred in irradiated NHPs and WAG/RijCmcr rats. NSRR is another key indication of lung injury, and the initial increase in NSRR determined the time-to-onset of overt lung injury and major signs of morbidity and mortality. Notable differences are also seen between animals that survived compared to non-survivors. In an MCM efficacy study with AEOL10150 (described later) (*52*), improvements in survival and SpO_2 were observed. These non-invasive measurements of lung function were confirmed histologically, and the tissues examined at the timed euthanasia points confirmed the progression of lung damage. These data also showed similarities in lung injury after WTLI compared to PBI/BM2.5 or PBI/BM5, suggesting that tissue damage outside of the lung may not have a great influence over lung injury (*15*). This observation is perhaps counterintuitive since one would expect that the early burst of cytokines and chemokines and damage to the GI tract and bone marrow would have a greater influence on damage to the lung.

In summary, the presentations in this session and subsequent discussions uncovered the challenges in modeling RILI. Minimal shielding (BM7.5 or lower, depending on species) allows for the contribution of other organ

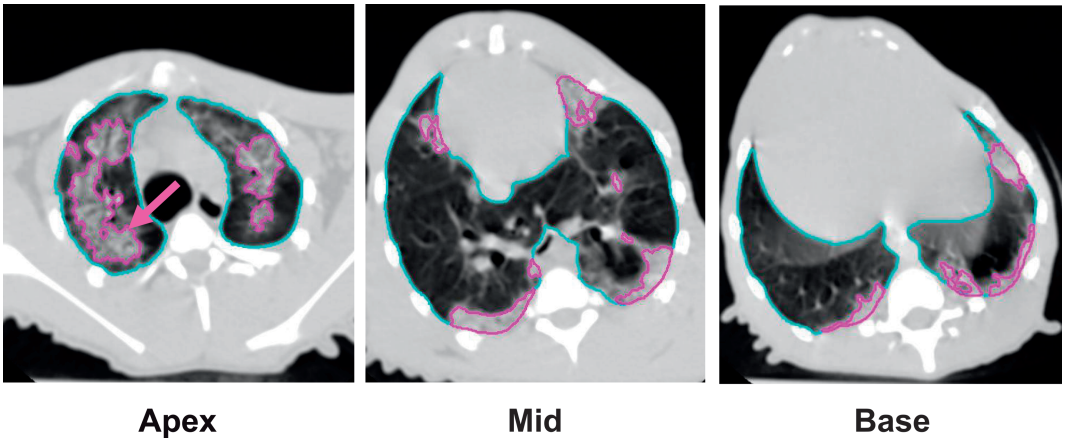

FIG. 2. Radiographs from rhesus macaques that received 11.5 Gy PBI with 5% of bone marrow sparing. Radiographs were taken 120 days postirradiation. Lungs show evidence of damage. These scans allow for the determination of total lung volume (blue outline) and percentage with lung damage (red outlines and arrow). This figure was presented at the meeting.

systems (including the heart in WTLI and kidney and heart in the bone marrow shielding models) to lung injury, and from a concept of operations perspective, is close to what an actual exposure could look like. The disease progression after irradiation is important to study to understand pathologies that can cause death (always a challenge since animal studies require euthanasia criteria that comply with accepted care and use standards) and the relationship, if any, between the inflammatory-based outcome, pneumonitis, and the repair-based outcome, fibrosis. It is possible that medical management of these multiple injuries could require a poly-pharmacy approach, or that some treatments, such as the ACE inhibitor enalapril, could protect against multiple injuries (*68*).

In addition, reproducibility among different sites is important. At the very least, facilities should use standard dosimetry and dosage-determining methods. When establishing a useful mortality dose-response relationship it is also important to monitor severity of lung injury through non-invasive measurements such as CT scan, breathing rate observations, and SpO_2. These clinical, cellular, and radiographic secondary end points allow for the tracking of disease progression and recovery; however, measurements and supportive care elements commonly used for patients, such as spirometry and administration of oxygen, are difficult to perform in animals. Additional biomarker measurements based on proteomics and metabolomics (see Session IV), could aid in tracking tissue damage as well. Lastly, there are questions about consideration of comorbidities and age. Pediatric and geriatric rat data (*62*) were presented at this workshop; however, these studies are difficult to perform in large animals because of much longer lifespans. Any consideration of comorbidities would depend on the availability of appropriate animal models.

SESSION IV: EXPERIENCES FROM CURRENT DRUG DEVELOPMENT FOR RADIATION-INDUCED LUNG INJURY

Development of AEOL 10150 as a Lung MCM

John McManus described his experience developing AEOL 10150 as a lung MCM. AEOL 10150 is a small molecular weight metalloporphyrin antioxidant with superoxide dismutase and catalase activity developed by Irwin Fridovich (Duke University) and Brian Day (National Jewish Health) to mimic the body's antioxidant defense systems. Drug development was first initiated in 2007, demonstrating significant activity against radiation, sulfur mustard and soman gas. AEOL 10150 was well tolerated in healthy human subjects.

In considering product development lessons learned, McManus discussed how the Animal Rule is not necessarily a shortcut to FDA approval or licensure and increased revenue. Depending on the status, development of a new MCM under the FDA Animal Rule can sometimes be more time-consuming, high risk, and more expensive compared to the traditional drug approval route. The FDA invites sponsors to hold dialogues early and often with the regulatory partners. Therefore, the FDA Office of Specialty Medicine-Division of Imaging and Radiation Medicine (FDA-DIRM) is willing to collaborate in defining models/species, resolving clinical holds, and providing input in the design of safety trials in healthy volunteers. FDA-DIRM also encourages testing of MCMs in patients who may benefit from the product. Sponsors should define, integrate and prioritize a primary commercial indication from the beginning of the development process. Any data obtained from clinical studies, especially those in related conditions (e.g., patients receiving radiation therapy) will be useful in determining safety, drug dose equivalent and possible outcome. Another aspect of drug development that should

be considered early are the chemistry, manufacturing and controls (CMC), which are often underappreciated by sponsors but represent a significant investment for both MCM sponsors and funding agencies. For example, a reformulation of AEOL 10150 that greatly reduced the cost of goods resulted in a development delay.

Perhaps most relevant to this workshop are laboratory animal model considerations. Mouse models are important for learning, while NHP models may also be required for FDA approval. Most preliminary studies of AEOL 10150 were conducted in a WTLI model in mice (*69*) and NHPs (*52, 70*). AEOL 10150 increased survival and reduced WTLI-induced lung damage and related biomarkers of damage in three different species: mouse (CBA, C57BL/6, and C57L/J strains) (*71*), rats (*72*), and NHPs (*70*). These studies demonstrated an association between radiation injury to the lung and response by AEOL 10150, suggesting that survival is driven by addressing the underlying radiation-induced mechanism of injury in these animals. These efficacy studies were performed as the animal models were being developed; given the urgency for approved MCMs to respond to the aftermath of a radiological event, there is a need to balance developing and refining animal models while advancing MCMs for approval.

Clinical experiences with radiation therapy, injury and resolution can provide key insights into drug development. Given the similar continuum of latency, fibrosis and pneumonitis in NHP and clinical subjects, lung cancer radiotherapy patients represent a human population for safety data and natural history. The molecular mechanisms of RILI span immediate and delayed cycles of apoptosis, inflammatory processes accompanied by vascular dysfunction, and tissue hypoxia that culminates in inflammation (pneumonitis) and fibrosis (*73*). Targeting inflammation, fibrosis, oxidative stress and vascular dysfunction that may result in multi-threat solutions and systemic efficacy, rather than focusing on a single organ alone, can be a better approach for MCM development. The identification of common pathways supports treating systemic injury, rather than focusing on systems, organs, or ARS/DEARE, and reveals models/products that can reduce development time, risk and cost. Clinical and research experience in other lung injuries, irrespective of insult such as chemicals, radiation, disease and trauma, can also provide important knowledge and potential solutions. Furthermore, lung injury is complicated, and a single MCM may not be sufficient in mitigating or treating the sequelae; therefore, a polypharmacy approach may be more realistic.

