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Commentary

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Overlapping Science in Radiation and Sulfur Mustard Exposures of Skin and Lung: Consideration of Models, Mechanisms, Organ Systems, and Medical Countermeasures: Overlapping science in radiation and sulfur mustard injuries to lung and skin

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Abstract

Purpose: To summarize presentations and discussions from the 2022 trans-agency workshop titled "Overlapping science in radiation and sulfur mustard (SM) exposures of skin and lung: Consideration of models, mechanisms, organ systems, and medical countermeasures."
Methods: Summary on topics includes: (1) an overview of the radiation and chemical countermeasure development programs and missions; (2) regulatory and industry perspectives for drugs and devices; 3) pathophysiology of skin and lung following radiation or SM exposure; 4) mechanisms of action/targets, biomarkers of injury; and 5) animal models that simulate anticipated clinical responses.
Results: There are striking similarities between injuries caused by radiation and SM exposures. Primary outcomes from both types of exposure include acute injuries, while late complications comprise chronic inflammation, oxidative stress, and vascular dysfunction, which can culminate in fibrosis in both skin and lung organ systems. This workshop brought together academic and industrial researchers, medical practitioners, US Government program officials, and regulators to discuss lung-, and skin- specific animal models and biomarkers, novel pathways of injury and recovery, and paths to licensure for products to address radiation or SM injuries.
Conclusions: Regular communications between the radiological and chemical injury research communities can enhance the state-of-the-science, provide a unique perspective on novel therapeutic strategies, and improve overall US Government emergency preparedness.

Study background

At the end of the American-Soviet Cold War in 1991, an era during which the threat of radiation and chemical attacks was ever-present, research and development of products for public health preparedness to address injuries resulting from these agents was mostly stalled. Driven by the Department of Defense, medical countermeasures (MCMs) research was focused primarily on protecting military personnel on the battlefield. However, following the events of September 11 2001, the US Government recognized the need to re-establish civilian-focused, and counterterrorism MCM development programs under the leadership of the Department of Health and Human Services (HHS), to ensure national medical preparedness in case of a chemical, biological, radiological, or nuclear (CBRN) incident.

In 2004, the National Institute of Allergy and Infectious Diseases (NIAID) was tasked with oversight of both the Chemical Countermeasures Research Program (CCRP) and the Radiation and Nuclear Countermeasures Program (RNCP). Both organizations are components of the broader civilian biodefense research effort within HHS and the National Institutes of Health (NIH). The overarching goal of the CCRP is to integrate cutting-edge research with the latest technological advances in science and medicine to enhance the nation's medical response

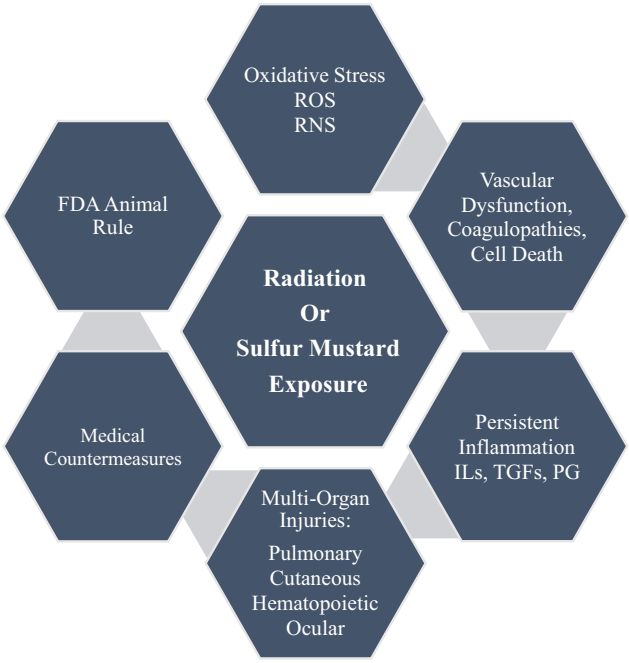

Figure 1. A representation of the overlap in radiation- and sulfur mustard-induced early and delayed multiorgan injuries, and the regulatory pathways for approval of medical countermeasures/ therapeutics. ROS/ RNS- reactive oxygen species/ reactive nitrogen species.

capabilities during public health emergencies involving the release of chemical threat agents. Many of these Department of Homeland Security (DHS)-identified chemical threats are not only potential agents of terrorism, but also may be released from transportation and storage facilities during industrial accidents or natural disasters. Similarly, the RNCP was directed to develop a robust research program to accelerate the development and deployment of bio-dosimetry devices and MCMs to assess and mitigate/ treat radiation injuries resulting from a radiological or nuclear public health emergency. Apart from a shared mission to protect the public from unanticipated health threats, there are several commonalities in the radiation and chemical injury research space (Figure 1), such as animal models, and endpoints; pathology of injury to skin and lungs; MCMs under study; and the Food and Drug Administration (FDA) Animal Rule (AR) licensure pathway to drive the regulatory licensure or approval of drugs (Subpart I-21 CFR Parts 314.6000.650) and biologicals (Subpart H-21 CFR Parts 601.90-95).

To further understand these similarities and learn from successes in each space, the NIAID RNCP and CCRP, in collaboration with the Biomedical Advanced Research and Development Authority (BARDA) Radiological and Nuclear Countermeasures Branch, Thermal Injury Program, and Chemical Countermeasures Program, hosted a 2- day workshop on January 13 - 14, 2022 titled "Overlapping Science in Radiation and Sulfur Mustard Exposures of Skin and Lung: Consideration of Models, Mechanisms, Organ Systems, and Medical Countermeasures." The goals of this workshop were to examine pathologies in pulmonary and cutaneous injuries following chemical or radiological/ nuclear insults; discuss animal models and MCMs under study in both fields; and identify existing gaps, challenges, and needs for translational application in both mission spaces. Given the enormous scope of chemical threat agents, only sulfur mustard (SM)-induced injuries to skin and lung were considered for this workshop. The workshop brought together academic and industrial researchers, medical practitioners, US Government program officials, and regulators to discuss facets of drug development, lung-, and skin-specific animal models, and biomarkers, as well as paths to licensure for products involving radiation or chemical injuries to the skin and lungs. An outcome of this workshop was data sharing between these research communities, identification of commonalities of injuries and novel intervention targets, and acknowledgment of the regulatory pathways for clearance or approval of MCMs focused on radiation, and/ or SM threats.

Session topics centered on: (1) an overview of the radiation and chemical countermeasure development programs and mission priorities; (2) regulatory and industry input on the regulatory pathways for the development of MCMs; (3) organ systems (primarily lung and skin), and pathophysiology following exposure to radiation or SM; (4) mechanisms of action/ targets of radiation or SM-induced lung and skin injuries, biomarkers, and mechanisms of injury; and (5) laboratory animal models in radiation and SM research that simulate anticipated clinical response. This report summarizes the talks presented by subject matter experts (Table 1), and the main points brought forward during panel and participant conversations. It is however, not a comprehensive review of all chemical threat agents, radiological incidents, or all organ systems, models, and MCMs. Where unpublished data is shared, the presenter's name is provided in parentheses (with their permission). Although each session had a separate panel discussion, to allow for better readability, content derived from all the sessions has been aligned and combined into a single overall discussion at the end of this report.

Session I: Setting the Stage

To bring context to the funding history for both radiation and chemical insults, the first presentations of the meeting covered the role of the different government funding agencies in the development of MCMs for both threats, spanning early, basic research through advanced development.

NIAID's history of shared interest between radiation and chemical defense portfolios (A DiCarlo, D Yeung)

There has long been a partnership between US Government funding agencies involved in MCM development for use during a public health emergency. This has included biological pathogens, as well as radiation and chemical injuries. In some instances, government funding has also been provided to address non-emergency exposures (e.g., to radiation or chemicals during cancer treatment, space flight, or in military situations). Since 2005, an annual budget appropriation has been provided to the NIH Office of the Director, executed by NIAID, and shared between NIAID's efforts to identify MCMs for radiation,[1] (through the RNCP) and chemical (through the CCRP) injuries.[2] Although funding was originally imagined to support areas of science with little overlap, over the years, these programs have identified shared scientific mission spaces, which has resulted in the groups joining together to leverage their investments in several scientific areas. Examples of overlap between the programs are identified as (1) similar considerations for how an emergency response would be undertaken (e.g., post-exposure treatments and response timelines, stockpiling needs, expectations of scarce resources, and the desire to develop MCMs with utility for multiple threats as well as existing, non-MCM clinical indications); (2) need for small and large laboratory models to determine product efficacy and

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Table 1. Workshop speakers and their areas of expertise[a]

Name	*Affiliation*	*Area of expertise*
Dina Andrews	Amgen Inc., Thousand Oaks, CA	Drug discovery, translational science, pathology
Peter Antinozzi	Argentum Medical, Geneva, IL	Regulatory affairs, cutaneous SM and radiation injury, devices
Vikhyat Bebarta	University of Colorado, Boulder, CO	Translational research, sulfur mustard models, mass casualty
Shampa Chatterjee	University of Pennsylvania, Philadelphia, PA	Radiation-induced lung injury, vascular dysfunction, MCM
Claire Croutch	MRI Global, Kansas City, MO	Sulfur mustard dermal injury, wound dressing, biotechnology
Brian Day	National Jewish Health, Denver, CO	Radiation and sulfur mustard, lung injuries, MCM development
Andrea DiCarlo	NIAID, NIH, Rockville, MD	Radiation threats, NHP, product development, and MCMs
Melanie Doyle-Eisele	LBRI, Albuquerque, NM	Models of radiation and sulfur mustard injury, lung, skin
Joe 'Skip' Garcia	University of Arizona, Tucson, AZ	Radiation and sulfur mustard, lung injury, immune-therapeutics
Allan Guan	CDRH, FDA, White Oak, MD	Medical devices, lab-on-a-chip, dermal wounds, and devices
Mary Homer	BARDA, HHS, Washington DC	Radiation, NHP, MCM testing, and advanced development
Carol Iddins	REACT/S, Oak Ridge, TN	Cutaneous radiation injury, clinical expertise
James James	University of Georgia, Athens, GA	Disaster medicine, public health preparedness
Lauren Jackson	University of Maryland, Baltimore, MD	Radiation-induced lung injury, animal models, MCM
Judith Laney	BARDA, HHS, Washington, DC	Chemical threats, MCM, advanced development
Debra Laskin	Rutgers University, Brunswick, NJ	Sulfur mustard lung injury, novel targets, MCM
Jeffery Laskin	Rutgers University, Brunswick, NJ	Sulfur mustard, cutaneous injury, novel targets
Kurt Lu	Northwestern University, Evanston, IL	Sulfur mustard skin injury, inflammation, MCM research
Libero Marzella	CDER, FDA, White Oak, MD	Regulatory development of MCM, animal rule
Andrea Powell	CTECS, CDER, FDA, White Oak, MD	Emergency health response, animal rule
Julie Ryan	University of Rochester, Rochester, NY	Cutaneous radiation injury, dermatology, clinical outcome
Livia Veress	University of Colorado, Aurora, CO	Sulfur mustard, pulmonary damage, airway thrombosis
Carl White	University of Colorado, Aurora, CO	Acute and chronic sulfur mustard injury, inhalation, airways
Dave Yeung	NIAID, NIH, Rockville, MD	Chemical threats, preparedness, models MCM

[a]All speakers had the opportunity to review this meeting's report prior to journal submission.

human safety protocols; (3) similar disease states (e.g., acute and chronic concerns such as fibrosis, inflammation, and chronic oxidative stress); (4) the same regulatory requirements for MCM development; and (5) similar organ systems of interest (e.g., lung, skin, bone marrow, and central nervous system).

