Disaster Medicine and Public Health Preparedness

www.cambridge.org/dmp

Animal Model Considerations for Medical Countermeasure Development for Radiation and Sulfur Mustard Exposures: Animal models for radiation and HD exposures

Isabel Lauren Jackson PhD[1] and Melanie Doyle-Eisele PhD[2]

[1]True North BioPharm LLC, Rockville, MD and [2]Lovelace Biomedical Research Institute, Albuquerque, New Mexico

Systematic Review

Cite this article: Jackson IL and Doyle-Eisele M. Animal model considerations for medical countermeasure development for radiation and sulfur mustard exposures: Animal models for radiation and HD exposures. *Disaster Med Public Health Prep*. **18**(e81), 1–7. doi: https://doi.org/10.1017/dmp.2023.180.

Keywords:
radiation; sulfur mustard; acute lung injury; fibrosis; medical countermeasures

Corresponding author:
Isabel Lauren Jackson;
Email: insight@truenorthbiopharm.com.

Abstract

Development of medical countermeasures (MCM) to mitigate and/ or treat the pulmonary complications associated with exposure to chemical, radiological, and/ or nuclear weapons is a national, public health preparedness posture priority in the United States (US). Pulmonary exposure to either sulfur mustard vapor or radiation causes oxidative damage, vascular injury, hyperinflammation, and pro-fibrotic signaling cascades that lead to life-threatening and potentially debilitating lung disease. There is no MCM currently approved by the US Food and Drug Administration (FDA) to mitigate and/ or treat lung injury caused by sulfur mustard or radiation exposure. Thus, there remains a major unmet public health need for development of threat-agnostic, host-directed therapeutics that target common pathophysiological mechanisms underlying the progression of acute and/ or late lung injury independent of the etiology of disease. This review describes the clinical manifestations and underlying mechanisms of sulfur mustard and radiation-induced lung injury and regulatory considerations for MCM development under the non-traditional Animal Rule pathway.

Background

Medical countermeasure (MCM) development for chemical, biological, radiological, and/ or nuclear (CBRN) threats first emerged as a global health security priority following the September 11, 2001 ("9/11") terrorist attacks in the United States (US) in which 2977 civilians were killed. In the years since the 9/11 attacks, the global threat landscape has markedly evolved and with it, the global public health strategy for emergency medical preparedness and response. The growing threat of deliberate or accidental deployment of CBRN threat agents due to fractures in traditional security alliances and countries' growing assertiveness to militarily advance foreign and defense policy goals has strengthened the global resolve to repurpose commercially approved and late-stage therapeutics as MCMs to mitigate, and/ or treat CBRN-related illnesses.[1]

It is impossible to predict when, where, or what agent may be deployed against US civilians, or military personnel in the future. The human body is logically designed to respond to insults in a predictable pattern irrespective of the causative agent. Disease pathologies arise when natural physiological responses to perturbations in bodily systems turn maladaptive.[2] Recognizing that pathophysiological mechanisms (e.g., coagulopathy, hyperinflammation, fibrosis, etc.) underlying the myriad of critical illnesses caused by CBRN threat agents share common therapeutically targetable pathways, there has been a strategic shift towards the development of threat-agnostic, host-directed MCMs to treat "Threat X," and away from targeting individual threats. To this end, tactical assembly of a strategic national stockpile (SNS) of vendor-managed, multipurpose agents will ensure an agile medical armamentarium strategically geopositioned to ensure rapid access to life-saving therapeutics in a public health emergency.

The Department of Homeland Security has identified approximately 200 chemical compounds as posing a credible health security threat to the US population.[3] Of the chemical compounds identified, sulfur mustard, a highly toxic, blistering (i.e., vesicant) agent, is considered the chemical warfare agent most likely to be used in combat due to the ease, and inexpensiveness of manufacturing. Indeed, sulfur mustard (military designation HD or H) is often called "The King of Battle Gases" as it has been deployed more often in military conflicts than all other chemical warfare agents combined.[4] Sulfur mustard is a radiomimetic alkylating agent that causes DNA damage and chromosomal aberrations leading to myelosuppression and long-term immune insufficiency, as well as ocular, dermal, respiratory, and neurological toxicity.[4]

Unlike sulfur mustard, nuclear weapons have been used only twice throughout the past century, both times by the US during World War II. Today, 9 countries including the US, United

SDMPH
SOCIETY FOR DISASTER MEDICINE & PUBLIC HEALTH

Kingdom, and France, as well as Russia, Israel, China, and Pakistan, possess over 13 000 nuclear weapons. This list also includes India and North Korea. Moreover, non-state sponsored terrorists including far-right extremists within the US have sought to sabotage nuclear facilities and acquire nuclear weapons to inflict mass casualties, foment civil unrest, and advance their extremist ideology.[5] Similar to sulfur mustard, ionizing radiation causes direct and indirect damage to DNA and other cellular macromolecules, leading to a biological avalanche effect that results in cellular dysfunction, and/ or cell death, vascular injury and coagulopathy, hyperinflammation, and pro-fibrotic signaling cascades.[6,7] Absorbed radiation doses are measured in Gray (y), which is equivalent to 1 joule of energy absorbed per kilogram of organ or tissue weight. At doses greater than 2 Gy to all or most of the body, individuals are at risk for developing acute radiation syndrome (ARS) and/ or the delayed effects of acute radiation exposure (DEARE). ARS and DEARE are characterized by multiorgan dysfunction that may lead to multiorgan failure and death without appropriate treatment intervention.