Development of BIO 300 as an MCM for Lung-DEARE

Michael Kaytor described Humanetics Corporation's experience with BIO 300, which is a proprietary, aqueous, nano-suspension, and a synthetic bioavailable form of genistein suitable for oral (PO), intramuscular (IM) or subcutaneous (SC) administration. The drug is shelf stable (three years at 15–30°C), with a good safety profile. Mechanistically, BIO 300 is a radiation modulator that acts as an agonist of estrogen receptor beta (ERβ). Receptor binding causes dimerization and migration to the nucleus, thereby modulating transcription either through co-activation of transcription modulators or direct DNA binding of transcription factors to ERβ elements or ER-like elements (*74*). Activated ERβ initiates a cellular program of increased cellular checkpoints and DNA repair proteins, and decreased proliferation and inflammation. Initially, BIO 300 was studied as a radioprotectant, and increased survival when administered PO, IM (*75*) or SC (*76*) prior to TBI in mice. Subsequently, BIO 300 mitigated radiation-induced lethality in WTLI C57L/J mice (*77*) with a strong correlation with the sphingomyelin lung biomarkers and survival efficacy (*78*). BIO 300 also reduced lung injury in a murine lung cancer xenograft model. BIO 300 safety, PK, and efficacy (prevention of grade 3+ pneumonitis, fibrosis and esophagitis) have been studied in a phase 1b/2a lung cancer trial in patients with non-small cell lung cancer. PD markers related to lung function were also evaluated.

Based on these data, Humanetics approached the FDA for study design concurrence on a protocol to test efficacy of BIO 300 in a randomized, blinded, placebo-controlled study using a WTLI NHP model, with 180-day survival as the primary end point, and body weights, blood indices, lung function and histology as secondary end points. Advice given by the FDA included:

- Drug efficacy must be understood in real-world, mass casualty scenarios and in the context of standard of care, including leukocyte growth factors.
- The drug development pathway should start with well-powered survival studies in small animals, followed by large animal studies with clinically relevant functional end points.
- Studies must include a measurable strong anchor of disease measure that is translatable between animals and humans.
- Relevant efficacy measures from human studies (in closely related indications) would be viewed as supportive and persuasive.

The FDA communicated that, since lung-DEARE is likely due to a multisystemic effect resulting from radiation exposure, a model that reflects this natural history of disease will be required to fulfill the Animal Rule requirements. Therefore, Humanetics redesigned the NHP study to incorporate a PBI/BM5 model, with all animals receiving Neupogen and one half also receiving BIO 300; the primary end point was survival and the secondary end points were lung function and histology. Exploratory end points examining BIO 300 PK/PD and effect of BIO 300 on multi-organ injury over the time-course of the study were also included.

The main challenge to lung-DEARE MCM development is the need for validated delayed injury models. Some issues

are inherent to model development due to the differences in medical management in rodents compared to NHPs and humans. For instance, use of antibiotics in the murine model shows no significant impact on survival (*79*), calling to question the advantage in including antibiotics in this model. On the other hand, for medical management of irradiated rats, NHPs and humans, antibiotics are recommended. Another non-clinical consideration is the trigger-to-treat criterion versus scheduled treatments. Assessment of clinically relevant end points for lung-DEARE is challenging due to limited functional tests, species-specific respiratory physiology, and the fact that CT scans might not be congruent with functional deficits (*80*). Furthermore, the extent of lung injury and natural history of lung damage varies with selection of mouse strains (*71*). Finally, one must determine when and at what dose leukocyte growth factors, which are considered the current human standard of care for ARS, will be given in these models.

Necessary animal end points, such as reduction in mortality and major morbidities, require validated models that align with the expected human condition. Currently, there are no validated rodent models to establish these parameters. Although there are long-term mouse studies in which the GI system was studied (*81, 82*), most mouse PBI model data span 30–60 days postirradiation; therefore, efforts to more fully establish the long-term PBI lung-DEARE models in rats and mice are ongoing. An aspect mentioned before is the difference in radiosensitivity and lung pathologies based on strain (*26, 83*). Based on WTLI data, Humanetics selected the C57L/J mouse strain, since it is susceptible to both pneumonitis and fibrosis, with a radiation-induced pathology similar to humans (*26, 57*), as described above. To demonstrate model relevance, developers need to demonstrate that animals transition through H- and GI-ARS using blood indices, clinical chemistry, biomarkers, and crypt and BM histopathology. Given the high rate of mortality of these pathologies, a major concern with the PBI model is having enough animals survive the acute radiation phase that can then develop lung-DEARE in sufficient numbers to obtain statistically significant results.

Development of IPW-5371 as a Lung-DEARE MCM

Barry Hart (Innovation Pathways) described a class of TGFβRI inhibitors, which are orally bioavailable, chemically stable, suitable for once-a-day dosing, have scalable synthesis, and are candidates for mitigation of radiation-induced fibrosis and idiopathic pulmonary fibrosis (IPF). TGFβ is the master switch for fibrosis and is implicated in systemic radiation injury involving multiple organs. In the lung specifically, TGFβ signaling is persistently increased after acute irradiation, resulting in fibrosis and lung function deficits that are characteristic of the delayed sequelae of exposure. TGFβ inhibitors mitigate lung fibrosis, increase lung function, and improve survival of animals exposed to thoracic radiation (*84, 85*). IPW-5371 is well-suited to be a lung-DEARE MCM, given that both the PO and IV routes of drug administration result in a dose-dependent increase of the MCM in plasma and the lungs, with drug concentration being higher in the lung. In a preliminary WTLI ''top-up'' exposure protocol using C57L/J mice, IPW-5371 administered orally for 6 or 20 weeks, starting 24 h postirradiation, increased 180-day survival when given for 20 weeks, with concomitant improvement of lung and cardiac function and reduced fibrosis (*86*). At the time the workshop was conducted, data for IPW-5371 indicated that there was activity against lung-DEARE; however, optimization studies are required to determine the fully effective drug dose and administration schedule. IPW-5371 also reduced cardiac effects and biomarkers of radiation injury in the liver and intestine weeks and months postirradiation, which adds to the multi-organ efficacy of the MCM.

To advance the MCM, Innovation Pathways had originally proposed testing IPW-5371 in mice and NHPs in pilot and pivotal WTLI models, in combination with supportive care and G-CSF; however, consultation with the FDA led to the following guidance:

- Models that seek to isolate the lung injury are not considered relevant to the multi-organ injury expected in a radiological disaster scenario, and their use is not recommended. While use of the WTLI model and ''top-up'' exposure protocol may be acceptable for early proof-of-concept studies, their use in the NHP is not recommended.
- Understanding MOA and PD markers is critical.
- Bioavailability concerns should be addressed.
- Standard therapy for H-ARS (e.g., a form of G-CSF or GM-CSF) should be included as part of supportive care.

This guidance resulted in the company changing course to conduct efficacy in mouse, rat and NHP PBI/BM-sparing models, in pilot and pivotal studies, using survival as the primary end point while also examining secondary end points associated with key signs of morbidity and lung insufficiency. PK and PD will be repeated in rats, and a radiation dose-response relationship with supportive care (including dexamethasone) is being developed to establish the model. Despite significant delays and funding challenges, the IPW-5371 development is now proceeding, following guidance from the regulatory agency and discussions with collaborators.

Development of Available Therapeutics for Analogous Pulmonary Conditions

Khalid Puthawala brought the focus from animal models and new MCMs to the analogous human pulmonary injuries and current treatments. Two pulmonary condition analogous to RILI or radiation therapy-induced lung injury (RTLI) are IPF and systemic sclerosis interstitial lung disease (SSc-ILD). The pathology of RILI or RTLI is described in Session I (D. Lederer and J. Garcia) above. This talk

focused on approved IPF drug development programs, regulatory considerations for these approvals, and insightful case examples of early and late phase products that may be considered as potential MCMs.