Considering the funded portfolios of both NIAID programs, it is clear that there are scientific approaches that could be operable in both areas, including biomarker assessment for triage, patient management, prognosis, and efficacy; investigation into molecular pathways to determine druggable targets and identify mechanisms of action of both threat agents and potential MCMs; and classes of treatment investigated, such as anti-apoptotics, anti-inflammatories, anti-oxidants, anti-fibrotics, and cytokines/growth factors.[3]

Both the RNCP and CCRP have had significant achievements in their relevant fields. For example, funding from the RNCP led directly to US FDA approval of 3 drugs to increase survival in patients acutely exposed to myelosuppressive doses of radiation, resulting in hematopoietic Acute Radiation Syndrome (H-ARS) (Neupogen®,[1] Neulasta®,[2] and Nplate®,[3] Amgen). In addition, the RNCP has met with hundreds of companies and has invested in more than 600 compounds to address radiation injuries to several different organ systems. The program has a robust pipeline of funding to address other areas of concern during a radiation emergency, including the development of de-corporation agents that are amenable to mass casualty use, and the identification of biomarkers of radiation injury to triage and guide patient management and predict severity of late radiation-induced health outcomes.[4] The CCRP, which focuses on discovery research and early development, has supported the development of many animal models,[5] including those addressing ocular,[6] hematologic,[4] and lung[5] injuries arising from SM exposure. The program has also provided guidance and funding to allow the transition of many products to BARDA for further development. Product transitions to date have included galantamine (FDA-approved treatment for Alzheimer's, under study as a neuroprotective nerve agent MCM)[6]; midazolam[7] (epilepsy drug to treat seizures resulting from nerve agent exposure)[7]; tissue plasminogen activator[8] (drug to break up clots in stroke patients, repurposed to address SM-induced airway blockage; R-107[5] (a novel nitric oxide donor compound), and a TRPV4 channel blocker[9] to address inhalational chlorine injuries. Other product transitions include tezampanel and ganaxolone[10] (as novel anticonvulsant and neuroprotectant for nerve agent toxicity)[8]; and INV-102[11] (Invirsa, a p53 modulator regulating DNA damage response for SM-induced eye injuries. Lastly, the CCRP works closely with the BARDA chemical countermeasures

[1]https://www.fda.gov/emergency-preparedness-and-response/about-mcmi/fda-approves-radiation-medical-countermeasure

[2]http://www.accessdata.fda.gov/drugsatfda_docs/label/2015/125031s180lbl.pdf

[3]https://www.niaid.nih.gov/news-events/niaid-funded-research-leads-approval-drug-acute-radiation-injury

[4]https://www.niaid.nih.gov/sites/default/files/radnucstrategicplan.pdf

[5]https://www.nei.nih.gov/grants-and-training/funding-opportunities/programs-and-research-priorities/counteract-ocular-therapeutics-program

[6]https://www.nei.nih.gov/grants-and-training/funding-opportunities/programs-and-research-priorities/counteract-ocular-therapeutics-program

[7]https://www.jpeocbrnd.osd.mil/Media/News/Article/2594007/fda-approval-of-anti-seizure-drug-provides-a-new-tool-for-protecting-americans/

[8]https://www.phe.gov/Preparedness/news/Pages/clot-busting-150923.aspx

[9]https://www.phe.gov/Preparedness/news/Pages/gsk-chem.aspx

[10]https://www.hhs.gov/about/news/2018/04/27/hhs-partners-develop-new-treatment-seizures-caused-nerve-agents.html

[11]https://www.medicalcountermeasures.gov/newsroom/2020/invirsa/

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program to administer *in vivo* screening programs to identify potential MCM candidates for SM-induced toxicities.

Although the chemical focus of the current meeting was SM, close to 200 chemicals and 12 toxidromes have been identified by the US Department of Homeland Security Probabilistic Analysis for National Threats Hazards and Risks (PANTHR) program,[12] as high consequence public health chemical threats of concern, including other pulmonary (e.g., chlorine and phosgene), and skin (e.g., nitrogen mustard, Lewisite, arsenicals)- targeted compounds; pharmaceutical-based agents (e.g., synthetic opioids), inhibitors of cellular respiration (e.g., cyanide and hydrogen sulfide); anticoagulants (e.g., brodifacoum and bromadiolone); and neurological-affective compounds (e.g., nerve agents or organophosphate pesticides). Similarly, the scope of work funded by the NIAID RNCP extends beyond just lung and skin models and MCM development, to the support of MCMs for hematopoietic (H-), and gastrointestinal acute radiation syndrome (GI-ARS), cutaneous radiation injuries (CRI, both alone and in combination with other trauma), cardiovascular, and kidney, as well as central nervous system damage. Both NIAID programs also have a primary interest in repurposing products that already have FDA approval/ licensure for another clinical indication.[9] To streamline this meeting, the programs elected to focus on similarities between radiation exposure and SM-induced injuries to the lung and skin; although arguably, the case could also have been made to include bone marrow myelosuppression. However, to keep the meeting focused and manageable in terms of length, these other areas of study were not included.

BARDA radiation and chemical programs for advanced product development (J Laney, M Homer)

The radiation and chemical programs implemented by the BARDA have historically been responsible for advanced development of candidate MCMs. The programs have recently taken a more systems biology approach to identify therapeutics for both radiation and chemical injuries. For example, the Chemical Countermeasures Program focuses on treating the injury, not the threat agent. Their stated mission is "improving the health outcome for all victims of chemical exposure." Aspects that are unique to their mission include the fact that there are many agents of concern, and exposure to these chemicals will be unpredictable and localized. In addition, due to the rapid action of many chemical agents and the impracticality to do pre-treatment, centralized stockpiling may not be feasible, hence necessitating forward deployment of treatments. Finally, there is a lack of available diagnostics, making early triage of specific injuries difficult. To address these challenges and ensure that chemical MCMs are available in an emergency, BARDA has developed a strategy that employs a threat-agnostic pipeline (with strong NIAID collaboration) and focuses on repurposing common drugs. In that way, drugs are already in place with end-users who are familiar with their use. Examples of their successful repurposing efforts have included Silverlon® wound dressing (Argentum Pharmaceuticals), which obtained FDA clearance in 2019 for vapor dermal SM injuries[13]; and coordinated funding with the Department of Defense and NIH leading to FDA approval of Seizalam (midazolam, Meridian Medical Technologies, Inc.) in 2018 for status epilepticus seizures (including those resulting from nerve agent exposure). Argentum's Silverlon received FDA's 510K clearance for radiation dermatitis and cutaneous radiation injury (CRI) subsequent to this workshop but are mentioned here for the sake of completeness.[14] Other drugs being considered for repurposing include those to address chlorine-induced lung injuries, opioid-induced respiratory depression, and both SM ocular and inhalational injuries. Finally, BARDA's chemical program has partnered with the University of Hertfordshire, UK, and first responders across the US to create the second edition of Primary Response Incident Scene Management (PRISM) Guidance for Mass Decontamination.[15] This valuable resource provides information on operational responses to chemical incidents, with new procedures that remove most contamination, even before first responders arrive while requiring no special equipment, or products.

The BARDA Radiological/ Nuclear Countermeasures Program has followed a similar approach within their portfolio. Awarded contracts, which include approaches addressing radiation-induced vascular injuries, sepsis, and coagulopathy, as well as fibrinolysis, and cell death are the largest part of the program, which also focuses on ischemic injuries and inflammation as key components of irradiation's bodily impact. The program continues to support both polypharmacy and repurposing approaches, building on a successful track record of working with companies to provide advanced development support and stockpile acquisition of necessary therapeutics for radiation injuries. BARDA funding led to the FDA approval of Leukine® to treat H-ARS (Partner Therapeutics, originally Sanofi),[10] and had a role in regulatory and procurement-related activities for the other 3 approved H-ARS approaches mentioned above. The program also looks to leverage across other threat areas, including mechanical trauma, thermal burn, and chemical injuries, as well as infectious diseases, to maximize government investments and allow for multi-utility approaches to be pursued. BARDA's current focus areas include radiation-induced platelet loss, endotheliopathies, and inflammation. BARDA is also tasked with ensuring sustainable supplies of both traditional and next-generation blood products, such as spray-dried blood plasma.[16] Recently, an interest in enabling technologies like tissue chip platforms led to co-funding of contracts on *in vitro* micro-physiological systems (MPS) with the National Aeronautics and Space Administration (NASA), given the overlapping missions of irradiation-induced health effects and their mitigation. The NIAID RNCP also supports a contractor with NASA for MPS development.

About the journal selection for a special issue (J James)

The Disaster Medicine and Public Health Preparedness Journal has had a long history of interactions with the CBRN communities, so it was an excellent choice for a journal to reach both radiation and chemical defense agencies and investigators. For example, in 2011, a special issue of the journal was published on nuclear preparedness, and the papers included therein continue to be important resources for researchers, funding, and regulatory agencies.[11–22] There have also been more than 50 manuscripts (as of this writing) published in the journal since 2007, which covered global radiation emergency preparedness, radiation scenario modeling, and other areas of interest.[23–27] Similarly, the journal has reported on findings and discussions surrounding

[12]https://www.dhs.gov/science-and-technology/panthr

[13]https://www.phe.gov/Preparedness/news/Pages/FDA-blister-injuries.aspx

[14]https://www.prweb.com/releases/silverlon_receives_fda_510k_clearance_for_radiation_dermatitis_and_cutaneous_radiation_injury/prweb19017793.htm

[15]https://www.medicalcountermeasures.gov/barda/cbrn/prism/

[16]https://www.phe.gov/Preparedness/news/Pages/barda-driedbloodplasma.aspx

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hazardous chemical materials, including but not limited to reports on preparedness,[28,29] training exercises,[30] case studies,[31,32] and review articles.[33,34] For these reasons, the meeting organizers began working with the journal at the very beginning of the process, leading to the publication of this meeting report and the special issue in which it resides.

In summary, all 4 NIAID and BARDA radiation and chemical medical defense programs routinely have contract and grant solicitations for the development of products to treat chemical and radiological injuries, and to ensure that there is a robust pipeline of approaches under consideration to address these threats. They intend to continue to coordinate to leverage government investments in both mission areas and provide guidance to enable multi-utility of approaches across the chemical and radiation spaces. Publication of this meeting report, and the special issue in which it resides, is a step along the way toward ensuring harmonization across the programs administered by these different government agencies.

Session II: Regulatory Issues –FDA & Industry

In this session, representatives from several parts of the U.S. FDA discussed different aspects of MCM development and regulation for products for a radiation and SM indications. These talks included an overview of the FDA's regulations commonly known as the Animal Rule, as well as insights into how to best approach the FDA for early MCM product development meetings for drugs, biologicals products, and devices. There was also a presentation of a case study for the first drugs to be approved under the AR for a radiation MCM indication, to provide a firsthand account of important aspects of the process.

MCM development under the FDA Animal Rule (A Powell)

There are specialized MCM groups within the FDA. These include the Office of Counterterrorism and Emerging Threats (OCET) (in the Office of the Commissioner); the CounterTerrorism and Emergency Coordination Staff (CTECS) within the Center for Drug Evaluation and Research (CDER); the Preparedness and Response Team in the Center for Biologics Evaluation and Research (CBER), and within the Center for Devices for Radiological Health (CDRH), the MCM group is known as All Hazards Readiness, Response, and Cybersecurity (ARC) (formerly known as Emergency Preparedness/ Operations and Medical Countermeasures EMCM). It is important to note that these groups do not have the regulatory authority over the MCMs; that authority lies with the regulatory review divisions.

In May 2002, the FDA published AR to allow (under very specific circumstances) the approval of new drugs and licensure of biological products when human efficacy studies are not ethical or feasible (21 CFR Parts 314.6000.650 for drugs and 21 CFR Parts 601.90-95 for biological products).[17] These regulations allow for product efficacy to be established based on adequate and well-controlled studies in animals "when the results of those animal studies establish that the drug product [*or the biological product*] is reasonably likely to produce clinical benefit in humans,"[18] and safety is evaluated "under preexisting requirements for establishing the safety of a new drug and biological products."[19]

For a product to be approved under the AR, all of the following criteria must be met: (1) there is a reasonably well-understood pathophysiological mechanism of the toxicity of the substance and its prevention or substantial reduction by the product; (2) the effect is demonstrated in more than 1 animal species expected to react with a response predictive for humans, unless the effect is demonstrated in a single animal species that represents a sufficiently well-characterized animal model for predicting the response in humans; (3) the animal study endpoint is related to the desired benefit in humans, generally the enhancement of survival or prevention of major morbidity; and (4) the data or information on the kinetics and pharmacodynamics of the product or other relevant data or information, in animals and humans, allows a selection of an effective dose in humans.[20] The FDA can also consider other information such as published human data.

As of January 2022, 16 products have been approved or licensed under the AR, with indications that can be grouped into 7 broad categories of severe, and life-threatening diseases or conditions resulting from exposure to CBRN agents.[21] In the labeling of these products, 7 have descriptions of animal efficacy studies in 2 species; 8, which also had relevant human efficacy data, have descriptions of efficacy studies in a single animal species; and 1 has descriptions of efficacy studies in more than 2 species. A critically important aspect of product development under the AR is the selection of the animal models in which the efficacy of the investigational product will be tested. The animal models should adequately reflect key elements of the human disease or condition and should be appropriate for use with the investigational product. FDA recommends that sponsors obtain agency concurrence on the animal models that will be used in efficacy testing of their investigational products. Animal model development is a resource intensive process. CDER's and CBER's Animal Model Qualification Program (AMQP) was created to support the development of product-independent animal models that will be used for testing multiple products under the AR.[22] This is a voluntary program, and the use of a qualified model is not required for AR approval. The AMQP provides a formal avenue to obtain FDA subject matter expert feedback on early animal model development. Qualified animal models are made publicly available.

FDA encourages early and ongoing communication, and sponsors should have a thorough understanding of the available guidance documents for developing products under the AR, such as the overarching guidance: 'Product Development Under the Animal Rule: Guidance for Industry,'[23] and any relevant indication-specific guidances.[24,25,26] FDA also developed a compliance program for the inspection of nonclinical laboratories conducting AR-specific studies, and it may be helpful for

[17]Federal Register Vol. 67, No. 105, 37988-37998, May 31, 2002

[18]21 CFR 314.610(a) for drugs and 21 CFR 601.91(a) for biological products

[19]Federal Register Vol. 67, No. 105, 37989, May 31, 2002

[20]The 4 criteria are direct quotations from 21 CFR 314.610(a) for drugs and 21 CFR 601.91(a) for biological products

[21]https://www.fda.gov/media/150191/download

[22]https://www.fda.gov/drugs/drug-development-tool-ddt-qualification-programs/animal-model-qualification-program-amqp

[23]https://www.fda.gov/regulatory-information/search-fda-guidance-documents/product-development-under-animal-rule

[24]https://www.fda.gov/regulatory-information/search-fda-guidance-documents/internal-radioactive-contamination-development-decorporation-agents

[25]https://www.fda.gov/regulatory-information/search-fda-guidance-documents/smallpox-variola-virus-infection-developing-drugs-treatment-or-prevention-guidance-industry

[26]https://www.fda.gov/regulatory-information/search-fda-guidance-documents/anthrax-developing-drugs-prophylaxis-inhalational-anthrax-guidance-industry

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stakeholders to understand how these studies will be inspected.[27] Regulatory review of product applications is an integrated, multidisciplinary review process under the director of the regulatory review division. A typical CDER review team for an MCM consists of reviewers and team leaders representing chemistry, manufacturing, and controls; pharmacology/ toxicology; clinical medicine; clinical pharmacology; statistics; regulatory project management; and other disciplines, as needed. CDER/ CTECS offers early informational meetings to academic investigators or sponsors of MCMs that will be regulated by CDER, to help them prepare for pre-Investigational New Drug (preIND) interactions with the review division. CTECS does not provide scientific or regulatory advice but does provide general information such as information about the AR, the regulatory review process, contact information for the appropriate review division, and expectations for preIND meetings, as well as meeting packages and useful resources.