This review will describe the similarities and dissimilarities in lung damage between sulfur mustard and radiation exposures, common pathophysiological mechanisms underpinning the development of clinical manifestations of lung disease, MCM research, and development across the pipeline from blue sky science to regulatory approval under the FDA Animal Rule (AR) regulatory pathway.

Clinical Perspectives

Clinical Manifestations of Sulfur Mustard -Induced Lung Disease

Sulfur mustard gas was first introduced on the battlefields of Belgium during World War I.[4] It is estimated that 400 000 of the 1.2 million soldiers exposed required long-term medical care. Widespread use in battle, including arial bombs, were used by Spain and France against the Berbers during the Rif war in the 1920s, and by Italy against the Ethiopians during the Italo-Ethiopian War from 1935 - 1936.[8] In more recent years, sulfur mustard was deployed by the Iraqi military against the Iranian military and civilians, as well as the Iraqi Kurdish minority, during the Iran-Iraq War from 1983 - 1988.[4] Sulfur mustard was also used against civilians during the Syrian Civil War from 2013 to 2018.[1] Although sulfur mustard exposure is acutely fatal in less than 5% of cases, long-term complications associated with immune dysregulation can cause life-threatening illness and debilitating disease among survivors.[9]

The temporal onset and severity of symptoms after sulfur mustard exposure are dependent on the route, total dose, duration of exposure, and proximity to the source, as well as environmental conditions, and usage of personal protective equipment.[10] Exposure may occur through ingestion (e.g., drinking or swimming in contaminated water, eating contaminated food, etc.) and through skin or eye contact and inhalation when sulfur mustard is released as a vapor. The most common acute complications associated with exposure are observed in the eyes, skin, lung, and bone marrow. Heavily exposed patients may also present with gastrointestinal (e.g., nausea, vomiting) and neurological symptoms. Long term immune insufficiency is considered a major cause of increased incidence of infection and cancers in survivors.[4]

The most common life-threatening complication of sulfur mustard inhalation is upper respiratory and lower respiratory injury.[4] Iranian physicians have extensively characterized molecular, functional, radiographic, and histopathologic sequelae accompanying acute and delayed lung injury among civilians and military exposed during the Iran-Iraq War.[4] Briefly, the severity of respiratory effects is dose-dependent and damage may extend from the nasal mucosa to the terminal bronchioles.[9] Upon inhalation, sulfur mustard reacts rapidly with the cells of the upper respiratory tract. As the particles traverse the upper airways, there is dilution due to filtration as the vapor reaches the bronchioles and alveoli.[11]

Heavy sulfur mustard exposure in chemical warfare is exceedingly rare. In these cases, mortality due to acute lung injury occurs rapidly, within days. Clinical experience with casualties from Bari Harbor during World War II and Iranian casualties from the Iraq-Iran War indicates acute respiratory symptoms may occur within 4 to 48 hours.[9,12] Symptoms included productive cough, sore throat, laryngitis, and head congestion; which abate rapidly in most cases. In the severely ill or terminal, symptoms progress to frank hoarseness and aphonia, lower respiratory tract infection, dyspnea, and cyanosis.[9,12] In those patients with lower respiratory tract infection, death occurs rapidly.[12] These early mortalities are associated with multi-organ failure and acute respiratory distress syndrome (ARDS).[9] Autopsy findings reveal congestion and inflammation, moderate to marked edema, ulceration, and epithelial denudation, as well as small, focal hemorrhages (Figure 1). Cast formation, due to epithelial denudation and fibrin, is observed in the lower trachea with extension into both bifurcations and bronchial ramifications.[12]

Symptomatic and pathological features of sulfur mustard-induced respiratory illness in long-term survivors is consistent with bronchiolitis obliterans and pulmonary fibrosis.[4] The progressive narrowing and scarring of the bronchioles lead to respiratory insufficiency and debilitating diseases that may become life-threatening. In a study by Emad and Rezaian, the primary respiratory complications observed in Iranian combat veterans 10- years after exposure were chronic bronchitis, pulmonary fibrosis, and large airway narrowing, as well as asthma, and bronchiectasis.[13] Chronic obstructive pulmonary disease (COPD) was the primary complication observed 16 to 20 years after exposure followed by bronchiectasis, asthma, large airway narrowing, and pulmonary fibrosis.[14]

Clinical Manifestations of Radiation-Induced Lung Disease

Clinically symptomatic pulmonary complications following acute total or partial body irradiation occur between the first and sixth month after exposure depending on the overall dose. Lung disease, characterized by acute pneumonitis between 1 to 6 months after exposure and chronic fibrosis months to years later, occur primarily in patients surviving ARS, or alternatively, patients who received the highest doses of radiation to the upper half body.