IPF is an orphan disease and has an unclear etiology, with a poor prognosis of 2–5 years median survival. SSc-ILD is a secondary syndrome of systemic sclerosis occurring in 5–500 cases/1 million; it is a progressive disease, with a prognosis comparable to RTLI. In both RTLI and SSc-ILD, inflammation is prominent; however, inflammation is less prominent and more fibrotic foci are present with IPF. RTLI and SSc-ILD present a characteristic ground-glass appearance under CT; in contrast, IPF is associated with basilar honeycombing and minimum ground glass (*87–89*). The two drugs approved for treatment of IPF are nintedanib and pirfenidone. Nintedanib is an inhibitor of the receptors for fibroblast growth factor, platelet-derived growth factor receptor, and vascular endothelial factor, thereby preventing fibroblast proliferation, migration and differentiation (*90*). The phase 2 and 3 studies used to support nintedanib approval were randomized, double-blinded, placebo-controlled, parallel group control studies of 52-week treatment duration, with a primary end point of reduced decline in forced vital capacity (FVC).[3,4] In all of the studies, patients in the treated arm showed a lesser FVC decline compared to the placebo arm. Pirfenidone, another drug approved for IPF, has anti-inflammatory and anti-fibrotic activity, and inhibits TGFβ production and activity; however, the exact mechanism of action as well as drug target are unknown (*91*). In three phase 3 studies comparing drug schedules[5,6] (72 weeks and 52 weeks), pirfenidone reduced FVC decline compared to placebo control. After approval for IPF, these possible MCMs are currently being tested in other pulmonary diseases such as progressive fibrosing ILD[7,8] and SSc-ILD.[9,10]

Discussion of regulatory considerations for approval of these two drugs centered around study population, trial design and end points. Given that IPF is an orphan disease, enrollment of large study populations is difficult, and retention/fallout is severely impacted by the severity of the disease and fatalities. Enrollment eligibility is contingent upon a positive IPF diagnosis based on standard criteria (*92*) with a high-resolution CT image-based diagnosis. A single, primary end point of FVC at 52 weeks is generally recommended, but secondary end points, such as mortality, hospitalizations, or acute exacerbations of IPF, are considered important. While mortality or exacerbation end points are attractive, they may not be feasible as primary end points due to the 52-week observation time and low frequency of events. The FDA has not recommended composite end points, such as FVC combined with mortality. The use of high-resolution CT and quantitative lung imaging is increasing; however, these approaches are not validated and there is no prior regulatory experience in this space.

In addition to the above-mentioned products, other products are currently under development. Pamrevlumab is a human recombinant DNA-derived mAb that binds to connective tissue growth factor and has demonstrated efficacy in a WTLI mouse model of radiation-induced fibrosis when initiated 16 weeks postirradiation (*93*). In phase 1 and 2 (*94*) trials in IPF patients, pamrevlumab demonstrated activity; phase 3 trials are ongoing. Autotaxin (ATX) is an enzyme that cleaves lysophosphatidylcholine (LPC) producing lysophosphatidate (LPA); ATX knock-out mice are protected against bleomycin-induced, LPA-mediated lung fibrosis (*95*) and inhibition of the LPA pathway reduced inflammation (*96*). Several ATX inhibitors are currently undergoing phase 2/3 studies in IPF patients (*97*). Another promising pathway involves serum amyloid P/pentraxin 2 (PTX2). Recombinant PTX2 blocks monocyte differentiation to pro-fibrotic macrophage and diminishes bleomycin-induced pulmonary fibrosis (*98*). In a recent phase 2 study in IPF patients, PTX2 reduced decline of FVC following 26-week therapy (*99*). Other examples of early-stage cases under investigation which have entered phase 1 clinical trials include: 1. JNKi, which is a C-Jun N-terminal kinase (JNK) inhibitor that interrupts transcription of implicated pathogenic fibrosis genes (*100*); 2. TGFβ siRNA, which targets TGFβ1 mRNA and reduces the expression of TGFβ protein (*101*); and 3. angiotensin receptor II blockers. The renin-aldosterone-angiotensin system is a key mediator of both lung and renal fibrosis (*102*), and treatment with losartan has been shown to reduce fibrosis (*103*). These data are consistent with rat model findings presented in Session III.

In summary, although no MCM for lung-DEARE has been approved by the FDA under the Animal Rule, several products (AEOL 10150, lisinopril, BIO 300, and IPW-5371) warrant further study. One challenge has been that, except for lisinopril, these products were tested in the WTLI model, which has fallen out of favor because it does not replicate the probable real-life exposure after a radiological incident. As new animal models are being refined, close support and collaboration among sponsors, funding and regulatory agencies is essential.

[3] Highlights of prescribing information. OFEV® (nintedanib) capsules, for oral use. Silver Spring, MD: U.S. Food and Drug Administration; 2018. (https://bit.ly/3ixBeWh)

[4] Medical Review(s). Nintedanib. Application number 205832Orig1s000. Report no. NDA 205832. Silver Spring, MD: Center for Drug Evaluation and Research, U.S. Food and Drug Administration; 2014. (https://bit.ly/3zjDDL0)

[5] Highlights of prescribing information. ESBRIET® (pirfenidone) capsules and film-coated tablets, for oral use. Silver Spring, MD: U.S. Food and Drug Administration; 2016. (https://bit.ly/3iOLOsj)

[6] Medical Review(s). Pirfenidone. Application number 022535Orig1s000. Report no. NDA 022535. Silver Spring, MD: Center for Drug Evaluation and Research, U.S. Food and Drug Administration; 2014. (https://bit.ly/3rtDvWK)

[7] NCT02999178.

[8] NCT01933334.

[9] NCT02597933.

[10] NCT02958917.

SESSION V: BIOMARKERS OF LUNG INJURY

Diagnostic, Predictive and Pharmacodynamic Biomarkers

A biomarker is a parameter associated with a disease/injury that can be objectively measured and evaluated to track disease progression. Biomarkers may be applied in animal models as secondary end points, as triggers for intervention, and/or for selection of the minimum effective dose in humans (*104, 105*). However, currently there is no FDA-qualified biomarker for radiation-induced lung injury. Maureen Kane provided an overview of the considerations involved in the process of discovery and qualification of suitable biomarkers for the evaluation of RILI in animal models by means of imaging modalities.

Acute radiation exposure to the lungs results in radiation pneumonitis and fibrosis as delayed effects. A biomarker would ideally enable early detection of these effects, be measurable in a readily accessible biofluid, and serve as an indicator of tissue repair. Targeted metabolomic analysis by liquid chromatography (LC) with tandem mass spectrometry (MS) enables high-throughput biomarker discovery. In the LC step, sample components are separated according to their affinity for the stationary phase. Subsequently, peptides are separated from other components (first MS step), fragmented, and sorted in order of their mass (second MS step). The whole process, including analysis of the data, can be completed in a few minutes.

Kane and coworkers used LC/tandem MS to identify biomarkers that correlated with survival and were responsive to the application of BIO 300 in a mouse model of RILI (*78*). Importantly, the plasma levels of three of the candidate biomarkers correlated with those in the lung, suggesting ease of measurement. Moreover, plasma levels of those biomarkers in mice were correlated with those in an NHP model. Plasma levels of several candidate biomarkers in the NHP assessed at day 1 were predictive of survival at day 180 postirradiation. Finally, the candidate biomarkers were, based on statistical analysis of their levels in NHP and human plasma, predicted to be feasible for clinical use. The next step is to establish the relationship between plasma levels of the candidate biomarkers and clinical end points in RILI.

Biomarkers for Predicting Onset of Chronic Conditions after Exposure to Radiation

It is not only proteins and lipids that may be used as biomarkers. Naresh Menon spoke on the utility of circulating microRNAs (miRNAs) as biomarkers of the onset of chronic effects after radiation exposure. Because a multitude of factors influence the clinical course after irradiation (*106*), ChromoLogic LLC has elected to focus on the discovery of miRNA biomarkers predictive of the outcomes. Such biomarkers could be applied to identifying radiation-exposed individuals who require treatment to prevent the onset of chronic effects such as pulmonary fibrosis. Because the profile of miRNAs differs among tissues and cell types, these profiles could be used to evaluate injuries in a tissue- or organ-specific manner. In addition, circulating miRNAs can be sampled by minimally invasive means and are stable in plasma.