Amgen's regulatory journey to drug approval for Acute Radiation Syndrome (D Andrews)

Amgen's success with the approval of Neupogen, Neulasta, and Nplate highlights the successful interactions between a sponsor, funding agency, and the FDA. Radiation exposure can result in ARS, where injury severity increases with increasing levels of exposure.[35] In response to the need for an MCM to treat H-ARS, Amgen partnered with NIAID and other agencies, and interacted with FDA to obtain approval for products to treat patients. In 2015, Neupogen (recombinant granulocyte-colony stimulating factor; G-CSF) (the first MCM), was approved by the FDA under the AR, in collaboration with NIAID and included in the National Strategic Stockpile. In the same year, Neulasta, a pegylated form of G-CSF was also approved to treat patients with H-ARS. In 2021, Nplate, a thrombopoietin receptor agonist indicated to treat patients with immune-mediated thrombocytopenia was also approved for the treatment of H-ARS.

These approvals were contingent on the criteria for approval under the AR described above, and it took 11 years for NIAID/ Amgen to develop Neupogen/ Neulasta as MCMs. The NIAID/ Amgen/ FDA interactions played a central role in this achievement, from influential feedback on: (1) animal model development (mouse and nonhuman primate (NHP) studies), (2) long-term patient safety concerns, and (3) pharmacological modeling of adult and pediatric doses in H-ARS. The key discussion points from the FDA joint meeting of the Medical Imaging Drugs Advisory Committee and Oncologic Drugs Advisory Committee leading to approval were: (1) mechanism of injury and repair in irradiated NHP translates to humans with H-ARS; (2) survival benefit in irradiated NHPs administered Neupogen,[36] or Neulasta,[37] translates to a survival benefit in humans; and (3) pharmacokinetic (PK)/ pharmacodynamic (PD) data from irradiated NHPs studies with Neupogen/Neulasta and Amgen's human patient data can identify an appropriate effective human dose through pharmacologic modeling. The committee was asked to deliberate and vote regarding Neupogen's clinical benefit in a radiation exposure emergency based on NIAID NHP efficacy study data and Amgen patient safety and efficacy data, resulting in a 17-1 vote in favor.

In 2021, no advisory committee was convened for Nplate approval. In contrast to prior product approvals, Nplate approval required only 5 years, with a more streamlined regulatory path based on the precedent set by Neupogen/ Neulasta. Pivotal NHP studies clearly demonstrated benefit in NHPs administered Nplate as well as comparable pharmacodynamics following exposure.[38] In addition to FDA's AR Guidance,[39] other guidelines that expedited radiation MCM approvals were the Standard for Exchange of Nonclinical Data (SEND) Implementation guide and the compliance program for the inspection of nonclinical laboratories conducting AR-specific studies.[28]

Based on these successful interactions, Amgen's recommendations for achieving drug approval under the AR regulations are: (1) invest in a well-defined and committed partnership/ collaboration with sponsoring government agencies that have appropriate biology and AR expertise, offer funding opportunities, and share in the mission to develop radiation MCMs for the US population; (2) develop a dedicated and experienced team of cross-functional subject matter experts with appropriate company sponsorship to meet project demands in time and resources; (3) seek early FDA agreement on overall non-clinical strategy especially when animal models are not well established; (4) maintain milestone meetings with the FDA to share progress/ results, design next steps, and ensure alignment on fulfillment of all the requirements of the AR prior to regulatory submission; (5) know and meet expectations under the FDA AR regulations in study designs and when authoring regulatory documents; and (6) publish/ share results to advance animal model development and promote additional drug approvals under the AR.

Development of drug products for treatment of Acute Radiation Syndromes (L Marzella)

For sponsors of SM-focused, or radiation MCMs, it is important to review the regulatory history of products approved for H-ARS and the scientific literature reports of studies of products under development for the treatment of skin and lung radiation syndromes. Also critical is the evaluation of available scientific knowledge for threat agents based on the analysis of fundamental mechanisms of organ injury and dysfunction caused by radiation or SM. Finally, consider the applicability of regulatory strategies for product development and approval adopted by developers of countermeasures for radiation threat agents.

Selection of a pharmacologic target is key. Characterization of the mechanism of action (MOA) of organ injury and dysfunction that is targeted by the MCM candidate is needed for animal efficacy studies. MOA for H-ARS, primarily myelosuppression with acute depletion of myeloid precursors, is well defined; approved MCMs stimulate proliferation, differentiation, and function of myeloid precursor cells.[40] Proposed MOAs for skin and lung radiation injuries can be more complex due to the potential for multi-organ dysfunction and a multi-stage clinical course with early acute edema and inflammation, delayed development of necrosis, followed by repair and fibrosis, hence the need for more complicated protocols.

It is important that the pathology in the selected animal system model the clinical condition. Natural history studies are necessary to establish the time course and manifestations of the injury caused by various levels of exposure to the threat agent, and the response to various doses of the MCM. CDER has relied on adequate and well-controlled efficacy studies in NHP models for the approval of H-ARS indications,[36,37] and data from rodent studies have provided key support for efficacy. Rhesus macaque is the standard NHP model for H-ARS and has been under development for lung

[27]https://www.fda.gov/inspections-compliance-enforcement-and-criminal-investigations/compliance-program-manual/bioresearch-monitoring-program-bimo-compliance-programs

[28]Chapter 48 Inspection of Nonclinical Laboratories Conducting Animal Rule-Specific Studies (fda.gov)

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injury. More recently, the lack of availability of rhesus related to COVID-19 is requiring validation of a cynomolgus macaque model in some instances. Porcine animal models are also under development for CRI.

Efficacy endpoints in animal models for radiation MCMs are primarily survival or reduction in major morbidity. It is advised that the conduct of studies in patients with organ injury and dysfunction caused by similar agents (e.g., chemotherapy) be done to obtain safety, and when possible supportive clinical efficacy data. Survival has been the primary animal efficacy endpoint for H-ARS and is under consideration for radiation-induced lung injury (RILI), but the utility of quantitative measures of lung function or anatomy is under evaluation.[41] For skin injury,[42] quantitative assessment of area and depth of skin injury and repair verified by histopathology is recommended as the primary animal efficacy endpoint, while clinically meaningful reductions in severe skin injury or improvement in healing, quality, and durability of repair, bridging to engraftment or reconstruction, and survival should be considered for safety assessment (e.g., in models of combined injury). Endpoints that characterize the recovery of organ injury or dysfunction, such as neutrophil or platelet counts that document recovery from myelosuppression, or positive microbial cultures, are secondary to survival and may be used to support of primary efficacy endpoint, trigger initiation of supportive care, and guide pharmacodynamic (PD) modeling for selection of human dose.

For the successful selection of an effective human dose of an MCM, it is important to conduct dose-ranging studies in animal models. Two approaches commonly used are the PK approach, where comparison of predicted drug exposure in affected humans to the animals receiving a fully effective dose, and the PD approach, where the drug exposure in humans results in a similar magnitude of PD marker in animal models. With these salient points, regulatory strategies for the development of drug products for radiation injuries may be similarly applicable to the development of products for SM-induced injury. A consult of the publicly available FDA drug approval packages and scientific literature will inform the product development pathway.

Repurposing Silverlon® dressings for treatment of cutaneous Sulfur Mustard (SM) injuries (P Antinozzi)

Another successful sponsor-FDA interaction is the repurposing of Silverlon dressing for the management of SM-induced cutaneous injury. Silverlon dressings are sterile, flexible, porous, and non-adherent, as well as knitted nylon plated with elemental silver and silver oxide.[29] The silver ions provide an antimicrobial barrier for the clinical management of various infected wounds (traumatic, surgical, first/ second degree thermal, and dermal ulcers) and vascular access. With BARDA support, Argentum has explored repurposing Silverlon dressings as an MCM for use in mass casualty incidents involving radiation and vapor SM exposure. The vapor SM indication was pursued as a non-inferiority study (silver sulfadiazine as comparator) against superficial and moderate partial-thickness wounds in Göttingen minipigs. Results of the study were used to support the eventual FDA clearance of Silverlon dressings in July 2019[30] as the first MCM indicated to manage SM-induced vesicant injuries not requiring skin grafting.[31] Ongoing efforts are geared toward obtaining similar FDA approval of Silverlon dressings as a radiation MCM. Nuclear detonations and dirty bombs presumably produce complex injuries comprised of blast injury, thermal burns, and radiation effects. Since Silverlon dressings are already indicated for traumatic wounds likely to result from blast injury, as well as both first- and second-degree thermal burns, if the product can mitigate CRI, then it may be an effective CRI MCM. As such, a 2-stage regulatory approach was undertaken where: Stage 1 utilized clinical data derived from radiotherapy patients supporting an indication for lower severity radiation injury,[32] and Stage 2 utilized preclinical data from a Yorkshire swine model to support a higher severity radiation injury indication. Silverlon dressings were granted Breakthrough Device designation in October 2021 for the proposed low and high severity radiation dermatitis indications from FDA's Center for Devices and Radiological Health (CDRH). As an update, after the meeting on which this report is based, Argentum obtained FDA clearance for the use of Silverlon in radiation dermatitis (e.g., experienced in the clinic following radiotherapy), and CRI.

Regulation of wound dressing devices and considerations for development of medical devices used for Sulfur Mustard and Cutaneous Radiation Injury (A Guan)

Wound dressing devices fall under the product jurisdiction of CDRH and are broadly described under 3 subcategories: (1) solid dressings; (2) gels, creams, and ointments; and (3) wound wash solutions. Wound dressing devices are regulated by the FDA as Class I (generally do not require premarket review and does not contain drugs, biologics, or animal-derived materials), II (generally requires 510(k) premarket review and cleared based on substantial equivalence to predicate devices based on intended use and technological characteristics), or III (premarket approval with reasonable assurance of safety and effectiveness as an intended wound treatment, skin substitute, life-supporting, or sustaining purpose and typically requires clinical data) devices. 510(k) submissions for wound dressings typically do not require animal wound healing study data, unless (1) the device is considered cytotoxic, which may delay the natural wound healing process; (2) a sponsor elects to conduct a wound healing study in lieu of an implantation study to evaluate the local tissue response after application of the device to the wound site; and (3) non-animal testing is not adequate to demonstrate substantial equivalence concerning safety and effectiveness compared to the predicate.

FDA recommends utilization of the swine model for wound healing studies due to anatomical and physiological similarities to humans, and because pigs also close partial-thickness wounds largely through re-epithelialization. While radiation dermatitis (RD) and CRI may present similarly, they are not exclusively interchangeable clinical conditions. CRI patients may experience signs and symptoms that are far more severe, less controllable, and potentially fatal compared to patients with RD. Wound dressing devices indicated for management of the signs and symptoms for RD may be considered as appropriate predicate devices for 510(k) review of a wound dressing intended for the same signs and symptoms in CRI patients, but a CRI indication may need to be supported by additional performance data. More specifically, wound dressing devices indicated for more severe symptoms and co-morbidities associated with CRI (wet desquamation, blisters, ulceration, and hemorrhage, as well as necrosis, and need for skin grafting) may not be appropriate for 510(k) review.

[29] https://www.silverlon.com/

[30] https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm?ID=K190343

[31] https://www.accessdata.fda.gov/cdrh_docs/pdf19/K190343.pdf

[32] https://clinicaltrials.gov/ct2/show/NCT04238728

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Session III - Organ Systems

In Session III, presenters explored organ systems that are most impacted by exposure to either radiation or SM exposures, namely the skin and lungs. The concept of combined injuries was also considered, as well as the ways that these 2-organ systems crosstalk to enhance the amount of damage caused by either threat agent.