Total body irradiation (TBI)- induced ARS is characterized by a dose-dependent increase in severity of myelosuppression and gastrointestinal (GI) injury leading to an increased risk for infection, hemorrhage, and death within the first 4 to 6 weeks after exposure. Radiation-induced myelosuppression and GI injury mirror has been reported in sulfur mustard exposed individuals. Four MCMs in the myeloid colony stimulating factor (CSF) class of drugs have been approved by the FDA under the non-traditional Animal Rule (21 CFR 314.600-650 for drugs; 21 CFR 601.90-95 for biologics; effective July 1, 2002) regulatory pathway to treat myelosuppression associated with ARS in adults and children. Empirical observations in humans and controlled studies in animal

https://doi.org/10.1017/dmp.2023.180 Published online by Cambridge University Press

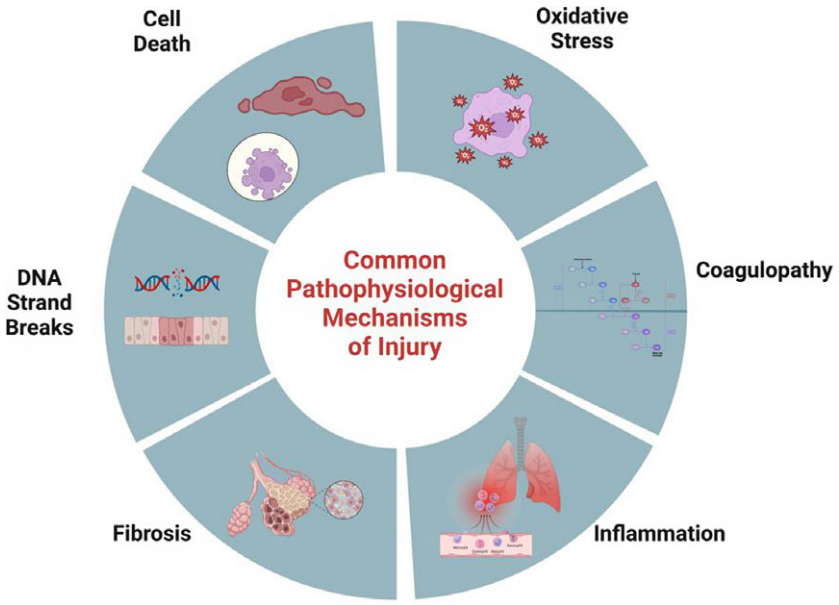

Figure 1. Shared pathophysiological mechanisms underlying the development of sulfur mustard (HD) and radiation-induced acute and late lung injuries. Development of threat agnostic, host-directed therapeutics targeting maladaptive systems biology responses to mitigate, and/ or treat acute lung injury, and delayed pulmonary complications of sulfur mustard, radiological, and/ or nuclear exposures can be pursued under the FDA Animal Rule regulatory pathway. This illustration was created with BioRender.com.

models demonstrate survival from ARS is possible with minimal to advanced care and cytokine therapies in a medical setting (e.g., CSF, antibiotics). However, ARS survivors are most notably at risk for developing DEARE, debilitating, and/ or life-threatening lung damage within weeks to months after exposure.[15]

DEARE-lung is characterized by acute, life-threatening pneumonitis or alveolitis occurring approximately 1 to 6 months post-exposure to radiation doses of 7.5 Gy or higher, and late fibrosis that may develop several months post-exposure. Fibrosis may progress up to 2 years post-exposure after which it generally stabilizes in the lung, although continued lung volume retraction may be observed up to 5 years after exposure.[16] Clinical signs and symptoms of radiation pneumonitis and/ or fibrosis include abnormal pulmonary function tests (e.g., DLCO, diffusing capacity for carbon monoxide; %FEV, percent change in forced expiratory volume), radiographic changes (e.g., ground glass opacification and increased lung density), and microscopic changes (e.g., increased alveolar wall thickness, mononuclear cell infiltration, proteinaceous alveolar, and interstitial edema, as well as collagen deposition and honey-comb scarring).

It is not uncommon for chronic lung fibrosis to present in the absence of acute pneumonitis, and the ability to dissociate fibrosis from pneumonitis through pharmacological intervention suggests these are 2 separate disease conditions.[17,18] As such, therapeutically targeting the mechanism of action to improve survival from radiation pneumonitis may require a different approach than mitigation and/ or prevention of debilitating lung injury arising from chronic, progressive fibrosis.

As at the time of writing this paper, there are no FDA approved MCMs to prevent, mitigate, and/ or treat sulfur mustard or radiation-induced lung injury. The lack of MCMs poses a major unmet medical need for which several academic researchers, early-stage biotech, and clinical-stage pharmaceutical companies are seeking to address through the development of novel therapeutics and repurposing (i.e., label expansion) of commercially available products.

