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MEETING REPORT

A Poly-Pharmacy Approach to Mitigate Acute Radiation Syndrome

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The National Institute of Allergy and Infectious Diseases, Radiation and Nuclear Countermeasures Program, was tasked by the United States Congress and the U.S. Department of Health and Human Services to identify and fund early-to-mid-stage development of medical countermeasures (MCMs) to treat radiation-induced injuries. In developing MCMs to treat various sub-syndromes (e.g., hematopoietic, gastrointestinal, lung), it is important to investigate whether a poly-pharmacy approach (i.e., drug cocktails) can provide additive benefits to mitigate injuries arising from the acute radiation syndrome (ARS). In addition, potential drug-drug interactions must be examined. For this reason, a workshop was held, which centered on understanding the current state of research investigating poly-pharmacy approaches to treat radiation injuries. The first session set the stage with an introduction to the concept of operations or support available for the response to a nuclear incident, as this is the key to any emergency response, including MCM availability and distribution. The second session followed the natural history of ARS in both humans and animal models to underscore the complexity of ARS and why a poly-pharmacy approach may be necessary. The third session featured talks from investigators conducting current MCM poly-pharmacy research. The meeting closed with a focus on regulatory considerations for the development of poly-pharmacy approaches or combination treatments for ARS.

INTRODUCTION

Exposure to high doses of penetrating radiation in a short period of time can lead to the acute radiation syndrome (ARS), which manifests as a full-body assault and includes damage to the hematopoietic system (heme), vasculature and major organs: lung, kidney, gastrointestinal (GI) tract, heart, and brain. Given the complexity of this injury, the likelihood of identifying one medical countermeasure (MCM) to serve as a ''magic bullet'' to rescue all systems and organs is small. A more probable scenario is a poly-pharmacy approach, where multiple MCMs, as well as supportive care measures, are administered to help minimize radiation damage and casualties.

On October 25, 2018, the National Institute of Allergy and Infectious Diseases (NIAID), Radiation and Nuclear Countermeasures Program (RNCP) held a workshop entitled, ''A Poly-Pharmacy Approach to Mitigate Acute Radiation Syndrome.'' This marked the first forum where poly-pharmacy was discussed as a way to address the multiple injuries anticipated in a radiation incident. For the purposes of this meeting, the term poly-pharmacy was defined as the use of multiple drugs that are medically necessary (N. Coleman) (*1*). A mass casualty radiation incident will likely lead to multi-organ injuries, i.e., various subsyndromes of ARS (heme and GI), the delayed effects of acute radiation exposure (DEARE), cutaneous radiation injuries (burns and open wounds) and blunt trauma, thereby necessitating a poly-pharmacy treatment approach. Pre-existing conditions may also require medical personnel to consider drug-drug interactions. By defining poly-pharmacy in the context of treatment of injuries after a radiation mass casualty public health incident, the emergency response and planning perspective must be considered.

In the first session of the workshop, details about U.S. Government communication plans, operational logistics, and existing infrastructure for the distribution and dispensing of MCMs were discussed. The second session detailed the natural history of the injuries, in which acute and delayed biological effects observed in small and large animal models were presented. This discussion provided a frame of reference for the timeline of progression of damage. In the third session, current NIAID-sponsored poly-pharmacy research was highlighted, demonstrating efforts underway and potential regulatory issues faced by product developers. In the fourth and final session,

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TABLE 1
Workshop Speakers and Presentations[a]