Cutaneous Radiation Injury (CRI) (C Iddins)

CRI is expected to occur in up to a third of individuals exposed in the context of a 10-kiloton detonation in an urban setting like New York City,[43] where it is estimated that of the 4 million that could be exposed, ~64 000 could receive a dose of > 6 Gy. Indeed, data from Chernobyl indicate that 54/134 documented cases had CRI as part of their exposure.[44] Although CRI alone does not result in mortality (not to be confused with Cutaneous Radiation Syndrome, a much larger and more significant injury), the morbidity with associated pathologies can manifest as disfigurement, recurring wounds and fibrosis, and unrelenting chronic pain requiring constant and lifelong medical interventions.[42] Therefore, it is critical to understand how to manage this injury in the event of a radiological or nuclear event. Clinical manifestation of CRI results from cellular damage including death of epidermal stem cells, DNA damage, ROS/ RNS, and an inflammatory response cycle.[45] The resulting pathophysiology of this injury can include vascular dilation, increased capillary permeability, microhemorrhage, and platelet consumption.[42] Neutrophil and lymphocyte infiltrates can result in perivascular edema, cell hypoxia, and cell death. CRI pathophysiology and injuries progress through early "prodrome" inflammatory responses, followed by "latent phase" anti-inflammatory response, accompanied by macrophage activation, and formation of reactive oxygen and nitrogen species, as well as elevated proinflammatory cytokine production. Ultimately, these chronic conditions result in the "manifest illness phase" characterized edema, erythema, and moist desquamation, as well as blistering and possibly, deep ulceration and necrosis. Longer term effects are characterized by fibrosis, poor wound healing, and scarring. These phases and latencies are contingent on the absorbed dose and duration of exposure.[45] At the site of injury, white blood cells, specifically mast cells, are a significant contributor to CRI pathology. Their degranulation and release of heparin and histamine can lead to coagulation and vessel permeability. It is this activation that can further recruit other pro-inflammatory white blood cells like neutrophils, monocytes, antigen-presenting cells, etc. Recruitment of these cells can also lead to sustained TFG-β1 release that can result in fibrosis, which depending on injury severity, can extend into the blood vessels and deeper tissues.[45]

Clinical assessment of CRI severity is done with scenario history including dosimetry or dose reconstruction along with patient history/ physical exam and serial photographs of the injury.[46] Imaging is another essential tool used to assess radiation injury, including magnetic resonance imaging (MRI), magnetic resonance angiography, and ultrasound.[47–51] However, the integration of imaging, bio-dosimetry, and clinical picture may provide the best assessment of wound severity.[46] There are also different grading systems in nature of the injury, including the Radiation Therapy Oncology Group (RTOG) grading system,[52] and METREPOL cutaneous grading system, which uniquely includes body surface area involvement as a quantitative element.[53] As part of their medical management strategy, The Radiation Emergency Assistance Center and Training Site (REAC/TS) relies on a modification of the 2009 National Council of Radiation Protection and Measurements (NCRP) clinical threshold guidelines.[33] REAC/TS has found these clinical grading and dose threshold tables to be informative in a clinical dose estimation even when other dosimetry is available.[54] Thus, there are standardized tools clinicians have for assessing CRI and their progression.

While there are similarities of CRI to thermal burns in determining injury severity (e.g., depth and body surface area involvement), unlike thermal burns, CRI severity depends on the type and quality of radiation, and dose rate.[55] Wounds may be localized or large and could involve deeper tissues and other organs. At higher radiation doses, wounds may have difficulty healing and remaining healed.[45] Case studies demonstrate the pathology and complexity of treatment for CRI. In August 2008, a worker's hand was exposed while changing out a Co source.[60] The individual's hand was only exposed for seconds and the estimated dose by re-enactment was 6 - 7 Gy. Clinical management included pentoxifylline, topical vitamin E, silver sulfadiazidazine cream, and standard burn therapies. With that regimen, the wound healed completely; however, 2 years later the digit was amputated, and retrospective dosimetry estimated that the actual dose to the finger ranged from 22.5 - 40 Gy.[45] In another case study, a large dorsal wound resulting from a fluoroscopically-induced necrotic lesion was treated with standard burn care in addition to hyperbaric therapy. Ultra-sound and Doppler imaging were performed to assess blood flow at the injury, and surgeons were able to excise the damaged tissue and provide the patient with negative pressure wound therapy. With the excision and extensive wound care, this large wound was able to heal completely.[45] Therefore, REAC/TS recommends that CRI care include protecting the area, providing non-steroidal anti-inflammatories, and pentoxifylline, antihistamines, as well as newer silver-based dressings, emollient moisturizers, and topical steroids as needed. In addition, surgical treatment may also be indicated. While grafts can be successful to treat CRI, they are also at risk for failure with wound recurrence if areas were exposed to doses of radiation resulting in necrosis. Other interventions for CRI can include use of investigational mesenchymal,[56,57] or adipose stem/ stromal cells,[58] as well as adipose-derived stromal vascular fraction,[59] and/ or growth factors, potentially utilized with more traditional modalities of dermal constructs, skin grafts, flap, and/ or amputations.[45]

There remains a need for clinical trials repurposing current therapies for similar injuries, for novel MCM research in animal models, and adaptation of bench to bedside advancements of research. CRI can be very complex, recurrent, and may not mimic thermal/ chemical burns. In the event of detonation of an improvised nuclear device, responders will be overwhelmed with these complex, long-term wounds/ injuries. Education and communication will be key in implementing long-term plans to address these injuries.

Sulfur Mustard: the skin and systemic connection (K Lu)

Dermal injury from SM exposure involves epithelial injury and immune activation. Mechanisms involved in SM and nitrogen mustard (NM) effects have been studied since World War II. These efforts led to the development of other analogs as chemotherapy agents (e.g., mechlorethamine, carmustine, uramustine, etc.). SM effects on the skin include blister formation, poor skin healing,

[33] https://ncrponline.org/publications/reports/ncrp-report-161/

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and re-epithelization. Injuries that are healed appear to be tinted and shiny indicating underlying fibrosis and limited function, even when closed.[60] These injuries can have potential chronic and long-term adverse effects like MSC senescence and squamous cell carcinoma development, both of which are indicative of immune alteration. This immune modulation involves activation of lymphocytes, especially myeloid cell interactions, and activation of macrophages expressing iNOS and TNF-α. SM exposure activates the immune system that is constantly surveying skin and the lumen of surface blood vessels, allowing the connection of this rich microenvironment to circulation.[61,62]

This activation of the immune system has led scientists to target multiprong, systemic approaches involving myeloid cells present in all organ systems of the body.[62] One therapeutic under study is high-dose vitamin D, which can induce quiescence of activated macrophages following exposure to these alkylating agents.[63] Data suggests that outside of its normal function in regulating the endocrine system and ensuring musculoskeletal health, vitamin D calms inflammatory macrophages by decreasing iNOS, promoting anti-inflammatory cytokines, and decreasing matrix metalloprotease (MMP) 9, which are all key players in injuries following SM exposure. In an example from a study of treatment with carmustine for lymphoma, a patient experienced a severe skin burning sensation that could have led to his removal from the study; however, administration of vitamin D3 mitigated the skin pain, allowing him to remain on the protocol.[61] SM studies using an animal skin model at Battelle have shown that vitamin D3 modulates immune activation and protects from chemical skin injuries - a single dose of vitamin D3 following a dermal exposure to SM rescued 40% of the animals. Administration of 1 dose of vitamin D or the iNOS inhibitor 1400W rescued animals, improved hematological parameters, and promoted wound healing.[64] A correlation was also seen between a decrease in both TNF-α and iNOS with these endpoints. SM/ NM-induced changes in these biomarkers are like those seen in CRI pathologies.

Pre-clinical and clinical studies using vitamin D have been conducted to better understand the relationship between genetic and proteomic changes in SM toxicity and response to possible treatments. Laboratory animal studies including mice,[65] Yorkshire pigs and rhesus macaques, have explored the impact of vitamin D on damaging exposures to ultraviolet radiation (UV), and SM, as well as NM. Human clinical studies have also explored vitamin D treatments following carmustine,[61] or NM exposure. A clinical trial of high-dose vitamin D3 against Valchlor™, an FDA-approved topical NM, in healthy volunteers[34] involved multiple observations, and biopsies, as well as blood draws. These results showed that most of the pathways involved in the injury and treatment mitigation were related to inflammation, including leukocyte, lymphocyte, and chemokine activation; patients also experience a decrease in erythema, swelling, and pain. The take home of the human studies is that although it seemed that skin has healed, it could break down repeatedly over time, as has been seen for radiation dermal injuries. NIAID funding of Northwestern's CounterACT Center supports a 3-prong approach for dermal injuries induced by SM/ NM, or chemotherapy agents' toxicity that involves superficial, mid-dermis, and systemic strategies. These strategies will be used to identify and develop combinatorial treatments to activate repair genes and suppress destructive inflammation, control other downstream processes (neo-angiogenesis and fibrosis), and for topical and systemic intervention for more critically ill patients.

SM lung injury and fibrosis: identifying targets and developing countermeasures (D Laskin)

Acute and chronic lung injuries from SM and NM exposure have been studied in several animal models developed to understand the pathogenesis of toxicity in the lung that would reflect their toxicity in humans.[66–70] With these models, several MCMs to target areas of toxicity are being developed. SM and NM exposure can result in pulmonary damage which is the major cause of morbidity and mortality. Pulmonary toxicity following SM inhalation has both an acute and chronic impact and follows similar pathogenesis to that seen in injuries resulting from irradiation. Acute effects are first seen in the upper airway followed by effects in the lower airway (e.g., acute respiratory distress syndrome [ARDS]). Mustard-induced chronic diseases are seen within 10 - 20 years following exposure and are primarily caused by persistent lung inflammation. Asthma, bronchitis, fibrosis, and chronic obstructive pulmonary disease (COPD), as well as bronchiolitis obliterans are some of the chronic diseases that are known to be caused by a single exposure to SM.[66]

Both mouse and rat models of SM injury by intratracheal inhalation and NM by intratracheal aerosolization have been developed.[71,72] These animals are exposed to a range of mustard doses and followed from 1 to 28 days post-exposure, with an assessment of lung tissue and lining, bronchoalveolar lavage (BAL) fluid, and immune cells, as well as pulmonary function analyses and live animal MRI/ CT scanning.[73] Histopathological effects of SM on the lung include early thickening of the epithelium and airways that become more prominent over time. These effects are pronounced at 16 days, with increased thickening of the alveolar areas, marked presence of immune-inflammatory cells, and increased sloughing of the upper epithelial lining. At 28 days there is congestion, massive amounts of inflammatory cells, and evidence of fibrosis.[66,68] SM-induced pulmonary fibrosis is present and confirmed by the presence of collagen deposition. In the pathogenesis of mustard-induced lung injury, there is an increase in extracellular matrix proteins and collagen seen early on. Later pathogenesis of fibrosis is evident, with dysregulated disposition of extracellular matrix proteins and collagen.[66,68,69,74] These non-clinical results confirm the acute lung injury caused by mustard exposure that progresses to chronic fibrosis, which is also seen in humans.

Histological studies have shown that SM toxicity is characterized by a persistent macrophage ($CD11b^+$) dominant inflammatory response. These inflammatory macrophages are present within 1 day post-exposure, and their size and numbers increase over time.[71] Further mechanistic studies focused on the contribution of distinct subsets of macrophages and the mediators they release on acute lung injury and chronic lung fibrosis induced by SM. These subsets develop from bone marrow precursor cells and are designated as M1 and M2 which develop in response to signals in the tissue microenvironment.[71,75] Upon exposure, M1 macrophages migrate into the site of injury and are the pro-inflammatory cells that are cytotoxic and release reactive oxygen species (ROS), reactive nitrogen species (RNS), tumor necrosis factor α (TNFα), and other chemokines to promote inflammation and get rid of dead cells, debris, and infectious agents.[66] As the inflammatory process progresses, mediators in the micro-environment form anti-inflammatory M2 macrophages that are involved in wound repair and promoting tissue. Dysregulation or excessive activity of

[34]http://www.cdek.liu.edu/trial/NCT02968446/

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either of these subsets of macrophages can complicate the healing process and cause further damage and disease. The levels of each subset of macrophages and their mediators directly correlated with the time course of acute and chronic injury seen in lung tissue.[69–77]

Involvement of these macrophages' subsets in pathogenesis of SM-induced lung fibrosis provides potential targets for mitigating damage. One approach is by targeting M1 macrophages and pro-inflammatory/ cytotoxic mediators specifically, rather than general anti-inflammatory agents. Some possibilities that have proven successful include N-acetylcysteine (NAC), aminoguanidine, 1400W, and pentoxifylline, as well as anti-TNFα antibody. MCMs toward RNS can be effective MCMs for SM-induced injuries as well. Preliminary studies using iNOS knock-out mice have shown protection from mustard toxicity. Administration of aminoguanidine, a specific iNOS inhibitor, further confirmed that blocking this mediator mitigates SM-induced congestion. TNFα is a known pro-inflammatory mediator and is released by stimulated M1 macrophages following SM exposure. Similarly to iNOS, TNFα knockout mice (TNFR1-/-) were protected from mustard toxicity. Other studies have shown that anti-TNFα antibody reduces SM-induced oxidative stress. Small, ventilated animals were used to assess lung elastance and compliance. The lung elasticity or the ability to exhale is reduced following NM exposure and was completely mitigated by an anti-TNFα antibody. BARDA continues to fund work on the anti-TNFα antibody, to advance the approach through the FDA approval process to treat mustard toxicity.[66,69–77]

As mentioned above, M2 macrophages accumulate early within the lung. As lung macrophages differentiate to M2 macrophages, following mustard exposure, their morphology changes. For example, M1 macrophages isolated 1 to 3 days after mustard exposure are round and small, and on day 7, there is a mixed population of M1 and M2 macrophages. By 28 days, all macrophages are enlarged, fibrotic, and foamy. Foamy macrophages responding to mustard exposures, both in lung tissue and isolated cells from BAL, are filled with lipids. NM has also been shown to dysregulate macrophage lipid transporters with increased lipid uptake. Obeticholic acid (OCA), FDA-approved for primary biliary cholangitis, restores lipid homeostasis in the liver, and prevents fibrosis. Studies using OCA have shown a decrease in NM-induced dyslipidemia, suppression of M2 macrophage activation, as well as suppression of foam cell formation, reduction of NM-induced histopathological changes in lung tissue, and protection against fibrosis.[68] The path forward includes continued testing of the efficacy of OCA as a countermeasure for SM-induced lung injury and fibrosis as well as developing methods for selective delivery of various countermeasures directly to M1 and M2 macrophages.