Based on data obtained from mouse and NHP models of radiation-induced injury, the ability of candidate acute miRNAs to predict late pneumonitis and lung fibrosis was evaluated in adult patients receiving radiotherapy for lung cancer. The analysis strategy comprised identification of a panel of approximately 300 differentially expressed miRNAs; these were narrowed down to approximately 20 based on the chosen outcome. Those 20 miRNAs were next entered into logistic regression models to identify the miRNA with the greatest predictive power for the outcome of interest (*107*). Finally, significance of the predictive score based on the miRNA with the greatest predictive power in normal (nonirradiated) humans was evaluated.

In NHPs, levels of a panel of six miRNAs measured at day 2 postirradiation were predictive of the development of neutropenia on day 6. Also, plasma levels of several miRNAs were predictive of pulmonary fibrosis, as diagnosed by lung CT at day 60. The potential utility of these biomarkers in humans exposed to radiation is suggested by the strong correlation of the levels of the miRNA biomarkers in the NHP and in humans. Based on these data, ChromoLogic aims to develop biomarkers that can be used to identify exposed patients at an early stage who are likely to develop chronic sequelae of that exposure, enabling intervention to reduce the morbidity and mortality rate. Clinical studies to this end are underway.

Evidential and Validation Considerations on Qualifying Imaging Biomarkers

Sue-Jane Wang provided an overview of evidential and validatory considerations of biomarkers for monitoring of RILI for the purpose of drug development. The Biomarker Working Group has established a compendium of harmonized terminology know as BEST (Biomarkers, EndpointS, and other Tools) to facilitate biomarker development and application. Under the FDA Animal Rule, biomarkers (molecular, histologic, radiographic or physiologic) alone should not be used as end points to establish efficacy (*12*). As one example, total kidney volume (TKV) assessed by magnetic resonance imaging, CT or ultrasound is used in clinical trials to predict the prognosis of patients with autosomal polycystic kidney disease. The TKV is affected by myriad factors related to the patient (physiologic and pathologic), to the imaging modality applied (means of image acquisition, analysis and interpretation) or to both.

The FDA has made available a template for a letter of intent to the Agency in which a sponsor can outline their plan to qualify a biomarker. The FDA also provides a *Framework for Defining Evidentiary Criteria for Biomarker Qualification* (*108*) and a draft guidance on *Biomarker Qualification: Evidentiary Framework* (*109*). The latter

document outlines that to support qualification of a biomarker of RILI, a sponsor should provide to the FDA the biological rationale, data in support of the relationship between the biomarker and a relevant clinical outcome, and data confirming that the analytical performance of the biomarker is appropriate for the context of use. The next step is to clinically validate the performance of the biomarker in terms of the outcome of interest.

Section 3011 of the Twenty-First Century Cures Act, entitled ''Qualification of Drug Development Tools,'' outlines a transparent public process managed by the FDA for biomarker development involving three stages: the letter of intent, the qualification plan, and the full qualification package. This process is indicative of the FDA's ongoing commitment to promote discovery and development of imaging and other biomarkers for RILI. Furthermore, the FDA believes that quantitative imaging biomarkers of RILI have potential, depending on the context of use, as adjuncts to conventional biomarkers, and encourages sponsors to make use of the biomarker qualification process.

SESSION VI: PANEL DISCUSSION/SUMMARY

To conclude the meeting, a panel of experts from academia, FDA, BARDA, and NIAID assembled to discuss major scientific questions in the study of lung radiation injury and seek consensus on a path forward for future research.

How Can We Standardize an Animal Model that Mimics the Clinical Condition?

The first discussion topic presented to the panel questioned the need for a single standardized animal model for use in lung radiation studies. To this point, the panelists generally agreed about the necessity of generating an accepted standardized model, stating that agreement among funding and regulatory agencies on the choice of model would enable pharmaceutical companies to evaluate MCMs more effectively for lung radiation. Without the use of a standardized model, it is difficult to determine efficacy for a given drug. The desired outcome is a model of disease that is standardized and reproducible between laboratories. Another panelist pointed out that in the current state of lung research, the scope of studies varies extensively to include multiple species, a wide range of radiation exposures, and different sets of measurements across studies. To move toward a unified model, experts will need to look closely at data already collected from various species and identify experimental end points that are both relevant to the clinical context and transferable between species. One attendee added that, although the predominant C57BL/6 mouse model is not perfect, it does mimic the human condition in that it exhibits both an acute and latent effect of radiation injury. Other laboratory models, such as the C3H and BALB/c mouse, may prove more sensitive to radiation, but do not mimic the human condition as closely.

A workshop attendee questioned whether a single standardized model would be feasible for lung radiation injury, given that the course of disease is variable between animal species, and may even be variable from human to human. For example, pleural effusions are rare in humans. In the clinic, the whole volume of the lung is never irradiated with a single high dose; therefore, the chance of observing similar effusions is limited. Nevertheless, pleural effusions have been noted as a consequence of thoracic radiation therapy, and this phenomenon differs based on both the sex and race of the patient. Some mouse models, on the other hand, do not manifest pleural effusion, and therefore may not be an ideal model for capturing all sequelae associated with lung injury in humans. In response to this comment, the panel acknowledged that pleural effusions are known to occur anecdotally in some patients (*110–113*) and more recently have been confirmed in a clinical trial (*114*), but the quantitative significance of these observations has not been properly evaluated. Anecdotal findings, such as pleural effusion, exemplify the need for a standardized model that can be adequately characterized. Ideally, the chosen model would allow quantification of effusion volume and its effect on pulmonary function and hemodynamics. The overall goal is to arrive at a model that, on its face, exhibits the features of the disease that the scientific community agrees are critical to understanding lung radiation injury (e.g., fibrosis, pneumonitis). For the purposes of product approval, efforts should focus on standardizing the model and characterizing it in terms of the time course of the disease, and the physiological effect of all changes that occur at the level of the lung.

During discussion of a standardized model, concerns were expressed about steroid use in a chosen model, given that steroid treatment is only given to patients as a last resort when all other treatment options have been exhausted, and may therefore not be appropriate to include in animal models. While steroid treatment has been widely used since the discovery of Cushing's disease in 1912, the mechanism by which steroids reduce inflammation remains poorly understood. In response to this comment, panel members provided counterarguments in support of steroid inclusion. One panelist stated that while the individual effect of steroids is not completely understood, steroid treatment serves the important purpose of extending the duration of drug studies by prolonging survival, and long-term studies are critical for accurately characterizing the full course of disease. To evaluate the possibility that steroids are responsible for reaching end points in small animal studies, it is critical to run non-steroid control arms in parallel with treatment arms. Another panelist noted that for early NHP models, their IACUC required the inclusion of steroids to prevent animal suffering due to lung injury. Moreover, if

researchers decide to omit steroids from future NHP studies, there exists no adequate replacement, unless a way to provide supplemental oxygen to animals can be found. In the time since this workshop was convened, the world has experienced the COVID-19 pandemic, and lung injury treatment has been a particular medical practice focus. Data from the RECOVERY trial has shown that dexamethasone treatment reduced mortality in patients that received invasive mechanical ventilation or oxygen-only treatment (*115*). These data support the concept of including dexamethasone in the medical management in large animal models of RILI, especially considering the similarities between radiation injury and COVID-19 (*116*).

What Quantitative Measurements of Lung Function/ Anatomy can be Used as Efficacy End Points?

On the topic of quantitative measurements to be used in animal studies, one attendee asked whether the FDA would require PD biomarkers to be rigorously qualified before attempting to establish a dose in humans. It was recommended that, as a first step to identifying a biomarker for dose selection, researchers should identify what stage of the regulatory process they are in (e.g., exploratory research, animal efficacy studies) before making final decisions on study design. In the current context of lung radiation injury, formal biomarker qualification is considered voluntary (not required); however, the FDA is generally open to the use of exploratory biomarkers as they can be very useful. For example, after lung irradiation, humans may experience inflammation and fibrosis, and radiologic indicators, such as biomarkers, could be used to assess disease severity. In particular, the combination of radiologic measurements with histopathology has great potential to generate reliable biomarkers. Development of measures of pulmonary function in animals could become end points for assessing drug efficacy.

All Models are Wrong… What Animal Models are Useful?