Regulatory Considerations for Development of Early to Late-Stage Therapeutics as MCM for Sulfur-Mustard and Radiation-Induced Lung Disease

In the United States, MCMs may be approved by the FDA under a non-traditional regulatory pathway known as the Animal Rule ("AR"). The pathway permits licensure of MCMs when it is neither ethical nor feasible to conduct clinical trials in humans. Under the AR, MCMs may be approved based on demonstrated efficacy in adequate and well-controlled studies: (1) with appropriate randomization and blinding, (2) in 1 or more animal models that recapitulate the disease in humans, (3) with robust safety data in humans, and (4) sufficient pharmacokinetic (PK)/ pharmacodynamic (PD) data to allow for adequate therapeutic dose selection in humans. When these criteria are satisfactorily met, regulators reasonably assume demonstrated efficacy in animal models will translate to clinically meaningful benefit in humans.

To support product approval under the AR, therapeutic efficacy studies are run in trials like Phase 1 – 3 trials conducted in humans, but in surrogate animal models. Successfully meeting the criteria for approval can be arduous and time consuming with an average development time of 5-7 years for therapeutics with prior commercial approvals, and even longer for novel therapeutics not previously approved by any regulatory agency for any indication (Figure 2).

Generally, early development work to identify appropriate dose/ dose-schedule selection and elucidate the therapeutic mechanism of action against the disease caused by the challenge agent (e.g., chemicals, radiation) is performed in small animal models such as the mouse, rat, guinea pig, or rabbit. Model selection is based on the desired clinically meaningful endpoint for efficacy, usually major morbidity (e.g., debilitating lung disease) or mortality. Advanced development of chemical and radiation countermeasures are generally performed in swine or non-human primates (NHP).[19–25] PK/ PD studies are conducted under Good Laboratory Practice (GLP) regulations using validated assays in healthy and challenged animals to establish the best dose and

https://doi.org/10.1017/dmp.2023.180 Published online by Cambridge University Press

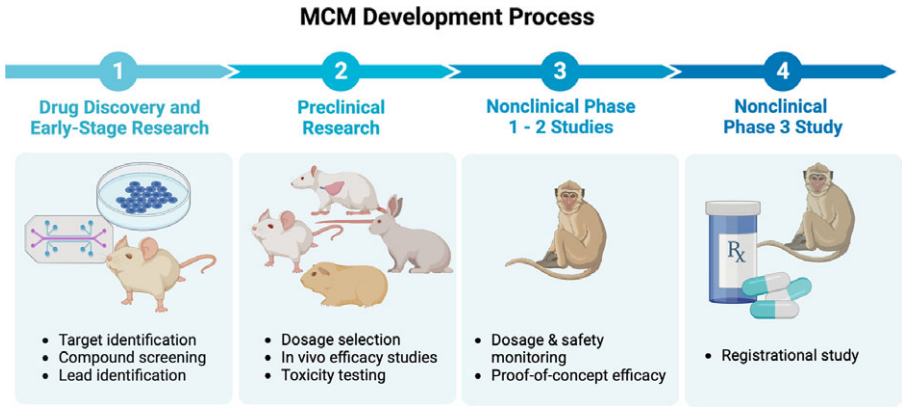

Figure 2. High-level overview of the medical countermeasure development process under the FDA Animal Rule regulatory pathway. Efficacy data in animal models demonstrating a significant improvement in mortality and/ or major morbidity as the primary endpoint must be supported by a robust database of safety in humans and sufficient PK/ PD data through PK - PD modeling and simulations to justify therapeutic dose selection in humans. This illustration was created with BioRender.com.

schedule for proof-of-concept efficacy evaluation in subsequent Phase 2, randomized, blinded, and placebo-controlled trials. Finally, the pivotal or registrational study is conducted in an adequate and well-controlled model to confirm the earlier evidence that a MCM is safe and effective. Approved MCMs for treatment of myelosuppression associated with ARS such as Neupogen®, Neulasta®, Nplate®, and Leukine® are considered current standard of care. Therefore, drug developers should include 1 of the approved MCMs in the registrational study design.

Animal Models of Sulfur-Mustard Induced Lung Disease

It would be highly unethical, and unfeasible, to evaluate MCM efficacy in humans due to the risk for debilitating morbidity, and mortality associated with chemical and radiological/ nuclear exposures. Therefore, it is intended that efficacy evaluation of MCMs is conducted in nonclinical trials in animal models in lieu of clinical trial in humans. As animal models serve as a human surrogate in these trials, the pathophysiological mechanism and natural history of disease progression and clinical manifestation must mirror that observed in humans. Registrational studies are required to be adequate and well-controlled, with appropriate randomization and blinding, and sufficiently powered to assess a clinically meaningful benefit against the primary endpoint (e.g., mortality/ morbidity).

The most common animal models used to interrogate the effect of chemical agents and/ or radiation on the lungs and screen promising MCM candidates for therapeutic efficacy are the mouse, rat, rabbit, and minipig, as well as swine (farm pig), canine, and NHP. Species/ strain and model selection is dependent on disease specificity and primary efficacy endpoint for evaluation. The rat, guinea pig, swine, and NHP are the species of choice for evaluation of MCMs to mitigate and/ or treat lung damage caused by sulfur mustard vapor exposure. Sulfur mustard is a highly reactive vesicating agent. Studies have shown spontaneously breathing swine exposed to sulfur mustard vapor in air at a dose of 100 - 150 mg/kg develop hypoxic lung injury with respiratory acidosis and necrosis and erosion of the tracheal epithelium within 6 hours post-challenge.[19] To avoid acute morbidity/ mortality resulting from nasopharynx toxicity (e.g., edema and necrosis), sulfur mustard is delivered via intratracheal installation into the lower respiratory tract to induce acute lung injury in the experimental setting. This artifact of the animal model impacts the ability to assess MCM efficacy against sulfur mustard-induced lung injury commonly observed in humans. Intratracheal administration allows deposition into the lower respiratory tract and avoids this common laboratory pitfall.