Speakers	Representing/affiliation	Title
C. Norman Coleman, MD	National Cancer Institute	Poly pharmacy: How might this need be considered for a major nuclear or radiological incident that could produce the acute radiation syndrome?
Steven A. Adams, MPH	ASPR	Strategic National Stockpile, support for the response to a nuclear incident
Christie M. Orschell, PhD	Indiana University School of Medicine	Defining the hematopoietic acute radiation syndrome and the delayed effects of acute radiation exposure in murine models
Thomas MacVittie, PhD	University of Maryland School of Medicine	The acute radiation syndrome in the nonhuman primate: Is ARS multi-organ injury (MOI) linked to the MOI characteristic of the delayed effects of acute radiation exposure?
J. Mark Cline, DVM, PhD, DACVP	Wake Forest School of Medicine	Multisystemic delayed effects of acute radiation exposure in nonhuman primates
George (Joe) Cox, PhD	Bolder Biotechnology, Inc.	Improving survival in a mouse H-ARS model using combinations of hematopoietic growth factors and an ACE inhibitor
Meetha Medhora, PhD	Medical College of Wisconsin	Enhanced mitigation of radiation-induced injuries by combining angiotensin-converting enzyme (ACE) inhibitors with other countermeasures
Juliann Kiang, PhD	AFRRI	Combined drugs with the pegylated-G-CSF therapy to enhance survival and against multi-organ injury after irradiation alone or combined with wound trauma
Yon Yu, PharmD	CDC	Regulatory considerations for emergency use of stockpiled medical countermeasures
Mario Sampson, PharmD	CDER, FDA	Clinical pharmacology considerations for products developed for acute radiation syndrome
Adebayo Laniyonu, PhD	CDER, FDA	Product development for acute radiation syndrome: Nonclinical considerations for animal rule efficacy studies

[a] Workshop participants had an opportunity to review this meeting report prior to journal submission.

regulatory considerations that impact the development and administration of multiple MCMs were discussed.

Altogether these sessions highlighted the need for a well-coordinated effort between U.S. Government officials, healthcare professionals and first responders, to approve, stockpile, triage, treat, and provide a continuum of care for exposed civilian populations. Of critical importance is an understanding of the availability and potential distribution of resources from the Strategic National Stockpile (SNS) to areas of urgent need. Discussions included: 1. prioritized use and functional interchangeability of currently available MCMs for the treatment of ARS in an emergency response; 2. potential interactions of newly developed MCMs to treat ARS or DEARE with current MCMs in the SNS; and 3. potential interactions of current MCMs with drugs that are taken routinely for pre-existing medical conditions, particularly in the context of immune-compromised populations.

MEETING PROGRAM OVERVIEW

As more MCMs are approved/licensed by the U.S. Food and Drug Administration (FDA), a poly-pharmacy approach for the treatment of ARS and DEARE must be considered.

The consideration of leukocyte growth factors (GFs) as the standard of care and the latest MCM research surrounding this concept are also presented. The target audience for the meeting and this report include U.S. Government emergency preparedness planning and funding agencies, as well as industry and academic researchers engaged in developing radiation MCM treatments. All invited speakers are listed in Table 1 and this meeting report provides a summary of their presentations, along with highlights of meeting discussions.

Response and Planning Considerations

After a radiation incident, thousands of people will likely need medical assistance; many will also be among the ''worried well,'' meaning that they are in good health, but may believe they were exposed to radiation, while others will require urgent care (N. Coleman) (*2*). Successful treatment of patients will depend on the level of advanced preparedness and planning, as well as efficient management of resources in a scarce environment, where patient needs may exceed the availability of medical resources (*3*). The shortage or lack of supplies may be further complicated by a destroyed physical infrastructure in severe damage zones and dangerous fallout extending beyond those zones (*4*). Injuries are expected to be complex; some individuals may experience radiation exposure only, while others may experience a combined injury of radiation and physical injuries (e.g., wounds and burns). Furthermore, injuries resulting from radiation exposure are multi-organ, and severity will likely be dose dependent. ARS is a complex injury, resulting in acute biological effects that manifest over subsequent days to weeks after exposure and delayed

biological effects that can occur months to years later. Patients will need to be triaged to assess the level of radiation, trauma or burn injuries to inform clinical management (*5*). Clinicians making treatment decisions will treat the presenting symptoms, but to help clinicians make specific treatment decisions for radiation-exposed patients, information is available on the U.S. Department of Health and Human Services' (HHS) Radiation Emergency Medical Management (REMM) website.[3]

The Radiation Triage, Treat, and Transport System (RTR)[4] is in place for operational management of the response after a nuclear detonation (*6*). Organized over space and time, RTR centers will transport people to assembly centers first, followed by medical centers, and then evacuation centers. At the RTR sites, the **S**ort, **A**ssess, **L**ife-saving intervention, **T**reatment/transport (SALT) mass casualty triage method will be applied, with sorting decisions modified depending on the incident (*7*). Triage categories will change depending on the scarcity of resources; thus, serial assessments will be needed to determine resource availability. Standards of care will vary on a scale from normal to poor, depending on location and time after the incident. Since resources will fluctuate daily and a scarce resource environment is probable, contingency standard-of-care plans should allow for substitute treatment options. Furthermore, an efficient response must include plans to co-locate these resources and maximize availability.