Evolution of lung injury after SM inhalation (C White)

The primary exposure route of toxic chemicals will determine the characteristics of the injury and signs and symptoms that manifest upon exposure in a lung injury model. For chemical threats like SM, nerve agents, and other toxic industrial chemicals, the inhalation route is the most important for mortality and morbidity. The epidermal route is also common, while exposure by ingestion/ injection is not generally representative of real world scenarios.[78] Exposure routes and downstream effects hold true for radiological and nuclear threats as well. SM causes a dose-dependent, multi-system acute injury, with similarities to ARS. The primary organs that are susceptible to acute SM-induced injuries are pulmonary, ocular, and skin. Secondary organs that may show acute SM toxicity are the central nervous system, gastrointestinal tract, liver, and kidney, as well as the heart. SM is also known to cause dysregulation in the immune response, hematologic/ coagulation, and hematopoietic stem cells. Chronic effects in the pulmonary system are the major cause of late disability and morbidity with a high probability of developing pulmonary fibrosis, bronchiolitis obliterans, recurrent pneumonia/bronchitis, and asthma (RADS), as well as COPD, large airway stenosis and/ or, bronchiectasis.[79,80] Chronic effects on the skin include hypertrophic scar and keloid formation along with deformity. Ulcerative keratitis is 1 of the major chronic diseases seen in SM-induced ocular injuries. The systemic dysregulation in immune and hematologic/coagulation responses may lead to leukemia and other malignancies. These effects have been seen in patients following the Iran-Iraq war, and in nonclinical studies in rats.[81]

Acute effects of SM inhalation involve injuries to both upper and lower airways. The major effects seen in the upper airway include sloughing and obstruction. Edema is also seen in the lower airways along with inflammation, epithelial destruction, sloughing, and fibrin cast (e.g., pseudo-membranes) formation that can lead to airway obstruction and mortality. The acute effects of SM inhalation exposure utilizing an intubated, anesthetized, ethanolic SM aerosol rat model that was developed by the US Army Medical Research Institute of Chemical Defense,[82] showed that SM inhalation exposure causes dose-dependent mortality due to tissue factor-dependent extra-vascular coagulation and airway thrombosis. Rats were exposed to a range of doses of SM with 24-hour survival ranging from 63 – 20% at lower SM concentrations, but with 100% lethality at higher inhaled dose (4.2 mg/ kg as inhaled intratracheal aerosol). The major cause of mortality following SM inhalation is the formation of fibrin-rich casts that form primarily in the conducting airways, causing choking and breathing difficulties. These lesions, as well as airway obstructions, resolve promptly after administration of the fibrinolytic drug tissue plasminogen activator (tPA) into the airways, demonstrating the crucial role of airway coagulation after acute SM inhalation. Pulmonary artery angiograms in rats following SM inhalation have an approximate 50% distal reduction in pulmonary arteries (pruning) within 24 hours of high doses of inhaled SM when compared to naïve rats, with further evidence of obstructive intravascular thrombosis.[83]

Studies with this SM aerosol-inhalation rat model have also looked at effects upto 6 months to study late pulmonary disease and fibrosis. Results have shown that SM causes progressive hypoxemia and mortality in a SM dose-dependent manner over 6-months. Rats exposed to inhaled SM have decreased oxygen delivery to the tissues and poor weight gain that correlate to changes in mortality over 180 days.[82]

Lung function is also affected by SM, with mixed obstructive and restrictive lung physiology observed. Lung compliance steadily declined, while fibrosis was also demonstrated by increase in tissue damping.[84] Bronchiolitis obliterans was present in the lesions obtained by airway microdissection, and there was a dose-dependent increase in airway collagen that correlated with mortality.[84] Results with interstitial collagen showed that SM exposure also caused parenchymal lung fibrosis, a form of interstitial lung disease that is typically irreversible. Pulmonary fibrosis was seen in SM-exposed rats in a dose- and time-dependent manner. Lung pathology showed evidence of patchy, heterogeneous areas of fibrosis that were especially predominant in sub-pleural region.[84] The coagulation pathway is also critical in

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fibrosis in early and late SM inhalation models. In the acute chemical inhalation models evaluated, Tissue Factor initiates the extrinsic coagulation pathway very early,[85–87] and in both acute and late models, the plasminogen activator inhibitor (PAI-1) enzyme inhibits fibrinolysis.[84,87,88] Furthermore, in chronic survival models after SM inhalation, protein levels of transforming growth factor (TGF) β1,and platelet-derived growth factor (PDGF)-A/B, as well as PAI-1 (the latter associated with coagulation in both humans and in rat models) significantly increased in lung homogenate, and BALF, as well as plasma, and lung. These results suggest that pro-fibrotic pathways are involved in rats and human SM-induced lung parenchymal and airway fibrosis.[84]

eNAMPT: A medical countermeasure target in radiation and chemical-induced inflammatory injuries (J Garcia)

Exposure to either ionizing radiation or toxic chemicals (such as SM) results in tissue injury and the release of multiple Damage-Associated Molecular Pattern (DAMP) proteins, which bind pathogen recognition receptors (PRRs) to initiate or amplify activation of inflammatory pathways that result in organ injury. eNAMPT or extracellular nicotinamide phosphoribosyltransferase, is a key DAMP that binds with high affinity to Toll-like receptor (TLR) 4, a critical PRR. eNAMPT-mediated TLR4 signaling results in NF-kB-dependent expression of pro-inflammatory and profibrotic genes/ proteins.[89] NAMPT is a cytozyme that also exhibits intracellular enzymatic activity and is involved in regulation of NAD metabolism (iNAMPT); however, eNAMPT secretion and activation of the evolutionarily-conserved eNAMPT/ TLR4 signaling pathway occurs in multiple human pathologies including bacterial infection,[90–92] trauma,[91,92] and COVID-19 infection.[91] It has been strongly implicated in pre-clinical models of both radiation- and SM- induced lung injury.[93,94] Released from epithelial cells, endothelial cells, and leukocytes to regulate innate immune responses following lung injury,[95,96] eNAMPT acts as a DAMP in the development of vascular and fibrotic injuries.[95] Human subjects with chest trauma, ARDS, sepsis, and COVID-19 pneumonitis, as well as radiation pneumonitis, all exhibit elevated plasma levels of eNAMPT compared to healthy controls.[90–92]

Based upon pre-clinical studies utilizing the eNAMPT - neutralizing, humanized immunotherapeutic ALT-100 mAb, eNAMPT expression is markedly elevated, and contributes significantly to the severity of acute, sub-acute, and chronic inflammatory lung injury.[93–95,97,98] For example, the anti-inflammatory ALT-100 mAb has proved highly protective in a rodent model of trauma-induced ARDS,[97] in an ARDS porcine model of septic shock with ventilator-induced lung injury,[97,98] and in a murine model of whole thoracic lung injury (WTLI)- induced radiation pneumonitis.[95] In each case, animals receiving the ALT-100 mAb exhibited reduced phosphorylation of NFkB, inflammatory indices, and histologic evidence of inflammatory injury compared to untreated animals, suggesting that blockade of eNAMPT/ TLR4 signaling alters disease pathophysiology. In pre-clinical WTLI models of murine and NHP lung fibrosis, eNAMPT lung tissue and blood expression at 12 weeks was markedly increased and mice receiving ALT-100 mAb exhibited reduced severity of lung fibrosis compared to untreated WTLI mice.[94] These studies highlighting the potential role of eNAMPT in sustaining chronic inflammation and fibrosis were supported by transcriptional analysis of RNA-sequencing data from lungs of irradiated animals.[94]

The utility of the eNAMPT-neutralizing mAb was also assessed in a partial body irradiation (PBI) murine model (6.75 Gy, C57BL/6 mice) with 5 percent bone marrow (PBI, 5% BM) sparing with specific examination of both survival and multi-injury progression. Compared to IgG-treated controls, PBI-exposed mice receiving the ALT-100 mAb given 24 hours post-PBI exhibited dose-dependent increased survival at 21 days post-exposure, reduced circulating cytokine levels, and importantly, reduced histopathological severity scores of lung, liver, kidney, and small intestine tissue injury.

Given commonalities in radiation- and SM-induced inflammatory lung injury, a rat model of SM exposure was used to query the role of eNAMPT in SM-induced pathology and inflammation. In limited studies of acute and sub-acute SM exposure models, increased eNAMPT and NOX4 expression in rat tissues was observed; ALT-100 given 2 hours post-SM exposure did not improve survival although a higher dose of ALT-100 mAb did yield a survival benefit compared to the SM-exposed group. In a model of chronic SM exposure, ALT-100 mitigated SM exposure-induced mortality, similar to ALT-100 results in radiation-induced lung fibrosis, and attenuated multiple inflammatory and fibrosis markers. Together these data are consistent with the premise that eNAMPT is a key DAMP involved in radiation- and SM- induced lung injury and fibrosis with ALT-100 mAb a potential MCM to mitigate both radiation and SM-induced lung injuries.

Session IV: Mechanism(s) of Action/ Targets

Session IV consisted of a series of focused talks on the mechanisms by which SM and radiation exposures lead to tissue injury and specific organs that are targeted by these insults. Key elements for advancement of model systems and tools, regulatory strategies, study design, overcoming experimental barriers, and making accurate comparisons between animal models and the human condition were topics discussed during this session.

Radiation- and SM- induced skin injuries, models, biomarkers, and regulatory strategies (P Antinozzi)

Argentum Medical has worked with research partners to develop the appropriate animal models, experimental approaches, and regulatory strategy to obtain FDA approval for Silverlon. The silver-nylon dressing has been shown to be safe and effective for the treatment of burn wounds in several animal species including rats, hairless guinea pigs, and Göttingen minipig models (of partial thickness, full thickness, thermal, and chemical burns). The company's regulatory strategy included 4 necessary milestones. The first was a Good Laboratory Practice (GLP) safety evaluation of Silverlon in a Göttingen minipig model of deep dermal wounds. The study showed wound healing with the dressing and no toxicity on the local or systemic level.[99] The second milestone was to determine the benefit of Silverlon in Yorkshire pigs after skin injury induced by ionizing radiation. The radiation source, a beta-particle emitting Strontium-90 (^{90}Sr) device designed by J Daniel Bourland PhD at Wake Forest School of Medicine, was employed for the task. ^{90}Sr is an isotope in fallout from nuclear weapons and nuclear accidents. The ^{90}Sr irradiator allows for reproducible and uniform dose delivery in a confined area, has an adjustable total dose, and can create multiple irradiated targets per animal.[100] The third milestone, based on Argentum's clinical activities, was a trial studying the ability of Silverlon to reduce radiation dermatitis in breast cancer patients. The 30-patient safety study was conducted at the University of

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Rochester to support Argentum's FDA submission. Trial investigators generated an unbiased, standardized computed skin toxicity score to document severity of radiation dermatitis in the patients.[35] The fourth milestone involved rescoring study images with clinical grading to develop software for the human clinical trial. Over 1000 images were evaluated by blinded dermatologists using the RTOG scale. In-person and computational scoring was found to be closely aligned and 98% of images received scores within 1 point between pairs of scorers. This database of images supported the development of a computational scoring method with an unbiased approach.

Skin models help define mechanism of action of SM (J Laskin)

The SM l alkylating agent and vesicant causes blistering on the skin and mucous membranes. The Rutgers University CounterACT Research Center of Excellence studies these target tissues to identify FDA-approved products that could be repurposed to treat SM injury. The Rutgers Center's goals also include drug formulation for optimal delivery to target organs (e.g., nanomedicines, controlled release, foams, etc.), elucidation of mechanisms of SM-induced toxicity and tissue repair, and development of rapid screening assays. In terms of drug delivery, the program formulates nanomedicines. Skin models are used to investigate mechanisms of SM damage, screen drugs, and assess product efficacy. Models include the Göttingen minipig, Guinea pig, human *ex vivo* full thickness skin constructs, and mouse ear and dorsal skin vesicant models. Göttingen minipig skin exposed to SM causes progressive epidermal damage, with complete epidermal degradation at later time points.[101] Eschar formation occurs by day 9, with epithelial cell regrowth and wound healing evident by day 14. Data generated in *ex vivo* human full thickness skin constructs and mouse skin models support that the initial SM response includes DNA damage, inhibition of cell growth, and apoptosis. Reactions with DNA form mono- and bi- functional adducts that interfere with cell cycle progression and new proteins can be synthesized when DNA is cross-linked.[102] Like SM, NM also inhibits cell cycle progression by modulating cell cycle proteins as shown in vitro with epithelial cells. NM has been shown to induce acetylation, ubiquitination, and cross-linking to p53 to elicit DNA damage in human keratinocytes. There are multiple and dynamic interactions that are occurring on a cellular level upon exposure to mustards. The DNA damage response pathway could help identify key proteins involved in the process of DNA repair and cell proliferation that occurs in response to a mustard insult.