A sulfur mustard exposure system for delivering controlled/ accurate, and quantifiable concentrations to the lower respiratory tract of the male Sprague Dawley rat was developed by Perry, Yeung, and colleagues.[26] In the Perry study, animals ($n = 4 - 5$/ group) were exposed to sulfur mustard vapor at a target dose of 0.30 mg/kg to 3.20 mg/kg. Study endpoints included overall survival on day 28, clinical exam, pulmonary function tests, and histopathology. Mortality (28- day) was observed at doses of 0.45 mg/kg or higher. In the Perry study, there were 2 waves of major morbidity/ mortality – an acute phase within the first 3 days post-challenge characterized by ALI (e.g., alveolar edema, alveolar epithelial necrosis, and alveolar hemorrhage, etc.), and a late phase approximately 22 - 28 days post-challenge, generally characterized by alveolar, septal/ interstitial, and pleural fibrosis more widespread than observed at earlier time points.[26] The Probit-estimated lethal dose for 50% of animals within the first 29 days was 0.80 mg/kg (95% confidence interval: 0.42, 1.18). Features of acute respiratory illness following inhalational exposure in the rodent model include a decrease in lung compliance and increase in resistance, fibrin cast formation within the bronchial tree, epithelial necrosis, and apoptotic cell death, as well as hemorrhage and necrosis of lymphoid tissue.[26]

Mortality and clinical pathology in Perry's model were consistent with that reported by Malaviya.[10] Microscopic exam of acute lung damage after sulfur mustard vapor inhalation revealed ulceration of proximal bronchioles and perivascular and peribronchiolar edema and proteinaceous alveolar exudate with entrapped inflammatory cells.[10] Limited studies have extended the follow-up time beyond 28 days in experimental models. Mishra et al. demonstrated sulfur mustard inhalation in Fisher 344 (F344) rats and cynomolgus macaques (i.e., NHP) induced acute and chronic inflammation, similar to that observed in humans.[27–30] Mishra followed cynomolgus macaques up to 60 days post- sulfur mustard inhalation. Those studies revealed an increase in Interleukin-17 (IL-17) positive cells in the areas of lung inflammation and fibrosis 60 days after sulfur mustard exposure.[30]

Swine are another common model for interrogating the effects of sulfur-mustard on the lower respiratory tract and MCM evaluation. The swine model presents several advantages to the

https://doi.org/10.1017/dmp.2023.180 Published online by Cambridge University Press

rodent model for sulfur-mustard induced lung toxicity. In the swine, physiological measurements are nearly identical to that in humans. The temporal progression to disease development in the swine model is more protracted compared to the rat, and cardiopulmonary and respiratory endpoints can be non-invasively evaluated longitudinally to monitor the therapeutic effect of candidate MCMs on clinical progression of disease. However, in this model, there are natural variations in cardiopulmonary output, which should be taken into consideration during data evaluation.

Animal Models of Radiation-Induced Lung Disease

For radiation-induced lung injury, the most common experimental models are the rodent (e.g., mouse, rat) and the NHP. Species and strain differences in pulmonary response to radiation are well-characterized.[18] The most widely accepted small animal species/ strains for MCM development are the C57L/J mouse (i.e., leaden coat color, The Jackson Laboratory) and the WAG/ RijCmcr rat, a strain proprietary to the Medical College of Wisconsin.[31–33] For advanced research and development, including registrational studies, the Chinese-bred rhesus macaque has generally been considered the most suitable species as it most closely resembles the anticipated pulmonary response in humans.[34] However, the 2020 global outbreak of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), the virus that causes Coronavirus Disease 2019 (COVID-19) led to a severe reduction in the availability of NHP due to China's ban on wildlife exports, and redirection of animals to vaccine and therapeutic research.[35] As a result, the development of a cynomolgus macaque model of radiation-induced lung injury has become a national priority to advance product development programs and is underway at this time.

Federal agencies, including the FDA, have weighed in on the most suitable radiation geometry for induction of DEARE in the lung. While several models have been used including whole thorax lung irradiation and total body irradiation with a "top-off" dose, the most widely accepted model is partial body irradiation with 2.5% - 5% bone marrow sparing in mice and the NHP, respectively.[33] This model more closely mirrors the human scenario in which animals progressing through ARS develop DEARE-associated multiorgan dysfunction/ failure weeks to months after exposure. Thus, these models allow efficacy evaluation in a disease course most likely observed in humans and in the context of evolving multi-organ injury. One challenge, however, is the high rate of lethality from ARS in these models. As a result, studies must be sufficiently powered to evaluate all-cause mortality and/ or major morbidity at the study endpoint, generally 180 days post-exposure in mice and NHP. Some studies extend follow-up beyond 180-days (e.g., 220 days) to evaluate the durability of response.