Careful analysis and planning over two years has led to the development of overarching ethical principles for triage, which optimize considerations for triage and treatment decisions according to patient-based issues (e.g., medical conditions, comorbidities, special populations, urgency for the response) and condition-based issues (e.g., efficacy of interventions, resource requirement, available resources) (*8*). In combination, these principles should help direct the triage category or treatment status to immediate, delayed, minimal or expectant. Of course, the triage conditions and categories must be re-evaluated frequently, since resources and conditions are expected to change (*9, 10*).

Ideally, patients will be sorted based on physical radiation dose (a traceable radiation dose on a physical target) and biological dose (biodose; a biological response in an irradiated organism). Importantly, every person will likely respond differently to a given radiation exposure. The proximity of the patient to the point of impact will be used to estimate the physical radiation dose; however, the actual individual biological effect (i.e., biodose) will require further assessment using biodosimetry techniques such as the dicentric chromosome assay (DCA). These results will be used by physicians to determine a treatment strategy in the context of resource availability (*11*). For all medical incidents, patients are managed by healthcare professionals using data, serial assessments, timely interventions and collaborative thinking. Currently, the MEdical TREatment ProtocOLs for Radiation Accident Victims (METREPOL) system, which focuses on symptomology as opposed to dose of radiation received, can help guide medical management after a radiation incident (*12*).

To better manage patient needs, a stepwise approach that incorporates re-evaluating medical triage and coordinated biodosimetry could improve diagnostics. Biodosimetry analyses could include protein and gene expression biomarker arrays (i.e., ''omics'' technologies) to assess individual levels of radiation exposure and resultant injury. As these technologies are developed, the feasibility of the type of sample, processing and analyzing time need to be appropriate for the operational environment. In addition, the actual biomarkers selected for an assay are critical; change in expression over time and potential confounding diseases must be considered. For example, researchers at the National Cancer Institute are working on an integrated approach using mRNA, miRNA, lncRNA and organ-specific biomarkers (N. Coleman). Since a variety of nucleic acids enter the blood circulation at different times after radiation exposure, these biomarkers could serve as an assay for the injury over time (*13*).

Strategic National Stockpile (SNS), Support for the Response to a Nuclear Incident

The SNS was created in the aftermath of the attacks on U.S. soil on September 11, 2001 under HHS from its predecessor, the National Pharmaceutical Stockpile, to serve as the nation's largest supply of pharmaceuticals and medical supplies for use during a public health emergency severe enough to cause a shortage of local supplies (S. Adams) (*14*).[5] Administered from 2004 by the U.S. Centers for Disease Control and Prevention (CDC), the SNS transitioned in October 2018 to the Office of the Assistant Secretary for Preparedness and Response (ASPR) within HHS. The SNS manages a ~$7 billion portfolio of medical materials, including pharmaceuticals (e.g., antibiotics, antidotes, antitoxins, antivirals and vaccines) and medical supplies. Although focused predominantly on chemical, biological, radiological, nuclear and explosive (CBRNE) threats, it also maintains prophylactics and therapeutics for emerging infectious diseases and pandemic influenza. The SNS also supports the tools for federal medical stations, which includes a suite of medical beds and medical supplies for victims of hurricanes. The SNS maintains these items through a network of strategically located repositories and commercial partnerships for storage, maintenance and rapid transport of material following an event. The response has been verified in real-life scenarios, such as the terrorist attacks in New York City on September 11, 2001, and in monthly exercises.

[3] www.remm.nlm.gov.

[4] https://www.remm.nlm.gov/RTR.htm.

[5] https://www.phe.gov/about/sns/Pages/default.aspx.

In the case of a nuclear incident, the SNS has a variety of therapeutic items available for radiation, including decorporation agents, granulocyte-colony stimulating factors (G-CSF, Neupogen®; pegylated G-CSF, Neulasta®) and granulocyte-macrophage colony-stimulating factor (GM-CSF, Leukine®). Also, of interest in a radiation response, the SNS holds anti-nausea drugs (ondansetron), pain medications (morphine, oxycodone), antibiotics (levofloxacin and amoxicillin), antivirals, antifungals, general pharmaceutical and medical supplies for blast and burns, electrolyte replacement, eye and wound care. Having this arsenal of treatments is critical for an effective response, but also underscores the fact that multiple drugs will be necessary. Furthermore, this poly-pharmacy approach will require clear instructions for use, and drug-drug contraindications must be considered.