Radiation skin injuries in the clinic: what we know, urgent gaps, and translation of MCMs (J Wolf)

Similarities in animal models of CRI described throughout the workshop can help address gaps in the field to improve the management of skin reactions seen in radiotherapy patients. Radiation dermatitis occurs in ~ 95% of patients receiving radiotherapy, with up to 30% of those skin reactions being severe. Acute reactions can present with mild erythema, followed by dried desquamation, then moist desquamation, and finally followed by ulceration. Chronic effects can occur weeks to years later.[103] At doses above 45 Gy, late and chronic radiation effects can include fibrosis, telangiectasia, and dermal atrophy. Reductions in these skin reactions may be possible by using intensity-modulated radiotherapy; however, radiation skin injury is still a concern. Lack of standardization of treatment and clinical rating of severity of radiation skin injury are also factors.[104]

Known mechanisms of radiation-induced skin injury include loss of basal keratinocytes, stem cells and Langerhans cells, impairment of the epidermal barrier with increased transepidermal water loss (TEWL), and endothelial cell/fibroblast damage, as well as stimulation of resident and circulating immune cells, chronic waves of inflammation, ROS, and antioxidant imbalance. To address the multitude of effects, extensive research has led to many therapeutic approaches; however, no effective agent has been identified to prevent the onset of injury. There is evidence of the benefit of antioxidants; however, findings are inconsistent, but topical steroids work well due to their broad-spectrum activity of anti-inflammation and immunosuppression. Treatment of the described skin reactions to reduce their progression is key but treatment for symptoms such as pain, itching, and burning is necessary as well.

Recently links have been made between the skin microbiome and radiation dermatitis. In 2021, Ramadan *et al.*[105] showed that a predominance of *Klebsiella pneumoniae*, *Pseudomonas aeruginosa,* and *Staphylococcus aureus* are associated with delayed healing of radiation dermatitis. Also shown was a tendency toward radiation dermatitis and delayed healing in patients with a raised proteobacteria/ firmicutes ratio. This suggests that microbiome profiling could inform treatment plans by indicating the progression of radiation skin injury.[105] There are notable similarities between SM and radiation skin injury. Both stressors cause damage to basal keratinocytes leading to erythema, blisters, hyperpigmentation, and pain, as well as itching, burning sensations, and desquamation. While wounds may heal, both injuries can produce chronic effects on pigmentation, telangiectasia, and pain. These similarities imply that potentially, treatments that work for 1 could be translated to help the other. To improve radiation skin injury treatment options, current gaps in the field must first be addressed.[42] These gaps include lack of consensus and standardization of effective treatment for the management of radiation skin injury, need for improved means of objective and quantitative severity measure of injury for all skin types, and identification and utilization of predictive markers and factors to identify individuals at high risk for severe skin reactions. A systematic review and Delphi Consensus Survey, conducted by the Multinational Association of Supportive Care in Cancer (MASCC) Onco-dermatology Study Group has evaluated standards of care in radiation dermatitis with the help of an expert panel. Their results indicate a lack of standardization for effective treatment or combination of treatments. Therefore, although there are clearly still gaps in understanding the underlying biology of radiation and SM-induced damage, there are several approaches under study to reduce the severity of these skin injuries, with many promising research findings.

Airway thrombosis as the leading cause of acute respiratory failure after SM inhalation (L Veress)

Airway thrombosis, characterized by the presence of fibrin airway casts composed primarily of white blood cells and fibrin networks, forms inside the airway lumen in response to conditions such as smoke inhalation, and airway burns, as well as asthma, H1N1 influenza, COVID-19 infection, and sickle cell disease. It also forms in response to pulmonary embolism, pulmonary lymphoma, and chemical inhalation.[106] These airway casts obstruct the airway and have a high mortality rate of 30 - 60%. This blockage impacts oxygenation and ventilation significantly, causing asphyxiation.

[35]Xie Y, Dhakal S, Ryan Wolf J. ABSTRACT: Onset and severity of radiation dermatitis: A retrospective chart */review. Radiation Research Society Annual Meeting (Virtual), 2021

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There is currently no standard of care but surgical interventions include manual removal via serial bronchoscopy, lobectomy, thoracic duct ligation, and Fontan fenestration, as well as heart transplant and heart function optimization. Pharmaceutical interventions include tPA, heparin, azithromycin, and sildenafil/ tadalafil.[106] A retrospective analysis of SM-exposed patients showed that 23% presented with airway casts. Of the patients with casts, 50% died and 20% of the survivors required emergent tracheostomy due to sudden airway occlusion by casts.[78] A case from World War I involved a patient with a 1-hour SM exposure who presented with vomiting and skin erythema within hours and eventually developed oral diphtheric necrosis. On day 7, the patient was in significant respiratory distress, and on day 11, the patient died. An autopsy revealed the larynx contained a large membrane of fibrin cast and smaller bronchi and bronchioles dilated and filled with fibrin and red cells.[107]

A model of airway thrombosis from SM inhalation was developed in male Sprague-Dawley rats in which SM vapor was administered, with multiple endpoints evaluated. The highest dose of SM (4.0 mg/ kg) had a 20% survival rate, and the lowest dose (3.7 mg/ kg) had a 62.5% survival rate. In the first few hours after exposure, there was no decrease in oxygen saturation; however, by hour 12, there was a dose-dependent drop. Rats developed fibrin-rich airway casts at 12 to 24 hours. The development of airway casts was dose-dependent at 12 hours, with the lower lobes developing the most severe obstruction across all doses. Notably, at the highest dose, the trachea began to develop significant obstruction. Overall, cast obstruction was dose-dependent, with higher doses leading to higher scores.[36] A female Yorkshire swine model was also developed due to their similar lung branching patterns to humans. This model allowed for the use of bronchoscopy to monitor real-time obstruction and used an intubated, anesthetized system, and similar endpoints to the rat model; study researchers developed a novel scoring system that allows for consideration of multiple endpoints. The score correlates with a dose-dependent decrease in oxygen saturation, with high doses producing both a high decrease and score and low doses producing both a low decrease score. There is hope that this scoring system can also be used for trigger-to-treat and early euthanasia criteria.[37] In the rat model, intravascular thrombosis formed in the smaller vessels, indicating endothelial activation. In both models, D-dimer and thrombin-antithrombin complexes were elevated. In summary, airway thrombosis/ airway fibrin casts can occur in various lung injury states, can cause significant respiratory compromise, and can be life-threatening. SM exposure causes acute mortality due to airway fibrin cast obstruction in humans and animals. To mimic a human response, rat and swine models have been developed for SM inhalation. The swine model specifically is ready for the development of rescue drugs, with appropriate human-relevant endpoints including bronchoscopy, monitoring, and serial biomarkers.

Radiation and Pulmonary Vascular Dysfunction: mechanisms and consequences (S Chatterjee)

Any radiation involving the thorax triggered inflammation in the lung tissue. This leads to radiation pneumonitis/ fibrosis that alters pulmonary function. Radiation-induced inflammation collapses alveoli and obliterates the pulmonary alveolar-vascular interface by the growth of connective tissue, interfering with the facilitation of gas exchanges done by the interface. The endothelial layer acts as the converging site for inflammation, meaning an activated endothelium is a prerequisite for inflammation. Neutrophils and other immune cells in the blood will only attach to endothelial cells when the endothelial layer is activated and produces cellular adhesion molecules and selectins. The average adult human has over 1000 m^2 of endothelial surface,[108] meaning that even a minor activation of the endothelium will have major ramifications. Radiation has several immediate effects on the human body, including DNA damage, protein modification, and lipid peroxidation, as well as cell death. It also has delayed effects, including a chronic inflammatory response. Studies were undertaken to determine whether low dose irradiation (< 1 Gy) would amplify inflammation and drive a pro-oxidant/ pro-inflammatory environment. One protocol looked at the intercellular adhesion molecule (ICAM-1). Under normal circumstances, ICAM-1 expression is low on the surface of endothelial cells. In response to a stimulus, these cells are activated, and ICAM-1 expression increases, facilitating the sticking of ICAM-1 molecule to the endothelial layer.[109]

Another molecule studied was NLRP3 inflammasome that in healthy cells shows low expression. The NLRP3 inflammasome can be activated by oxidative stress, leading to caspase 1 activation, IL-1 β formation, and cell death by pyroptosis. It is now considered an amplification factor, leading to a cascade of inflammation once expressed. Two models were created to study these effects. One was a reductionist approach using a flow chamber to recreate endothelium as it would be *in vivo* experiencing radiation. The other model used an integrative approach, using human, precision-cut lung slices, and allowing the organ to be studied in its native architecture. A dose-dependent increase of both ICAM-1 and NLRP3 expression was induced by radiation over 24 hours post-exposure, which continued beyond 24 hours.[110] Notably, NLRP3 expression at even low levels of radiation stimuli was comparable to the NLRP3 expression at high levels of pathological stimuli. These studies show that even low doses of radiation can increase inflammation and suggests a long-term effect on the endothelium. More studies are being done to understand the conditions under which these inflammation signals are retained for 72 hours and beyond, as well as examine the presence of oxidative stress markers.

Session V: Animal Model Challenges

The final session of the meeting consisted of 5 presentations, during which the challenges that developers have faced in using laboratory animals to faithfully simulate anticipated human responses were brought forward for discussion. Also addressed were advantages of certain models over others, and a comparison of those used to mimic SM and radiation injuries.

Oxidative stress: an intersection between radiation and SM lung injury (B Day)

SM and 2-chloroethyl ethyl sulfide (CEES) are alkylating, cross-linking and blistering agents, that can cause serious, and widespread injury to lungs (airway obstruction, edema, and hemorrhaging), skin (blistering and inflammation) and eyes (blindness). AEOL-10150 is a catalytic antioxidant meso-porphyrin with a combination of superoxide dismutase (SOD) and catalase activities that has been shown to diminish SM and CEES-induced lung injury.[111] In a rat model, anesthetized animals were nose-cone exposed CEES for 15 minutes, and AEOL-10150 was administered subcutaneously starting 1 hour after.[112] Rats

[36]BARDA Contract HHS-O100201500020C, CLIN 3
[37]BARDA Contract HHS-O10020150020C, CLIN 1

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exposed to CEES had elevated markers of lung edema and oxidative stress attenuated by the antioxidant. Similarly, in a SM model where rats were anesthetized, intubated, and exposed to SM, AEOL-10150 given after SM exposure increased survival at 48 hours (36% for SM alone, 73-88% survival with AEOL-10150).[113] Lung airway obstructions created by the SM were also reduced in the AEOL-10150 treated rats, and the product mitigated SM-induced lung oxidative stress.

Similarly, laboratory models to study radiation injury included a hemithorax rat exposure and a fractionated radiation-induced lung injury.[114] In the hemithorax model, rats were exposed to a single dose (28 Gy) to the right hemithorax, and AEOL-10150 was administered with a mini-osmotic pump 1 day after exposure for 10 weeks at doses of 1, 10, and 30 mg/ kg/ day.[115] AEOL-10150 mitigated radiation-induced lung injury and fibrosis as well as radiation-induced lung oxidative stress. In the fractionated irradiation model, daily fractions of 8 Gy were administered to the right hemithorax for 5 consecutive days (40 Gy total) to adult female Fisher-344 rats. In the treatment arm, AEOL10150 was administered subcutaneously 15 minutes prior to irradiation, and continued for 30 days post-irradiation. AEOL-10150 mitigated radiation-induced lung injury, radiation-induced lung fibrosis, and radiation-induced lung oxidative stress. These data suggest common oxidative stress mechanisms in many threat agents and indicate antioxidants could be developed as a MCM to combat these lung toxidromes.

Recapitulation of human pulmonary response in small and large animal models of DEARE-Lung After TBI with bone marrow sparing (L Jackson)

The lung is a late responding tissue, which upon irradiation manifests clinical signs and symptoms months and years post-exposure. The early period following irradiation is marked by tremendous activity that finally culminates in the clinical manifestation. RILI manifests as 2 specific sequelae, pneumonitis, and fibrosis. In the clinic, radiation pneumonitis occurs 1- 6 months after exposure and is marked by alveolar wall thickening, edema, and inflammation, with persistent cough and/ or lung failure. Radiation fibrosis develops after months to years with collagen deposition, scarring, and lung retraction leading to inefficient gas exchange and respiratory distress.[116] The ability to dissociate these 2 pathologies suggests that they are not inextricably linked. These conditions are recapitulated in various mouse models of RILI, when MCMs are shown to mitigate fibrosis without impacting the pneumonitis phase and vice versa.[117] Development of MCMs under the AR requires researchers to link the pathophysiological outcome of small and large animal models with clinical manifestation of the disease, and establish that the outcome in animal models is sufficiently representative of the anticipated responses in the human population. The goal is to develop and validate models that link in temporal onset, dose response, and pathology to non- NHPs, and humans.