In both the rodent and NHP models, the primary clinically relevant endpoint has traditionally been overall survival.[34,36] In the NHP, secondary endpoints include respiratory function (e.g., non-sedated respiratory rate), radiographic imaging (e.g., computed tomography), dexamethasone courses, and microscopic abnormalities on hematoxylin and eosin (H&E)- stained tissue sections and semi-quantitative analysis of lung fibrosis (e.g., Masson's trichrome-stained tissue sections).[34,36] Exploratory endpoints often include onset, duration, recovery from Grade 3 and Grade 4 neutropenia, and thrombocytopenia, as well as severe acute anemia, clinical chemistry (e.g., renal function), coagulopathy (e.g., fibrinogen, D-dimer), and histological abnormalities in other organs (e.g., gastrointestinal tract, spleen, kidneys, liver).[34,36]

Nonclinical MCM Development: Challenges and Opportunities

Drug development programs seeking marketing approval for MCMs under the AR are faced with non-traditional challenges. These include stringent and often unfamiliar regulatory hurdles, test system challenges inherent to animal research, integration of efficacy endpoints, and blood-based biomarkers to bridge animal data to clinical expectations. Therefore, it is recommended that companies meet early and often with the FDA to discuss the general investigation plan and regulatory path to approval.

The FDA product development under the Animal Rule guidance for industry does not specify a requirement for animal efficacy studies to be conducted in strict compliance with Good Laboratory Practice (GLP) regulations. However, the FDA does require assurance of the quality and integrity of raw data/ supporting documentation, facilities, and equipment, as well as results submitted to the agency in support of approval. The agency maintains authority to conduct inspections to verify data quality and integrity. With that in mind, it is generally assumed that registrational studies will be performed in compliance with the GLP except where reasonable limitations exist due to the nature of a challenge agent (e.g., sulfur mustard). Therefore, it is recommended that any deviations from the GLP regulations be discussed with the agency early during the development program.

Animal research and the safety of personnel operating in laboratory environments, including contract research organizations, are governed by the Office of Laboratory Animal Welfare (OLAW), US Department of Agriculture (USDA), and Occupational Safety and Health Administration (OSHA). Also, all animal research protocols require approval by the Institutional Animal Use and Care Committees (IACUC), which may interpret the OLAW regulations differently and impose their own requirements for limiting pain and distress in research animals. As a result, supportive care, and medical management of animals, as well as the protocol-specified criteria for humane euthanasia of animals may differ among independent laboratories.

Finally, clinical translation requires a suitable test system that reproducibly reflects the clinical symptoms and pathology associated with the disease in humans. Data integrity and reproducibility require control of biological (e.g., age, sex, weight), physical (e.g., controlled sulfur mustard or radiation dose delivery and assurances of the accuracy of dosing), and environmental variables (e.g., light/ dark cycles, humidity, food, and water), all of which may influence study outcomes. In radiation models, for example, sex and age differences significantly influence the pulmonary response including the relationship between the radiation dose and overall survival, temporal onset of respiratory dysfunction and mortality, and the severity of disease course. In contrast, overt sex, and age-differences in response to sulfur mustard exposure have not been observed based on the limited data available.

Primary and secondary endpoints selected for MCM efficacy evaluation should correlate with the clinically meaningful benefit desired in humans. In some cases, particularly in rodent models, identifying acceptable clinical correlates can be challenging. Non-invasive respiratory measurements using available technology often generates raw data that is highly variable (i.e., "noisy") due to rodent activity (e.g., sniffing) that creates difficulty in data interpretation particularly when the therapeutic effect is subtle. All these challenges underscore the necessity to meet with the FDA early and often in MCM development programs to gain alignment

https://doi.org/10.1017/dmp.2023.180 Published online by Cambridge University Press

on model suitability and study endpoints for the desired clinical indication.

Conclusions

This review is a high-level summary of the clinical manifestations and molecular mechanisms underlying acute lung injury and late pulmonary complications from chemical (i.e., sulfur mustard), radiological, and nuclear exposures, and regulatory considerations for MCM development programs. Acute pulmonary complications occur on the order of days following sulfur mustard exposure or weeks following radiation exposure. Delayed complications such as fibrosis occur months to years after sulfur mustard or radiation exposure and are characterized by chronic, often debilitating, lung injury. The acute and delayed complications can be considered 2 separate conditions that likely will require different therapeutic interventions. Currently, there is no FDA approved product to mitigate and/ or treat these injuries to improve the likelihood of survival or mitigate long-term, debilitating disease among survivors.

Acknowledgements. Doyle-Eisele and Jackson wish to thank the sponsors of the Trans-Agency Workshop including the Radiation/ Nuclear Countermeasures Program within the National Institute of Allergy and Infectious Diseases (NIAID)/ National Institutes of Health, Biomedical Advanced Research and Development Authority (BARDA), and US Food and Drug Administration (FDA) for the invitation to speak at the Trans-Health and Human Services (HHS) Workshop on " Overlapping Science in Radiation and Sulfur Mustard Exposures of Skin and Lung: Consideration of Models, Mechanisms, Organ Systems, and Medical Countermeasures" held from January 13 to 14, 2022.