After a public health emergency, delivery of material from the SNS to the designated areas for distribution and dispensing will depend on a seamless delivery platform. To ensure this platform is emergency ready, the CDC has funded public health preparedness initiatives to test receiving material from the SNS and has created pipelines for delivery into areas of need and dispensing supplies to patients. U.S. States that have received funding are charged with the development of all-hazards public health response plans and must identify multiple locations for material distribution and complete validation of these sites. MCM distribution and coordination is mediated by the CDC, as most of it is maintained as vendor-managed inventory. The CDC will use commercial transport to send the material to state-identified primary and secondary recipients, stage and store sites or alternate remote staging areas, while state authorities will coordinate local distribution. The delivery timeline for handoff of materials from the SNS to local authorities is anticipated to take less than 24 h; however, if coming from a vendor, longer times are expected. Once at the state level the length of distribution time can vary based on local procedures, policies and resources. The sub-distribution and integration of the material into the health-care system will be led by state and local jurisdictions. Given the uncertainty due to the distribution of causalities and disrupted communications, initial material distribution will follow a ''push'' model; in this model, higher-level-informed decisions will guide the need for materials, rather than a specific set of requests from a given hospital and/or jurisdiction, the latter of which may require more time. Without a clear idea of what is needed on the user's end, jurisdictions will have to employ whatever is available, further underscoring the need to understand how the therapeutics in the SNS complement or contraindicate one another, since treatment will likely require a poly-pharmacy approach. As communications improve, the focus will shift to a more strategic distribution to areas of greater need.

State and local response strategies for radiation and nuclear emergencies are less mature than for biological threat scenarios. While a robust response strategy should be in place for all scenarios, competition for finite planning resources poses a challenge. On the other hand, the resource burden can be reduced by addressing common issues such as limited access to the impacted area and the ability to mobilize material and people. Plans that have been developed for some hazards (such as anthrax) can be modified for other scenarios like radiation injury. Certainly, some cannot be easily adapted for a radiation emergency, such as in the case of uncertainties associated with radiation exposure levels and the need to set up multiple RTR centers. ASPR will continue to consider these challenges individually and address these particular needs as it continues to advance public health preparedness.

Natural History of Disease

Radiation injury can involve multiple organs, and different treatments may be needed for injuries to the various organ systems. By extension, this systemic dysfunction necessitates the consideration of poly-pharmacy approaches. To understand injuries to the various organ systems, investigators have looked at the natural history of radiation injury in two of the more well-characterized animal models: mouse (*Mus musculus*) and rhesus macaque (*Macaca mulatta*), as required by the Animal Rule (*15*).

Data were presented showing the effect of total-body irradiation (TBI) in mice and the delayed effects of radiation in surviving animals (C. Orschell). In the acute phase after radiation exposure, death is due to a breakdown in the immune system, causing hemorrhage and infection (*16, 17*), although later stages (weeks or months after exposure) are characterized by injuries to multiple organs. A compilation of data, from over 1,500 mice across seven years of hematopoietic (H)-ARS studies, showed residual damage to a number of organs. While radioresistant stem cells presumably led to the recovery of neutrophils, lymphocytes, erythrocytes and platelets, the counts did not reach the level seen in age-matched, nonirradiated mice (*18, 19*). This lack of recovery can be attributed to residual bone marrow damage. Other evidence of long-term radiation damage included increased chance of thymic lymphoma, reduction in lean mass gain, fibrosis/collagen deposition in heart, lung and kidney (*20, 21*), increases in anxious behavior and shortened lifespan. These long-term mouse studies showed that recovery from H-ARS was not complete, suggesting that treatments for the late-term syndromes will entail a poly-pharmacy approach.