The statistician George Box said, ''All models are wrong, but some are useful.'' In other words, all statistical models describe a phenomenon with some inherent level of uncertainty. Despite this inevitability, laboratory models, including animal models, remain useful tools for predicting the outcome of an event, as long as the model is carefully tailored to the hypothesis it is intended to address. The FDA provided advice on the validity of using PBI to model lung injury in humans, explaining that researchers have used a variety of partial-shielding models (i.e., WTLI, PBI-BM5-7.5) for small animals, but guidance is less clear for NHP studies, given the serious complications from GI and hematologic insult. One panel member explained that the first large animal model of GI-ARS proposed to the FDA constituted total-abdominal irradiation (TAI) (*117*), which was not considered representative of the human condition. Subsequent iterations of the model introduced partial shielding, intended to preserve bone marrow integrity. During testing, it was found that approximately 5% of an NHP's bone marrow was the maximum amount that could be spared, while still inducing potentially lethal hematopoietic ARS (*15*). For both WTLI and GI-ARS, the goal was to develop a model that reliably presented all relevant factors involved in the human condition, which these models largely accomplished. While the FDA is open to modifying the current model, if necessary, the aim is to first standardize a PBI model for small rodents and then make adjustments before moving on to NHPs. In the context of lung injury, PBI models include multiple organ involvement, resulting in a multifactorial injury. Planned models should be as developed as possible before approaching agencies that fund more advanced development, such as BARDA. The scope of model development should be narrowly focused during the early stages to maximize the likelihood of identifying predictive biomarkers, and an approximate 5% bone marrow sparing PBI model in NHPs (2.5% in mice) is valuable because it allows scientists to evaluate the effect of a product in addressing MOI.

CONCLUSION

Members of the panel were given the opportunity to voice their final thoughts regarding future directions for lung injury research. The overall list of recommendations is provided in Table 2; advice that would be relevant to the development of any particular MCM should be provided by the FDA. Because each product is different and preliminary efficacy data may have been obtained using a variety of animal models, early interactions with the FDA, especially when determining future testing in animal models, is advised. Early advice received from the FDA can be very useful, as it can lead to critical thinking about a product, not only with regards to long-term end points, but also early sequelae of radiation injury. Standardization of euthanasia criteria and medical management is a high priority, as these can substantially reduce the level of uncertainty when proceeding under the Animal Rule. Drug developers are also encouraged to take advantage of multicenter studies, which can reduce costs and expedite testing. For example, when validating a H-ARS minipig model, BARDA opted to fund combined studies across multiple institutions, and the results of the study were ultimately beneficial. In closing, small animal studies are useful as initial proof of concept for developing a lung-directed MCM. Ideally, these efforts would be followed by confirmatory studies in large animals and would include nonirradiated and irradiated PK/PD work, lung assessment, imaging and biomarkers, while also limiting the number of NHPs studied. This approach would ultimately facilitate product development under the Animal Rule, which generally requires drug validation in more than one animal species.

TABLE 2
Recommendations from the Workshop

1. Investigators doing animal work should understand the human clinical experience, to best model details of their studies.
2. Rodent animal models that use focal irradiation may be useful for exploring efficacy, but the adequate and well-controlled studies that are intended to support licensure will need to include radiation exposure such that other organ systems (e.g., bone marrow, GI tract) are involved. Although animal models that employ focal lung irradiation may be useful, they do not reflect actual exposures anticipated during a radiological or nuclear incident.
3. More animal model development, especially for partial bone marrow shielding (2.5–7.5%) is needed.
4. Investigators should use pulmonary function measurements and find relevant biomarkers to predict level of radiation injury to the lungs and follow aspects of recovery.
5. Proper radiation dosimetry, particularly in PBI models, is important to reduce variation among experiments and facilities.
6. Companies and funding agencies should consult with the FDA in designing animal studies that will be used to support marketing applications.
7. Potential interactions of a product with expected radiation medical management, such as leukocyte growth factors, should be considered during medical countermeasure development.
8. Use of steroids is an open question because of differences in clinical use vs. traditional laboratory animal veterinary practice. Their use should be temporary, tapered and indicated by pre-determined triggers.

ACKNOWLEDGMENTS

We thank the meeting speakers (Table 1) and conference attendees for providing their expertise and insight during both the presentations and discussion sessions. We also acknowledge the conference moderators (Session I: Banu Karimi-Shah and Libero Marzella, FDA; Session II: Paul Price, NIAID and Rosemary Roberts, FDA; Session III: Zulmarie Perez Horta, NIAID and John Esker, BARDA; Session IV: Merriline Satyamitra, NIAID; Session 5: Sue Jane Wang, FDA and David Cassatt, NIAID; and the Session VI Panel Discussion: Alex Gorovets, FDA). We recognize the efforts of other FDA members of the planning committee (Betsy Ballard, Karen Bleich, Ronald Honchel, Kyong Kang, Suhail Kasim, Danial Krainak, Adebayo Laniyonu, Frank Lutterodt, Yanli Ouyang, Khalid Puthawala, Lisa Skarupa, Cynthia Welsh, Kunyi Wu and Zishan Zhao). We are grateful for the assistance of the medical writers Ellen Wertheimer, Graeme O'May, and Stephen Page of DRT Strategies. Finally, we thank RNCP, NIAID colleagues (Andrea DiCarlo, Brynn Hollingsworth, Carmen Rios, Lanyn Taliaferro and Thomas Winters) for their critical review of the manuscript prior to submission. This article reflects the views of the authors and should not be construed to represent FDA's views or policies.

Received: June 21, 2021; accepted: July 21, 2021; published online: August 3, 2021

REFERENCES

1. Giuranno L, Ient J, De Ruysscher D, Vooijs MA. Radiation-induced lung injury (RILI). Front Oncol 2019; 9:877.

2. Gross NJ. Pulmonary effects of radiation therapy. Ann Intern Med 1977; 86:81–92.

3. Van Dyk J, Keane TJ, Kan S, Rider WD, Fryer CJ. Radiation pneumonitis following large single dose irradiation: A re-evaluation based on absolute dose to lung. Int J Radiat Oncol Biol Phys 1981; 7:461–7.

4. Jin H, Yoo Y, Kim Y, Kim Y, Cho J, Lee YS. Radiation-induced lung fibrosis: Preclinical animal models and therapeutic strategies. Cancers (Basel) 2020; 12.

5. Ghita M, Dunne V, Hanna GG, Prise KM, Williams JP, Butterworth KT. Preclinical models of radiation-induced lung damage: Challenges and opportunities for small animal radiotherapy. Br J Radiol 2019; 92:20180473.

6. Ueki N, Matsuo Y, Togashi Y, Kubo T, Shibuya K, Iizuka Y, et al. Impact of pretreatment interstitial lung disease on radiation pneumonitis and survival after stereotactic body radiation therapy for lung cancer. J Thorac Oncol 2015; 10:116–25.

7. Rajan Radha R, Chandrasekharan G. Pulmonary injury associated with radiation therapy – assessment, complications and therapeutic targets. Biomed Pharmacother 2017; 89:1092–104.

8. Kong FM, Ten Haken R, Eisbruch A, Lawrence TS. Non-small cell lung cancer therapy-related pulmonary toxicity: An update on radiation pneumonitis and fibrosis. Semin Oncol 2005; 32:S42–54.

9. Baranov A, Gale RP, Guskova A, Piatkin E, Selidovkin G, Muravyova L, et al. Bone marrow transplantation after the chernobyl nuclear accident. N Engl J Med 1989; 321:205–12.

10. 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. Br J Radiol 2005; 78:13–16.

11. Williams JP, Jackson IL, Shah JR, Czarniecki CW, Maidment BW, DiCarlo AL. Animal models and medical countermeasures development for radiation-induced lung damage: Report from an NIAID workshop. Radiat Res 2012; 177:e0025–39.

12. Guidance for industry: Product development under the Animal Rule. Docket No. FDA-2009-D-0007. Silver Spring, MD: U.S. Food and Drug Administration; 2015.

13. Garofalo M, Bennett A, Farese AM, Harper J, Ward A, Taylor-Howell C, et al. The delayed pulmonary syndrome following acute high-dose irradiation: A rhesus macaque model. Health Phys 2014; 106:56–72.