Author contribution. MDE and ILJ wrote and revised the manuscript based on their respective presentations at the Trans-Health and Human Services (HHS) Workshop on "Overlapping Science in Radiation and Sulfur Mustard Exposures of Skin and Lung: Consideration of Models, Mechanisms, Organ Systems, and Medical Countermeasures" held from January 13 to 14, 2022.

Funding support. The authors do not have any specific contract or research grant from any funding agency in the public, commercial, or not-for-profit sectors to report.

Abbreviations. ARS, Acute Radiation Syndrome; CBRN, Chemical, Biological, Radiological, Nuclear; CFR, Code of Federal Regulations; DEARE, Delayed Effects of Acute Radiation Exposure; FDA, Food and Drug Administration; Gy, Gray (equivalent to 1 joule of energy absorbed per kilogram of organ or tissue weight); HD, Sulfur Mustard; MCM, Medical Countermeasure; US, United States

References

1. **Rimpler-Schmid A, Trapp R, Leonard S, *et al.*** *EU preparedness and responses to chemical, biological, radiological, and nuclear (CBRN) threats*. European Parliament, Juillet; 2021.
2. **Maslove DM, Tang B, Shankar-Hari M, *et al.*** Redefining critical illness. *Nat Med.* 2022;28(6):1141-1148. doi: 10.1038/s41591-022-01843-x
3. **Yeung DT, Harper JR, Platoff GE Jr.** The National Institutes of Health Chemical Countermeasures Research Program (NIH CCRP): a collaborative opportunity to develop effective and accessible chemical medical countermeasures for the American people [published online ahead of print, 2020 Jun 22]. *Drug Dev Res.* 2020;10.1002/ddr.21707. doi: 10.1002/ddr.21707
4. **Darchini-Maragheh E, Balali-Mood M.** Delayed complications, and long-term management of sulfur mustard poisoning: recent advances by Iranian researchers (Part I of II). *Iran J Med Sci.* 2018;43(2):103-124.
5. **Roecker S, Roth N.** Why al-Zawahiri's death should focus attention on nuclear terrorism—foreign and domestic. *Bulletin of the Atomic Scientists.* 2022. https://thebulletin.org/2022/08/why-al-zawahiris-death-should-focus-attention-on-nuclear-terrorism-foreign-and-domestic/
6. **Bentzen SM.** Preventing or reducing late side effects of radiation therapy: radiobiology meets molecular pathology. *Nat Rev Cancer.* 2006;6(9):702-713. doi: 10.1038/nrc1950
7. **Schaue D, Kachikwu EL, McBride WH.** Cytokines in radiobiological responses: a review. *Radiat Res.* 2012;178(6):505-523. doi: 10.1667/RR3031.1
8. **Grip L, Hart J.** *The use of chemical weapons in the 1935–36 Italo-Ethiopian War*. SIPRI; 2009:8.
9. **Balali-Mood M, Hefazi M.** Comparison of early and late toxic effects of sulfur mustard in Iranian veterans. *Basic Clin Pharmacol Toxicol.* 2006;99(4):273-282. doi: 10.1111/j.1742-7843.2006.pto_429.x
10. **Malaviya R, Abramova EV, Rancourt RC, *et al.*** Progressive lung injury, inflammation, and fibrosis in rats following inhalation of sulfur mustard. *Toxicol Sci.* 2020;178(2):358-374. doi: 10.1093/toxsci/kfaa150
11. **Aliannejad R. *Comment on "Bronchiolitis Obliterans and Pulmonary Fibrosis after Sulfur Mustard Inhalation in Rats" by* McGraw MD, Dysart MM, Hendry-Hofer TB, *et al.*** *Am J Respir Cell Mol Biol.* 2018;59(2):273-274.
12. **Alexander SF.** Authors alone are responsible for opinions expressed in their contributions. *The Military Surgeon.* 1947;101(1):1-17.
13. **Emad A, Rezaian GR.** The diversity of the effects of sulfur mustard gas inhalation on respiratory system 10 years after a single, heavy exposure: analysis of 197 cases. *Chest.* 1997;112(3):734-738. doi: 10.1378/chest.112.3.734
14. **Hefazi M, Attaran D, Mahmoudi M, *et al.*** Late respiratory complications of mustard gas poisoning in Iranian veterans. *Inhal Toxicol.* 2005;17(11):587-592. doi: 10.1080/08958370591000591
15. **Baranov AE, Selidovkin GD, Butturini A, *et al.*** Hematopoietic recovery after 10-Gy acute total body radiation. *Blood.* 1994;83(2):596-599.
16. **Boerma M, Davis CM, Jackson IL, *et al.*** All for one, though not one for all: team players in normal tissue radiobiology. *Int J Rad Biol.* 2022;98(3):346-366.
17. **Movsas B, Raffin TA, Epstein AH, *et al.*** Pulmonary radiation injury. *Chest.* 1997;111(4):1061-1076. doi: 10.1378/chest.111.4.1061
18. **Dabjan MB, Buck CM, Jackson IL, *et al.*** 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(9):936-949. doi: 10.1038/labinvest.2016.76
19. **Fairhall SJ, Jugg BJ, Read RW, *et al.*** Exposure-response effects of inhaled sulfur mustard in a large porcine model: a 6-h study. *Inhal Toxicol.* 2010;22(14):1135-1143. doi: 10.3109/08958378.2010.527398
20. **Farese AM, Cohen MV, Katz BP, *et al.*** Filgrastim improves survival in lethally irradiated nonhuman primates. *Radiat Res.* 2013;179(1):89-100. doi: 10.1667/RR3049.1
21. **Hankey KG, Farese AM, Blaauw EC, *et al.*** Pegfilgrastim improves survival of lethally irradiated nonhuman primates. *Radiat Res.* 2015;183(6):643-655. doi: 10.1667/RR13940.1
22. **Jugg BJ, Hoard-Fruchey H, Rothwell C, *et al.*** Acute gene expression profile of lung tissue following sulfur mustard inhalation exposure in large, anesthetized swine. *Chem Res Toxicol.* 2016;29(10):1602-1610. doi: 10.1021/acs.chemrestox.6b00069
23. **Bunin DI, Bakke J, Green CE, *et al.*** Romiplostim (Nplate®) as an effective radiation countermeasure to improve survival and platelet recovery in mice. *Int J Radiat Biol.* 2020;96(1):145-154. doi: 10.1080/09553002.2019.1605465
24. **Clayton NP, Khan-Malek RC, Dangler CA, *et al.*** Sargramostim (Rhu GM-CSF) improves survival of non-human primates with severe bone marrow suppression after acute, high-dose, whole-body irradiation. *Radiat Res.* 2021;195(2):191-199. doi: 10.1667/RADE-20-00131.1
25. **Weber W.** Development of a sulfur mustard non-human primate inhalation exposure model. *Society of Toxicology*. San Francisco, CA; 2021.
26. **Perry MR, Neal M, Hawks R, *et al.*** A novel sulfur mustard (HD) vapor inhalation exposure model of pulmonary toxicity for the efficacy evaluation