Several species have been used to model RILI (e.g., rodents,[118] hamsters,[119] and rabbits,[120] as well as pigs,[121] dogs,[122] NHP,[123] etc.) demonstrating that animal models need to closely resemble the human lung response to radiation, pathogenesis, and relevant clinical endpoints. Typically, survival is the primary endpoint,[124] while organ function, and imaging, as well as histopathology serve as secondary endpoints for major morbidities. Primary study outcome and secondary endpoints are considered to determine if the strain is appropriate as a model. Even among the mouse strains, development of RILI variation is documented for survival times, lung weights, and airway congestion.[118] For instance, C57L/J, CBA/J, and C3H/HeJ strains are characterized to be pneumonitis prone; C57BL/6 strain is fibrosis prone, while the BALB/6 strain is both pneumonitis prone, and has a DNA repair defect.[118] Using 6 strains and radiological imaging to look at lung damage, pathobiology of RILI following WTLI demonstrated significant variations based on survival, lung weights, and breathing frequencies well as lung density, and volume.[125] From this work and given the threshold dose, 95% incidence dose and temporal onset,[118] the use of C57L/J strain is recommended to best translate murine findings to higher species like NHPs, and humans.[126,127]

Based on FDA recommendations, there has been a shift in preferred models from WTLI to PBI (5% bone marrow sparing), to recapitulate a multiorgan impact and administration of growth factors more accurately on the efficacy of lung-MCMs in NHPs and C57L/J for RILI. Medical management/ supportive care was scaled from the NHP model to the rodent model; this was determined in coordination with NIAID, the industry partner, and FDA. One major difference in the supportive care regimen is that dexamethasone is not preferred for the mouse, although it is often used in the NHP as a trigger to treat modality (L Jackson). In summary, the mouse model, with certain preferred strains and exposure geometries, continues to be a good simulation of many aspects of RILI seen in larger animals and humans, and thus, remains an option for preclinical development on lung MCMs for radiation injuries.

Pivotal, GLP non-inferiority study to evaluate Silverlon dressings against superficial and moderate, partial-thickness SM Vapor wounds in Göttingen minipigs (C Croutch)

Silverlon obtained a 510(k) FDA indication for a burn dressing product in 2013. Since 2003, Silverlon has been widely used by the US Army in combat settings in Afghanistan and Iran.[128,129] Silverlon was tested serendipitously on a patient with a SM wound following sulfamylon debridement, and in the 8 weeks post-treatment, the wound healed. From this success, pilot studies were created to develop a model for SM burns in hairless guinea pigs (HGP) and Göttingen minipigs.[130] SM wounds were created – a superficial and a moderate dermal wound depending on the length of exposure – and animals were observed for 48 hours. Endpoints included histopathology, modified Draize scores, TEWL, and ultrasound, as well as digital images of wounds, and colorimetry, among other endpoints. To achieve consistent results, a histopathology scoring matrix was established, and a method was developed so that bandages remained moist, and in place during healing; debridement was standardized with 2 methods (laser and sulfamylon). Based on these data, FDA recommended that only the minipig model was needed for GLP studies, as it is more robust and produces more consistent results than the hairless guinea pig. Histopathological scores informed group sizes for GLP study, and these scores were the primary endpoint. The FDA also recommended a 'non-inferiority' study rather than an efficacy model and preferred a saline wet-to-wet debridement instead of those originally proposed. The GLP objective that was decided through consultation with the FDA was to assess non-inferiority of Silverlon when compared to gauze with 1% silver sulfadiazine (SSD) for the treatment of SM-induced dermal lesions. The primary endpoint was the histopathology composite score of dermal wounds obtained at the end of the study. Study outcomes showed that Silverlon outperformed SSD in every treatment arm and more wounds healed with the Silverlon bandages. Therefore, Silverlon was found to not be inferior to the SSD treatment, and

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was in fact better, leading to an FDA approval of the device in 2019.[38]

Based off the success from these studies, recommendations to implement during the development process are: (1) include a strong regulatory group as part of the team, (2) engage in frequent and clear regulatory communications, (3) refine the product label early in development, (4) include a clinician with experience in human injury and working knowledge of the animal model, (5) select animal models and study designs that reflect the human injury, and (6) generate as much preliminary data as possible, to help the pivotal GLP study succeed.

Dealing with characteristics outside of your control: genetics to animal behavior (M Doyle-Eisele)

Outside of the inherent challenges that arise from using animal models, there are other challenges that overlap across institutes and organizations, specifically overarching issues such as regulatory, and test system, as well as clinical translation, assays, and supportive care challenges. Starting with regulatory issues; a contract research organization, such as Lovelace Biomedical Research Institute, conducts a large variety of studies that are highly regulated. While regulatory challenges are typically discussed in terms of what is needed for submission, other challenges exist. For instance, occupational safety (safety of facility, technical staff, and animals a CRO works with) can lead to procedural or protocol changes and this can differ by facility. In addition, the number of study animals based off statistical analyses may be unrealistic. Also, utilization of purpose bred versus wild-caught animals can result in varying outcomes; restrictions on specific species, despite being the best model that characterizes the human condition, can also make study implementation challenging. Another potential hurdle is facility-specific Institutional Animal Care and Use Committee's (IACUC) requirements that add a distinct layer of complication by changes in policy that can impact anything from a simplistic housing requirement to complex supportive care modalities.

Test system challenges can arise due to issues with animal availability, animal species consideration, and logistical issues such as shipping delays. For example, in 2013 there were considerable delays in Göttingen minipig research due to increased toxicology and radiation studies, confounded by decreased breeding across the US. In another instance, a shift from rhesus to cynomolgus macaques for MCM testing for radiation indications has been necessitated due to the severe scarcity of the rhesus monkeys, but it is unclear what this will mean for current work and approved models; particularly because variations in species (physical or genetic variability) can result in unexpected and non-reproducible results.[131]

While the objective of clinical development is to achieve reproducible and biologically relevant data, the elements that go into this (test system, challenge material, test article, and endpoints) can have variability, which creates unanticipated challenges during development.[132] Clinical translation challenges include: (1) manifestation of injury cannot always be directly monitored in animals, (2) endpoints are not always practical, (3) animals cannot comply with dosing instructions, and (4) inadequate infrastructure and trained workforces. Assay challenges include pivotal immunological endpoints that are missing or unavailable, lack of availability of assay reagents, and batch-to-batch variability. When comparing human to animal endpoints that are critical for clinical translation, there is a gap between expectations and what is feasible and practical.

Supportive care challenges include occupational safety for staff, placebo treatments (if no standard of care is approved), IACUCs mandating analgesia, and monitoring protocols that can alter outcomes, as well as standards of care that are not readily available to research institutes because of clinical need. The ultimate goals of pre-clinical studies are to accurately model the desired biological effect of a drug in animals, to predict treatment outcomes in patients, and characterize all toxicities associated with a drug to predict adverse events in humans. Therefore, animal models must be carefully designed to mimic what happens in humans while balancing feasibility.

Advances in translational large animal lung injury SM models for mass casualty (V Bebarta)

One of the primary approaches to responding to any chemical injury is to focus on the toxidrome. Toxidrome is defined as the syndromic effect of a toxin, and a threat agnostic approach is to treat the injury, not the threat.[133] Given that scenario, the current research focus is on oxidative stress and how it impacts mammals (largely pigs), as assessed by lung inflammatory cell accumulation, and increased TNF-α, as well as other pro-inflammatory cytokines, lung matrix metalloproteinases, and DNA damage. Several animal species have been studied in SM research: large animals include dogs, sheep, NHPs, and pigs. Among these, swine is the best model to use because of the similarities to the human condition with regard to drug dosing, anatomy, and physiology (cardiac, pulmonary, and immunology). Furthermore, their clinical similarities to humans,[134] including a similar airway size, allows for use of the same devices as those used clinically, and the ability to perform serial blood draws/ large tissue samples. Currently, swine are used in other chemical MCM development, such as against SM pulmonary, and eye/cutaneous injuries. They are generally accepted by the FDA for these indications. One main drawback to the model is that swine are hypercoagulable compared to humans.[135]

An effective animal model must consider the preexposure, exposure, and post-exposure phases. In the pre-exposure phase, several factors are important such as handler expertise, animal weight, and subspecies, as well as vendor, prep work conducted, and anesthesia. Other factors include air/ oxygen used, and ventilation. During exposure, it is important to consider dose variability, total dose in bag/ plenum, and duration of exposure, as well as ventilation. In the post-exposure phase, it is important to consider off-gassing, recovery of the animal, and husbandry, as well as monitoring. Key outcomes measured include PK and general toxicology; clinical, behavior, and physiological; airway cast; system inflammation, blood cell counts, lung edema, and injury markers, as well as survival. Euthanasia criteria are crucial for rigor and reproducibility. Scenarios for real chemical responses have involved consideration of SM mass causality events for the Colorado/ Mountain plains region.[39] In the event of SM exposure, most patients will present with mild symptoms, although some may have severe concerns. Early decontamination is critical - supportive care should work for most patients in acute care;

[38]https://www.accessdata.fda.gov/cdrh_docs/pdf19/K190343.pdf

[39]~https://www.phe.gov/Preparedness/planning/PDHRCA-FOA/Pages/mountain-RDHRS.aspx#:~:text=The%20Mountain%20Plains%20Regional%20Disaster,(RDHRS)%20demonstration%20site%20project.

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however, trauma evaluations are also needed. Field drugs need to be used within 1 hour post-exposure to be effective, and developers need to keep in mind the importance of untrained personnel being able to deploy/ use the developed drugs.

Discussion

To maintain the meeting structure and to maximize opportunities for participants to dialogue with subject matter experts, questions were developed by the meeting planners for each session. These question/ answer periods were held at the end of each meeting session. Content from each question/ answer period has been captured here and subdivided into topic areas for clarity. Of note are some overarching themes evident throughout the question/ answer sessions, such as the use of the AR for the development of MCMs and how it is not required for devices. The ways in which different organ systems and mechanisms of action/ targets are impacted by radiation or SM exposures were another area of extensive discussion, as well as the complexity of developing appropriate laboratory animal models for radiation or SM exposures.

Regulatory Pathway

The US FDA AR pathway is a requirement for radiation mitigating drugs and biological products on track for eventual approval. Substantial evidence of drug/ biologic efficacy in the appropriate animal models is necessary for this consideration. Conversely, the development of devices does not fall under the purview of the AR. While substantive evidence for device clearance can come from animal models, they do not follow the AR for regulatory approval. Regulations for clearance and approval of devices allow for the use of different types of evidence, including animal data, but the same AR regulations for drugs, and biologics do not apply for devices. Truly, the AR levels the playing field for products that are reviewed by CDER and CBER that cannot be studied clinically due to ethical reasons. Although the AR does not apply to the review of medical devices/ diagnostics, CDRH will determine what kind of animal data would be appropriate to support a submission that may include bench testing, animal testing, and clinical data. Hence, animal data are considered performance information that can be supportive of device clearance. The CDRH will also consider if there are ethical issues in obtaining clinical data, which depends on the indications for use for the product. There is merit in using animal models and the AR, and the 2 should not be conflated with each other. These distinctions hold true for both radiation and SM-MCM development space and the radiation bio-dosimetry and SM devices (diagnostics, dressings) space. Interesting, while the AR is not applicable to devices, there is nothing to prevent a sponsor from proposing animal studies under the AR to test a device; however, that is contingent on the ethical issues and what they propose for CDRH review.

Product sponsors usually ask what a good starting point is for "early and often" interactions with the FDA. For example, would meeting in advanced of a proof-of-concept study in a large Yorkshire pig or NHP be appropriate for sponsors and government funding agencies to discuss the intended model and endpoints with the FDA? If there is a traditional product being developed, sponsors are encouraged to interact with the FDA (CDER/ CBER) primarily via "milestone" meetings that are available to all interested parties. For those areas that are related to public health challenges, all FDA Centers have formal programs to increase the level of interactions with the sponsors. Chemical and radiation injuries fall under the public health challenge category and the FDA does focus its energy and prioritize the development of these products. In addition to the formal face-to-face or virtual meetings, sponsors can ask for a written request-only meeting, where sponsors can ask the FDA to provide written response to specific questions. Pre-Investigational New Drug (IND) meetings allow the sponsors to approach the FDA and discuss development plans for their products.

If a sponsor of an MCM is unsure of the regulatory review division for the product and its proposed use, they can contact the specialized MCM group in the appropriate center to obtain contact information for the review division. If a sponsor is unsure of how their product should be classified (i.e., drug, biological product, device, or combination product), and thus unsure of the center that has regulatory jurisdiction, the sponsor can submit a Request for Designation.[40] FDA Centers work together, especially when reviewing products for the same indication or when a consultation is needed for part of a combination product application. For example, if a proposed product is a drug that has an associated delivery system (e.g., an associated autoinjector), CDER would have regulatory jurisdiction for the product, but CDRH would be consulted on the associated delivery system portion of the application.

FDA encourages obtaining concurrence on the animal models in which the efficacy of an investigational product will be tested (i.e., the design of adequate and well-controlled pivotal efficacy studies) prior to study initiation. Review divisions will provide input on the protocols for the natural history, and adequate/well-controlled efficacy studies. Protocols are eligible for evaluation under Special Protocol Assessment (SPA) provisions, although protocols for natural history studies are not eligible for SPA (A Powell).[41] As SPA is a cumbersome process, it might be more efficient to ask the FDA iteratively regarding protocol and study design.

The AMQP discussed above qualifies animal models for a specific context of use, the details of which are made public. When a qualified animal model is used within its specified context of use, FDA does not have to re-evaluate the adequacy of the model for representing key elements of the human disease or condition, or its appropriate use in regulatory applications (e.g., as a treatment or prophylaxis). This potentially reduces product development time. A sponsor, however, would still need to establish that the qualified animal model is a suitable test system for the investigational product. Because the AMQP provides a formal avenue to obtain FDA subject matter expert feedback on early animal model development, qualifying an animal model through the AMQP may have the greatest impact in therapeutic areas where there have been no AR approvals and limited model development.