14. Fish BL, Gao F, Narayanan J, Bergom C, Jacobs ER, Cohen EP, et al. Combined hydration and antibiotics with lisinopril to mitigate acute and delayed high-dose radiation injuries to multiple organs. Health Phys 2016; 111:410–9.

15. MacVittie TJ, Farese AM, Parker GA, Bennett AW, Jackson WE 3rd. Acute radiation-induced lung injury in the non-human primate: A review and comparison of mortality and co-morbidities using models of partial-body irradiation with marginal bone marrow sparing and whole thorax lung irradiation. Health Phys 2020; 119:559–87.

16. MacVittie TJ, Farese AM, Parker GA, Jackson W 3rd. The time course of radiation-induced lung injury in a nonhuman primate model of partial-body irradiation with minimal bone marrow sparing: Clinical and radiographic evidence and the effect of neupogen administration. Health Phys 2019; 116:366–82.

17. Parker GA, Li N, Takayama K, Farese AM, MacVittie TJ. Lung and heart injury in a nonhuman primate model of partial-body irradiation with minimal bone marrow sparing: Histopathological evidence of lung and heart injury. Health Phys 2019; 116:383–400.

18. MacVittie TJ, Bennett A, Booth C, Garofalo M, Tudor G, Ward A, et al. The prolonged gastrointestinal syndrome in rhesus macaques: The relationship between gastrointestinal, hematopoietic, and delayed multi-organ sequelae following acute, potentially lethal, partial-body irradiation. Health Phys 2012; 103:427–53.

19. DiCarlo AL, Jackson IL, Shah JR, Czarniecki CW, Maidment BW, Williams JP. Development and licensure of medical countermeasures to treat lung damage resulting from a radiological or nuclear incident. Radiat Res 2012; 177:717–21.

20. Checkley W. Neuregulin-1 as a potentially novel biomarker in

acute respiratory distress syndrome. Eur Respir J 2013; 41:259–61.

21. Thompson BT, Chambers RC, Liu KD. Acute respiratory distress syndrome. N Engl J Med 2017; 377:562–72.

22. Wirsdorfer F, Jendrossek V. The role of lymphocytes in radiotherapy-induced adverse late effects in the lung. Front Immunol 2016; 7:591.

23. King TE Jr., Pardo A, Selman M. Idiopathic pulmonary fibrosis. Lancet 2011; 378:1949–61.

24. Lederer DJ, Martinez FJ. Idiopathic pulmonary fibrosis. N Engl J Med 2018; 378:1811–23.

25. Koch A, Gulani J, King G, Hieber K, Chappell M, Ossetrova N. Establishment of early endpoints in mouse total-body irradiation model. PLoS One 2016; 11:e0161079.

26. Jackson IL, Vujaskovic Z, Down JD. A further comparison of pathologies after thoracic irradiation among different mouse strains: Finding the best preclinical model for evaluating therapies directed against radiation-induced lung damage. Radiat Res 2011; 175:510–18.

27. Mathew B, Huang Y, Jacobson JR, Berdyshev E, Gerhold LM, Wang T, et al. Simvastatin attenuates radiation-induced murine lung injury and dysregulated lung gene expression. Am J Respir Cell Mol Biol 2011; 44:415–22.

28. Garcia JG, Liu F, Verin AD, Birukova A, Dechert MA, Gerthoffer WT, et al. Sphingosine 1-phosphate promotes endothelial cell barrier integrity by edg-dependent cytoskeletal rearrangement. J Clin Invest 2001; 108:689–701.

29. Meyer NJ, Huang Y, Singleton PA, Sammani S, Moitra J, Evenoski CL, et al. GADD45a is a novel candidate gene in inflammatory lung injury via influences on Akt signaling. FASEB J 2009; 23:1325–37.

30. Mitra S, Sammani S, Wang T, Boone DL, Meyer NJ, Dudek SM, et al. Role of growth arrest and DNA damage-inducible alpha in Akt phosphorylation and ubiquitination after mechanical stress-induced vascular injury. Am J Respir Crit Care Med 2011; 184:1030–40.

31. Mitra S, Wade MS, Sun X, Moldobaeva N, Flores C, Ma SF, et al. GADD45a promoter regulation by a functional genetic variant associated with acute lung injury. PLoS One 2014; 9:e100169.

32. Mathew B, Takekoshi D, Sammani S, Epshtein Y, Sharma R, Smith BD, et al. Role of GADD45a in murine models of radiation- and bleomycin-induced lung injury. Am J Physiol Lung Cell Mol Physiol 2015; 309:L1420–9.

33. Quijada H, Bermudez T, Kempf CL, Valera DG, Garcia AN, Camp SM, et al. Endothelial eNAMPT amplifies preclinical acute lung injury: Efficacy of an eNAMPT-neutralising mab. Eur Respir J 2020; 57:2002536.

34. Moreno-Vinasco L, Quijada H, Sammani S, Siegler J, Letsiou E, Deaton R, et al. Nicotinamide phosphoribosyltransferase inhibitor is a novel therapeutic candidate in murine models of inflammatory lung injury. Am J Respir Cell Mol Biol 2014; 51:223–8.

35. Choi YW, Munden RF, Erasmus JJ, Park KJ, Chung WK, Jeon SC, et al. Effects of radiation therapy on the lung: Radiologic appearances and differential diagnosis. Radiographics 2004; 24:985–97; discussion 98.

36. Karpathiou G, Giatromanolaki A, Koukourakis MI, Mihailidis V, Sivridis E, Bouros D, et al. Histological changes after radiation therapy in patients with lung cancer: A prospective study. Anticancer Res 2014; 34:3119–24.

37. Hayashi T, Morishita Y, Kubo Y, Kusunoki Y, Hayashi I, Kasagi F, et al. Long-term effects of radiation dose on inflammatory markers in atomic bomb survivors. Am J Med 2005; 118:83–6.

38. Preston DL, Shimizu Y, Pierce DA, Suyama A, Mabuchi K. Studies of mortality of atomic bomb survivors. Report 13: Solid cancer and noncancer disease mortality: 1950–1997. Radiat Res 2003; 160:381–407.

39. Baranov AE, Selidovkin GD, Butturini A, Gale RP. Hematopoietic recovery after 10-Gy acute total body radiation. Blood 1994; 83:596–9.

40. Jacob J, Bartholmai BJ, Rajagopalan S, Kokosi M, Nair A, Karwoski R, et al. Automated quantitative computed tomography versus visual computed tomography scoring in idiopathic pulmonary fibrosis: Validation against pulmonary function. J Thorac Imaging 2016; 31:304–11.

41. Werner R, Hofmann C, Mücke E, Gauer T. Reduction of breathing irregularity-related motion artifacts in low-pitch spiral 4D CT by optimized projection binning. Radiat Oncol 2017; 12:100.

42. Plathow C, Li M, Gong P, Zieher H, Kiessling F, Peschke P, et al. Computed tomography monitoring of radiation-induced lung fibrosis in mice. Invest Radiol 2004; 39:600–9.

43. Kim HJ, Brown MS, Chong D, Gjertson DW, Lu P, Kim HJ, et al. Comparison of the quantitative CT imaging biomarkers of idiopathic pulmonary fibrosis at baseline and early change with an interval of 7 months. Acad Radiol 2015; 22:70–80.

44. Brennan D, Schubert L, Diot Q, Castillo R, Castillo E, Guerrero T, et al. Clinical validation of 4-dimensional computed tomography ventilation with pulmonary function test data. Int J Radiat Oncol Biol Phys 2015; 92:423–9.

45. Vinogradskiy Y, Koo PJ, Castillo R, Castillo E, Guerrero T, Gaspar LE, et al. Comparison of 4-dimensional computed tomography ventilation with nuclear medicine ventilation-perfusion imaging: A clinical validation study. Int J Radiat Oncol Biol Phys 2014; 89:199–205.

46. Tsipis K. Understanding nuclear weapons. London: Wildwood House; 1985.

47. Thrall KD, Mahendra S, Jackson MK, Jackson W 3rd, Farese AM, MacVittie TJ. A comparative dose-response relationship between sexes for mortality and morbidity of radiation-induced lung injury in the rhesus macaque. Health Phys 2019; 116:354–65.