of candidate medical countermeasures. *Inhal Toxicol.* 2021;33(6-8): 221-233. doi: 10.1080/08958378.2021.1951401

27. **Aghanouri R, Ghanei M, Aslani J, *et al.*** Fibrogenic cytokine levels in bronchoalveolar lavage aspirates 15 years after exposure to sulfur mustard. *Am J Physiol Lung Cell Mol Physiol.* 2004;287(6):L1160-L1164. doi: 10.1152/ajplung.00169.2003
28. **Emad A, Emad Y.** Levels of cytokine in bronchoalveolar lavage (BAL) fluid in patients with pulmonary fibrosis due to sulfur mustard gas inhalation. *J Interferon Cytokine Res.* 2007;27(1):38-43. doi: 10.1089/jir.2006.0084
29. **Emad A, Emad Y.** Relationship between eosinophilia and levels of chemokines (CCL5 and CCL11) and IL-5 in bronchoalveolar lavage fluid of patients with mustard gas-induced pulmonary fibrosis. *J Clin Immunol.* 2007;27(6):605-612. doi: 10.1007/s10875-007-9114-y
30. **Mishra NC, Rir-sima-ah J, Grotendorst GR, *et al.*** Inhalation of sulfur mustard causes long-term T cell-dependent inflammation: possible role of Th17 cells in chronic lung pathology. *Int Immunopharmacol.* 2012;13(1):101-108. doi: 10.1016/j.intimp.2012.03.010
31. **DiCarlo AL, Jackson IL, Shah JR, *et al.*** Development and licensure of medical countermeasures to treat lung damage resulting from a radiological or nuclear incident. *Radiat Res.* 2012;177(5):717-721. doi: 10.1667/rr2881.1
32. **Fish BL, MacVittie TJ, Gao F, *et al.*** Rat models of partial-body irradiation with bone marrow-sparing (leg-out PBI) designed for FDA approval of countermeasures for mitigation of acute and delayed injuries by radiation. *Health Phys.* 2021;121(4):419-433. doi: 10.1097/HP.0000000000001444
33. **Satyamitra MM, Cassatt DR, Marzella L.** A trans-agency workshop on the pathophysiology of radiation-induced lung injury. *Radiat Res.* 2022;197(4):408-414. doi: 10.1667/RADE-21-00153.1
34. **MacVittie TJ, Farese AM, Parker GA, *et al.*** 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(5):559-587. doi: 10.1097/HP.0000000000001346
35. **Federal Register USA**. *Nonclinical considerations for mitigating nonhuman primate supply constraints arising from the Covid-19 pandemic: guidance for industry; availability*. Food and Drug Administration; 2022. https://www.federalregister.gov/documents/2022/02/24/2022-03915/nonclinical-considerations-for-mitigating-nonhuman-primate-supply-constraints-arising-from-the
36. **MacVittie TJ, Farese AM, Parker GA, *et al.*** 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(3):366-382. doi: 10.1097/HP.0000000000000968

https://doi.org/10.1017/dmp.2023.180 Published online by Cambridge University Press