It is possible for approvals to be based on reduction of major morbidity without survival benefits. For example, in H-ARS, 1 of the manifestations of radiation injury is the myeloablation of precursor cells resulting in neutropenia and thrombocytopenia. Although neutrophils and platelets can be readily measured, it is difficult to use these parameters as the primary endpoints to determine whether there is a substantial benefit of the therapy. In the context of radiation-induced thrombocytopenia, it can be very difficult to achieve major morbidity (e.g., major bleeding).

[40] https://www.fda.gov/combination-products/jurisdictional-information
[41] https://www.fda.gov/regulatory-information/search-fda-guidance-documents/special-protocol-assessment-guidance-industry

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Similarly, in the context of neutropenia, it can be challenging to assess and quantify infections. Hence, survival and mortality are more straightforward outcomes that are reproducible and can be consistently evaluated. Another consideration is that if the disease manifestation is likely to include survival, then survival automatically becomes a major outcome. While the FDA is open to considerations on major morbidity as a primary outcome, it may be a more challenging endpoint to support. In the context of RILI, the FDA is open to functional endpoints for the lung, but there is a survival component observed in these models. For CRI, mortality is not an endpoint and hence, meaningful recovery of the radiation or chemical burn, full-thickness injury and recovery in repair and re-epithelialization are acceptable as primary endpoints.

Therapeutic Windows to Mitigate IR-and SM-Induced Inflammation, Fibrosis, and Pain

It is generally acknowledged that a radiation emergency response will not allow for administration of a medical countermeasure much earlier than 24 - 48 hours after an incident; however, the manifestations of SM are more rapid and dramatic. Although it is best to intervene in some acute chemical toxicities within minutes to hours, there appear to be 2 phases to the body's SM damage response. The first phase involves activation of acute inflammatory responses, which might be responsive to products like anti-TNF; however, at later time points, it is important to continue anti-fibrotic interventions, to effectively interfere with evolution of late complications. This concept of a multiple hit treatment is also understood in radiation research; however, the focus there is addressing the different organ systems that might succumb to the initial exposure, and the resulting waves of inflammation. In animal models, the SM damage response is dose-dependent: some exposure levels may be immediately lethal, but other doses could take hours to days to manifest. Instead of going after single factors, the chemical research community has focused interventions on early diversion of immune trafficking and targeting inflammatory cells and their cargo.

In terms of skin damage, there is evidence that even decades later, irradiated skin can still be a problem for the patient, with relapsing and re-opening of wounds; however, for SM cutaneous injuries, this is less of an issue. There isn't a recurrence of damage, although there are clinically very few cases on which to base this observation. There are some situations where there is a breakdown of the skin at a site distant from the 1 that was exposed to the chemical, but later effects normally encompass development of squamous cell carcinoma. One common trait identified for both radiation- and SM- induced skin lesions is the prominence of pain that can be a problem in long-term recovery. Vitamin D has been shown to help with mustard pain control, suggesting that it might also work in radiation lesions. In irradiated skin, approaches such as cellular therapies have also shown great promise in managing radiation-induced skin pain and could potentially be studied for chemical injuries.

Common Pathologies Between IR-and SM-Induced Inflammation and Fibrosis

It was also noted that there are common injuries for both threat agents; with involvement of the immune response, and more specifically, macrophages in SM, and radiation injuries. Similarly, myeloid lineages are susceptible to radiation, and their down-regulation, both in numbers and in function, is linked to the toxicities seen with exposure. There are also multiple macrophage classes affected by SM exposure, and the best approach to minimizing damage is to address each in turn in their appropriate timelines. With fibrosis as the overlapping pathology, previous work to address radiation-induced skin injuries has focused primarily on the acute phase, and the need to promote healing. Although fibrosis is a common late effect, there has been little focus on these late impacts of SM exposure (although radiation-induced fibrosis in the lung is well studied). Anecdotal experiences with human industrial exposures suggest that the best way to mitigate late radiation-induced skin fibrosis is early excision (representing an aggressive therapy in some situations). Other products, such as those that interfere with the TGF-β1 pathway, may also be efficacious. Similar to what has been seen for radiation, in SM skin exposures, anatomical location of the exposure is key; with injuries in the periocular region, as well as elbows and hands presenting a significant concern. Late scleroderma that leads to contraction is also common.

Targets/ Cytokines Influencing Inflammation Play a Role in Lung Injuries After Radiation or SM Exposure

It is important to maintain an appropriate and balanced immune response to avoid infections following either radiation or SM exposure. For this reason, work in both fields has targeted specific aspects of the immune response. Research into targeting TLRs has shown promise for radiation and chemical insults, but these kinds of studies must be undertaken with care. For example, in 1 clinical trial targeting sepsis using anti-TLR4 antibodies, some patients that received the therapy did worse than vehicle-treated patients. There are other approaches, such as those that do not block the receptor but target eNAMPT. Neutralization of the eNAMPT enzyme activity can modify the immune response, but still allow the body to respond to infection.

The concept of managing inflammation (the body's natural response to infections) in such a way that the immune response is not incapacitated is universal. The need to appropriately control inflammation is also a problem with COVID-19, where it is not so much the virus, but the body's response to the virus that leads to the clinical presentation and lethality. Similarly, in clinical populations undergoing chemotherapy with high dose alkylating agents, there are often severe side effects that are managed with dexamethasone or prednisone. Managing the body's response by mitigating some, but not all inflammation is key to a successful outcome. In addition, there are different levels of infections, ranging from a local contaminated wound to full-blown sepsis. Hence, the use of anti-inflammatory methods and their impact on the immune response is case-dependent. The response of the innate immune system as a significant part of the body's response occurs following both radiation and SM exposures. In another example, when trying to suppress innate immunity, it is possible to target a receptor such as translocating chain-associated membrane protein 1 (TRAM1), which plays a role in amplifying inflammation. By switching from negative to positive inflammation, excess suppression of immune response can be avoided.

Common Triage Criteria to Assess Injuries

Since not all radiation damage is obvious, it is critical to understand and identify biomarkers of injury and its resolution; however, there is not necessarily an equivalent research area for discovery of non-invasive biomarkers in the chemical research space. Most human literature on SM exposure is from conflicts that occurred in the 1990s, so it has been a challenge to identify biomarkers of injury in

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the established animal models. Nonetheless, in recent studies where it was not anticipated that signatures would be detectable, the advent of amplification proteomics has allowed for the identification of serum elements that could correlate with the severity of skin injury.

Combination Therapies

SM injuries are progressive, requiring multiple therapies to address each phase; therefore, any successful approach to fully address associated injuries will require the use of several products. As suggested above, it may be necessary to use an anti-inflammatory as soon as possible after exposure, whereas an anti-fibrotic would be needed later. Similarly, the anticipated use of treatments to address hematopoietic injuries (e.g., FDA-approved growth factors and platelet-promoting products) alongside other drugs targeting injuries to other organs, such as the gastrointestinal tract and lung, is also anticipated for radiation exposure scenarios.

Mechanisms of Action/ Targets

Overlapping skin and lung injury mechanisms between radiation and SM exposure have been observed. Regarding DNA damage from SM exposure, the harm is immediate, but the true damage takes time to occur. There are existing treatments that can be repurposed and leveraged from radiation to SM treatment or vice versa but knowing how to address injury on the molecular level is complex. Once there is more clarity on the mechanisms driving injury from either SM or radiation, a polypharmacy approach with antioxidants, DNA repair agents, etc., is anticipated. Curiosities in damage have been observed as well. For example, with nitrogen mustard exposure, adducts react quickly at various time points, but do not tend to be maintained; however, some studies suggest that adducts do not instantly form but appear over time. It was noted that SM takes time to get into the cells *in vitro* and may be possibly stored within the cells, but the reason why nitrogen adducts may take many hours before becoming observable is unknown.

The impact of radiation and SM on immune mechanisms adds another layer of complexity. As seen in radiation-induced skin injury, there appears to be a reoccurrence of injury after SM. The involvement of the immune system that can cause reoccurrence is evident and under investigation. For example, in clinical trials, skin samples from patients 8-weeks post-irradiation have a normal outward appearance; however, there is extensive inflammation evident on the cellular level. The cellular makeup is quite different from the acute neutrophil myeloid profile observed in the first week post-treatment compared to 8 weeks, where an increase of T cells and NK cells are observed. Single-cell RNA sequencing is being used to identify the type of immune memory component in the skin at these timepoints. Similarly, patients may return to the clinic with radiation dermatitis months or even years later with inflamed skin. There is a cycle of immune cell trafficking back into these areas of previously irradiated skin. While there may be re-epithelization of the skin after wounding, it is no longer the same as the normal epidermis in the surrounding, uninjured skin. Why and how the skin remembers that it has been exposed to SM or radiation is an intriguing question worthy of exploration.

Recovered tissue is not normal as seen in unprovoked tissue, therefore, it is likely that a similar phenomenon would be seen in the lungs after these kinds of insults. Findings from certain *in vivo* studies show that SM-induced lung injury is cyclic, and while there may be an apparent lull, active processes are occurring at each stage. The immune cells are actively shifting; for example, macrophages go from a proinflammatory state to a profibrotic state that may lead to reinjury or re-establishment of the pathway at many later timepoints. Both SM and radiation injuries are multifaceted occurring just after the acute event. It was noted that clinicians would benefit from a roadmap illustrating what they may expect to see when it comes to the "acute to late pathway" of injury from SM and radiation to the skin and lungs. This roadmap should provide clinicians with a timeline of the injury sequelae to guide treatment decisions and interventions.

Specific Examples for Different Use of a Single Therapy for Radiation or SM-Induced Skin Damage

Regarding how treatment methods differ when using Silverlons dressing to treat wounds from radiation versus SM, they are similar in that the dressing is applied when an injury is observed. In the case of radiation therapy, the timing of the insult is a known factor. This is advantageous because the dressing can be made available to patients in advance and can be applied just after radiation treatment. There are many types of skin complications that this dressing could potentially be used for (deep, superficial, etc.), but additional label extensions would need to be sought.

Other Discussion Points

Important topics addressed surrounding skin injuries include: (1) much of the skin data presented were from Caucasian patients; data on other skin pigments and sun-damaged skin needs to be obtained; (2) UV exposure from the sun could cause complications with radiation, so patients undergoing radiation therapy are advised to avoid sun exposure and use sunscreen; while the head and neck tend to receive more sun exposure, radiation therapy for head and neck cancer is usually given at much higher doses than other malignancies, such as breast cancer.

The impact of the microbiome on radiation therapy was also considered. It is known that microbiome profiles can vary across different regions of the skin, and there are composition shifts seen in the microbiota after radiation therapy. The differing microbiota and their abundance across the skin is a complex area of study. With respect to sampling a wound and introducing a secondary injury, there are methods to circumvent this issue. Usually, skin swabbing is performed on open wounds (or a biopsy is an option), which unlike tape-stripping, should not re-injure the area. Interestingly, assessing the microbiome could be a means of field profiling as a potential triage tool, but this technique would have to indicate clear and distinct stages to consider changes in the immune system. Other assessments could be used as well, such as determining TEWL to assess wound progression. Recovered skin is no longer normal skin and other outside factors impact the microbiome (e.g., pH and humidity), therefore, studies will have to be done to determine the progression, and stages of injury in relation to the microbiome profiling, and the correlation to underlying immune defects.

The use of G-CSF in animal models was discussed. It was noted that G-CSF triggers an increase in immune cells being released from the bone marrow, but an increased neutrophil count could induce a greater inflammatory response. Observations made in SM animal models and the literature suggest that SM exposures of the lung and skin cause a dip in neutrophils along with lymphopenia that are predictive of mortality due to sepsis. Whether G-CSF administration would be favorable in these scenarios is not clear. Too many neutrophils in the airway and blood could cause a higher

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degree of inflammation leading to tissue damage; therefore, it is a balancing act.

Finally, discussants emphasized the need for proper medical management of the laboratory animals, which is critical for maintaining study rigor, and reproducibility, and relies on having trained and experienced staff, clear guidelines, and proper equipment. There is a further need for shared pathophysiological targets and biomarkers, and participants agreed that these are critical to identify, develop, and to potentially apply a MCM to another disease state. Finally, the group reiterated the need to select appropriate strains of animals. They discussed how different strains can have differences in tolerance and outcomes during a study. If a study must include a new animal strain, researchers should re-characterize the model for that strain. The panel also highlighted that involving the FDA in strain selection can be important when conducting pivotal studies.

Conclusions

As shown by the subject matter experts through presentations and extensive discussion, there are significant similarities between injuries caused to the cutaneous and pulmonary systems of the body by chemical agents (specifically SM) and radiation exposures. Areas of overlap include mechanisms of action driving the damage to these tissues, animal models employed in each area to assess these insults, and MCMs with potential for multi-utility in both mission spaces. A primary goal of the meeting that the organizers believe was met was to determine the degree of overlap between these 2 seemingly distinct threat areas and discuss ways to leverage collective knowledge and effort. The US government agencies sponsoring the meeting intend to continue this dialogue among investigators and leverage research investments in each threat area for the benefit of both research communities.

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