48. Foster RD, Speiser MP, Solberg TD. Commissioning and verification of the collapsed cone convolution superposition algorithm for SBRT delivery using flattening filter-free beams. J Appl Clin Med Phys 2014; 15:4631.

49. Marks LB, Yorke ED, Jackson A, Ten Haken RK, Constine LS, Eisbruch A, et al. Use of normal tissue complication probability models in the clinic. Int J Radiat Oncol Biol Phys 2010; 76:S10–9.

50. Stewart FA, Akleyev AV, Hauer-Jensen M, Hendry JH, Kleiman NJ, Macvittie TJ, et al. ICRP publication 118: ICRP statement on tissue reactions and early and late effects of radiation in normal tissues and organs–threshold doses for tissue reactions in a radiation protection context. Ann ICRP 2012; 41:1–322.

51. Allio T. Product development under FDA's animal rule: Understanding FDA's expectations and potential implications for traditional development programs. Ther Innov Regul Sci 2016; 50:660–70.

52. Garofalo MC, Ward AA, Farese AM, Bennett A, Taylor-Howell C, Cui W, et al. A pilot study in rhesus macaques to assess the treatment efficacy of a small molecular weight catalytic metalloporphyrin antioxidant (AEOL 10150) in mitigating radiation-induced lung damage. Health Phys 2014; 106:73–83.

53. Jackson IL, Xu P, Hadley C, Katz BP, McGurk R, Down JD, et al. A preclinical rodent model of radiation-induced lung injury for medical countermeasure screening in accordance with the FDA animal rule. Health Phys 2012; 103:463–73.

54. Dabjan MB, Buck CM, Jackson IL, Vujaskovic Z, Marples B, Down JD, A survey of changing trends in modelling radiation lung injury in mice: Bringing out the good, the bad, and the uncertain. Lab Invest 2016; 96:936–49.

55. Travis EL, Down JD, Holmes SJ, Hobson B. Radiation pneumonitis and fibrosis in mouse lung assayed by respiratory frequency and histology. Radiat Res 1980; 84:133–43.

56. 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; 10:243–51.

57. 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; 173:10–20.

58. Jackson IL, Baye F, Goswami CP, Katz BP, Zodda A, Pavlovic R, et al. Gene expression profiles among murine strains segregate with distinct differences in the progression of radiation-induced lung disease. Dis Model Mech 2017; 10:425–37.

59. Kma L, Gao F, Fish BL, Moulder JE, Jacobs ER, Medhora M. Angiotensin converting enzyme inhibitors mitigate collagen synthesis induced by a single dose of radiation to the whole thorax. J Radiat Res 2012; 53:10–7.

60. Ghobadi G, van der Veen S, Bartelds B, de Boer RA, Dickinson MG, de Jong JR, et al. Physiological interaction of heart and lung in thoracic irradiation. Int J Radiat Oncol Biol Phys 2012; 84:e639–46.

61. Medhora M, Gao F, Glisch C, Narayanan J, Sharma A, Harmann LM, et al. Whole-thorax irradiation induces hypoxic respiratory failure, pleural effusions and cardiac remodeling. J Radiat Res 2015; 56:248–60.

62. Medhora M, Gao F, Gasperetti T, Narayanan J, Khan AH, Jacobs ER, et al. Delayed effects of acute radiation exposure (Deare) in juvenile and old rats: Mitigation by lisinopril. Health Phys 2019; 116:529–45.

63. Medhora M, Haworth S, Liu Y, Narayanan J, Gao F, Zhao M, et al. Biomarkers for radiation pneumonitis using noninvasive molecular imaging. J Nucl Med 2016; 57:1296–301.

64. Cohen EP, Hankey KG, Bennett AW, Farese AM, Parker GA, MacVittie TJ. Acute and chronic kidney injury in a non-human primate model of partial-body irradiation with bone marrow sparing. Radiat Res 2017; 188:661–71.

65. Prado C, Kazi A, Bennett A, MacVittie T, Prado K. Mean organ doses resulting from non-human primate whole thorax lung irradiation prescribed to mid-line tissue. Health Phys 2015; 109:367–73.

66. Prado C, MacVittie TJ, Bennett AW, Kazi A, Farese AM, Prado K. Organ doses associated with partial-body irradiation with 2.5% bone marrow sparing of the non-human primate: A retrospective study. Radiat Res 2017; 188:615–25.

67. de Faria EB, Barrow KR, Ruehle BT, Parker JT, Swartz E, Taylor-Howell C, et al. The evolving Mcart multimodal imaging core: Establishing a protocol for computed tomography and echocardiography in the rhesus macaque to perform longitudinal analysis of radiation-induced organ injury. Health Phys 2015; 109:479–92.

68. Gao F, Fish BL, Moulder JE, Jacobs ER, Medhora M. Enalapril mitigates radiation-induced pneumonitis and pulmonary fibrosis if started 35 days after whole-thorax irradiation. Radiat Res 2013; 180:546–52.

69. Murigi FN, Mohindra P, Hung C, Salimi S, Goetz W, Pavlovic R, et al. Dose optimization study of AEOL 10150 as a mitigator of radiation-induced lung injury in CBA/J mice. Radiat Res 2015; 184:422–32.

70. MacVittie TJ, Gibbs A, Farese AM, Barrow K, Bennett A, Taylor-Howell C, et al. AEOL 10150 mitigates radiation-induced lung injury in the nonhuman primate: Morbidity and mortality are administration schedule-dependent. Radiat Res 2017; 187:298–318.

71. Jackson I, Vujaskovic Z, Down J. Revisiting strain-related differences in radiation sensitivity of the mouse lung: Recognizing and avoiding the confounding effects of pleural effusions. Radiat Res 2010; 173:10–20.

72. 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; 67:573–80.

73. Cline J, Dugan G, Bourland J, Perry D, Stitzel J, Weaver A, et al. Post-irradiation treatment with a superoxide dismutase mimic, MnTnHex-2-PyP5+, mitigates radiation injury in the lungs of non-human primates after whole-thorax exposure to ionizing radiation. Antioxidants (Basel) 2018; 7:40.

74. Warner M, Huang B, Gustafsson JA. Estrogen receptor beta as a pharmaceutical target. Trends Pharmacol Sci 2017; 38:92–9.

75. Ha CT, Li X-H, Fu D, Xiao M, Landauer MR. Genistein nanoparticles protect mouse hematopoietic system and prevent proinflammatory factors after gamma irradiation. Radiat Res 2013; 180:316–25.

76. Day RM, Barshishat-Kupper M, Mog SR, McCart EA, Prasanna PG, Davis TA, et al. Genistein protects against biomarkers of delayed lung sequelae in mice surviving high-dose total body irradiation. J Radiat Res 2008; 49:361–72.

77. Jackson IL, Zodda A, Gurung G, Pavlovic R, Kaytor MD, Kuskowski MA, et al. BIO 300, a nanosuspension of genistein, mitigates pneumonitis/fibrosis following high-dose radiation exposure in the C57L/J murine model. Br J Pharmacol 2017; 174:4738–50.

78. Jones JW, Jackson IL, Vujaskovic Z, Kaytor MD, Kane MA. Targeted metabolomics identifies pharmacodynamic biomarkers for BIO 300 mitigation of radiation-induced lung injury. Pharm Res 2017; 34:2698–709.

79. Plett PA, Sampson CH, Chua HL, Joshi M, Booth C, Gough A, et al. Establishing a murine model of the hematopoietic syndrome of the acute radiation syndrome. Health Phys 2012; 103:343–55.

80. Marks LB, Fan M, Clough R, Munley M, Bentel G, Coleman RE, et al. Radiation-induced pulmonary injury: Symptomatic versus subclinical endpoints. Int J Radiat Biol 2000; 76:469–75.

81. Booth C, Tudor G, Tonge N, Shea-Donohue T, MacVittie TJ. Evidence of delayed gastrointestinal syndrome in high-dose irradiated mice. Health Phys 2012; 103:400–10.

82. Booth C, Tudor GL, Katz BP, MacVittie TJ. The delayed effects of acute radiation syndrome: Evidence of long-term functional changes in the clonogenic cells of the small intestine. Health Phys 2015; 109:399–413.

83. Down JD, Yanch JC. Identifying the high radiosensitivity of the lungs of C57L mice in a model of total-body irradiation and bone marrow transplantation. Radiat Res 2010; 174:258–63.

84. Anscher MS. Targeting the TGF-beta1 pathway to prevent normal tissue injury after cancer therapy. Oncologist 2010; 15:350–9.

85. Zhang P, Cui W, Hankey KG, Gibbs AM, Smith CP, Taylor-Howell C, et al. Increased expression of connective tissue growth factor (CTGF) in multiple organs after exposure of non-human primates (NHP) to lethal doses of radiation. Health Phys 2015; 109:374–90.

86. Rabender C, Mezzaroma E, Mauro AG, Mullangi R, Abbate A, Anscher M, et al. IPW-5371 proves effective as a radiation countermeasure by mitigating radiation-induced late effects. Radiat Res 2016; 186:478–88.

87. Leslie KO. My approach to interstitial lung disease using clinical, radiological and histopathological patterns. J Clin Pathol 2009; 62:387–401.

88. Wuyts WA, Cavazza A, Rossi G, Bonella F, Sverzellati N, Spagnolo P. Differential diagnosis of usual interstitial pneumonia: When is it truly idiopathic? Eur Respir Rev 2014; 23:308–19.

89. Solomon JJ, Olson AL, Fischer A, Bull T, Brown KK, Raghu G. Scleroderma lung disease. Eur Respir Rev 2013; 22:6–19.

90. Wollin L, Wex E, Pautsch A, Schnapp G, Hostettler KE, Stowasser S, et al. Mode of action of nintedanib in the treatment of idiopathic pulmonary fibrosis. Eur Respir J 2015; 45:1434–45.

91. Takeda Y, Tsujino K, Kijima T, Kumanogoh A. Efficacy and

safety of pirfenidone for idiopathic pulmonary fibrosis. Patient Prefer Adherence 2014; 8:361–70.

92. Raghu G, Remy-Jardin M, Myers JL, Richeldi L, Ryerson CJ, Lederer DJ, et al. Diagnosis of idiopathic pulmonary fibrosis. An official ATS/ERS/JRS/ALAT clinical practice guideline. Am J Respir Crit Care Med 2018; 198:e44–e68.

93. Bickelhaupt S, Erbel C, Timke C, Wirkner U, Dadrich M, Flechsig P, et al. Effects of CTGF blockade on attenuation and reversal of radiation-induced pulmonary fibrosis. J Natl Cancer Inst 2017; 109.

94. Gorina E, Richeldi L, Raghu G, Fernandez Perez E, Costabel U, Albera C, et al. PRAISE, a randomized, placebo-controlled, double-blind phase 2 clinical trial of pamrevlumab (FG-3019) in IPF patients. Eur Respir J 2017; 50:OA3400.

95. Huang LS, Fu P, Patel P, Harijith A, Sun T, Zhao Y, et al. Lysophosphatidic acid receptor-2 deficiency confers protection against bleomycin-induced lung injury and fibrosis in mice. Am J Respir Cell Mol Biol 2013; 49:912–22.

96. Brindley DN, Benesch MG, Murph MM. Autotaxin–an enzymatic augmenter of malignant progression linked to inflammation. In: Murph M., editor. Melanoma–Current clinical management and future therapeutics. London: InTech Open; 2015. p. 297–324.

97. Zulfikar S, Mulholland S, Adamali H, Barratt SL. Inhibitors of the autotaxin-lysophosphatidic acid axis and their potential in the treatment of interstitial lung disease: Current perspectives. Clin Pharmacol 2020; 12:97–108.

98. Murray LA, Rosada R, Moreira AP, Joshi A, Kramer MS, Hesson DP, et al. Serum amyloid P therapeutically attenuates murine bleomycin-induced pulmonary fibrosis via its effects on macrophages. PloS One 2010; 5:e9683.

99. Raghu G, van den Blink B, Hamblin MJ, Brown AW, Golden JA, Ho LA, et al. Effect of recombinant human pentraxin 2 vs placebo on change in forced vital capacity in patients with idiopathic pulmonary fibrosis: A randomized clinical trial. JAMA 2018; 319:2299–307.

100. Grynberg K, Ma FY, Nikolic-Paterson DJ. The JNK signaling pathway in renal fibrosis. Front Physiol 2017; 8:829.

101. Fouquerel E, Li J, Braganza A, Yu Z, Brown AR, Wang X-H, et al. Use of RNA interference to study DNA repair. Genotoxicity and DNA repair. In: Sierra L, Gaivao I, editors. Genotoxicity and DNA repair. Methods in pharmacology and toxicology. New York: Humana Press; 2014.

102. Wang J, Chen L, Chen B, Meliton A, Liu SQ, Shi Y, et al. Chronic activation of the renin-angiotensin system induces lung fibrosis. Sci Rep 2015; 5:15561.

103. Couluris M, Kinder BW, Xu P, Gross-King M, Krischer J, Panos RJ. Treatment of idiopathic pulmonary fibrosis with losartan: A pilot project. Lung 2012; 190:523–7.

104. Qualification process for drug development tools guidance for industry and FDA staff. Docket No. FDA-2010-D-0529. Silver Spring, MD: U.S. Food and Drug Administration; 2020.

105. Biomarkers Definitions Working Group. Biomarkers and surrogate endpoints: Preferred definitions and conceptual framework. Clin Pharmacol Ther 2001; 69:89–95.

106. Waselenko JK, MacVittie TJ, Blakely WF, Pesik N, Wiley AL, Dickerson WE, et al. Medical management of the acute radiation syndrome: Recommendations of the strategic national stockpile radiation working group. Ann Intern Med 2004; 140:1037–51.

107. Vlachos IS, Zagganas K, Paraskevopoulou MD, Georgakilas G, Karagkouni D, Vergoulis T, et al. DIANA-miRPath v3.0: Deciphering microRNA function with experimental support. Nucleic Acids Res 2015; 43:W460–6.

108. Framework for defining evidentiary criteria for biomarker qualification. North Bethesda, MD: Biomarkers Consortium Evidentiary Criteria Writing Group, Foundation for the National Institutes of Health; 2016. (https://bit.ly/3wYbOX3)

109. Biomarker qualification: Evidentiary framework. Docket No. FDA-2018-D-4267. Silver Spring, MD: U.S. Food and Drug Administration; 2018.

110. Bachman AL, Macken K. Pleural effusions following supervoltage radiation for breast carcinoma. Radiology 1959; 72:699–709.

111. Morrone N, Gama e Silva Volpe VL, Dourado AM, Mitre F, Coletta EN. Bilateral pleural effusion due to mediastinal fibrosis induced by radiotherapy. Chest 1993; 104:1276–8.

112. Rodriguez-Garcia JL, Fraile G, Moreno MA, Sanchez-Corral JA, Penalver R. Recurrent massive pleural effusion as a late complication of radiotherapy in Hodgkin's disease. Chest 1991; 100:1165–6.

113. Whitcomb ME, Schwarz MI. Pleural effusion complicating intensive mediastinal radiation therapy. Am Rev Respir Dis 1971; 103:100–7.

114. Zhao J, Day RM, Jin JY, Quint L, Williams H, Ferguson C, et al. Thoracic radiation-induced pleural effusion and risk factors in patients with lung cancer. Oncotarget 2017; 8:97623–32.

115. Horby P, Lim WS, Emberson JR, Mafham M, Bell JL, Linsell L, et al. Dexamethasone in hospitalized patients with Covid-19. N Engl J Med 2021; 384:693–704.

116. Rios CI, Cassatt DR, Hollingsworth BA, Satyamitra MM, Tadesse YS, Taliaferro LP, et al. Commonalities between COVID-19 and radiation injury. Radiat Res 2021; 195:1–24.

117. Vigneulle RM, Rao S, Fasano A, MacVittie TJ. Structural and functional alterations of the gastrointestinal tract following radiation-induced injury in the rhesus monkey. Dig Dis Sci 2002; 47:1480–91.