While much can be learned by using mouse models, a nonhuman primate (NHP) model has also been developed to reflect the standard of care expected for radiation victims. The effects of medical management and GFs in a rhesus macaque model were discussed (T. MacVittie). Unlike rodent models where animals are treated equally, the NHP model uses a pre-established trigger-to-treat plan to administer antibiotics, antiviral, antifungal, anti-pyretic, anti-diarrheal and anti-emetic agents as needed (*22*). In

addition to these pharmaceuticals, animals received fluids, nutritional support and blood products as necessary when they met certain criteria. These are in line with recommendations from a SNS Radiation Working Group that convened after the events of September 11, 2001, to consider medical management recommendations (*23*). Using accidental radiation exposures as well as data from animal studies, the SNS Working Group recommended a series of antimicrobial, antiemetic, and analgesic agents as well as infusion of blood products, GF injections and stem cell transplant. By itself, this level of supportive care represents a poly-pharmacy approach that provides a high level of protection from acute effects such as infection and hemorrhage.

Various NHP studies over the last 25 years have shown the effect of leukocyte GFs on blood counts and survival. These GFs are indicated for treatment of ARS since they trigger expansion, growth and differentiation of radioresistant bone marrow stem cells to generate the various hematopoietic lineages, including neutrophils and megakaryocytes (*24*). In fact, the body naturally begins production of GFs and cytokines after irradiation (*24*), and radiation accident victims who experienced life-threatening radiation exposure (e.g., Chernobyl liquidators) exhibited spontaneous recovery of neutrophils and platelets (*25*). Similarly, accelerated platelet recovery in NHPs has been observed after administration of recombinant megakaryocyte growth and development factor (a truncated form of thrombopoietin) with and without G-CSF (*26*). In subsequent studies, treatment with pegylated or non-pegylated G-CSF was shown to increase survival and accelerate neutrophil recovery in irradiated NHPs (*27, 28*). These studies were used to support FDA approvals of Neupogen (filgrastim, approved March 2015) and Neulasta (pegfilgrastim, approved November 2015) for the mitigation of H-ARS.[6] With the 2015 approvals, and the 2018 approval of Leukine® (sargramostim),[7] GFs will need to be considered alongside other pharmaceuticals that may be administered as part of a medical management regimen. Research into new MCMs will need to take these treatments into account.

Just as the acute phase of radiation injury in NHPs requires a poly-pharmacy approach, so do the later phases. To better understand these late effects, observations and treatments for NHPs at the Wake Forest School of Medicine (WFSM) Radiation Survivor Cohort were presented (J. M. Cline). This valuable cohort consists of NHPs that received various radiation doses and geometries, and underwent long-term observation to elucidate the late-stage consequences of radiation exposure. Animals were obtained from a number of different institutions and received TBI ranging from 6.5 to 8.4 Gy, with varying types of medical management in the immediate, acute phase of their postirradiation timeline. Many of the animals received some form of supportive care, which often involved poly-pharmacy treatments (e.g., antibiotics and other therapeutics). At WFSM, the animals are observed twice daily, and are monitored with annual CT scans. In addition, blood chemistry and hematology, urine output, kidney function, cardiac function, gastrointestinal endoscopy and stool quality, and metabolic, nervous and respiratory system function are examined annually.

Compared to age-matched controls, irradiated monkeys have an increased risk of developing type 2 diabetes (*29*), which is linked to radiation-induced muscle fibrosis and extracellular matrix remodeling (*30*). Similar to what was observed in long-term atomic bomb survivors (*31*), irradiated NHPs also have increased incidence of heart disease, in particular, myocardial degeneration and fibrosis and systemic inflammation (*32*). Cognitive function was also studied in the cohort, and irradiated animals showed deficiencies in cognitive flexibility (*33*). Radiation-induced brain injury in these animals was characterized by white-matter neuroinflammation, T-cell activation and increased expression of complement factors (*34*). Other immune system dysfunction has been noted in the animals. Although leukocytes recover in irradiated NHPs, the immune system has exhibited deficiencies, including unexpected bacterial infection, reduced lymphocyte counts in some subsets, and blind spots in the immunological repertoire. Research is continuing to examine other long-term effects, such as renal dysfunction and increases in neoplasms. These natural history animal studies, taken together with data from patients exposed to high doses of radiation, demonstrate that long-term effects are likely to occur since GFs increase survival but only address the short-term effects of radiation. As a result, a poly-pharmacy approach will be needed to manage this multi-organ injury that will persist for years after initial recovery from radiation exposure.

Poly-Pharmacy Approaches to Mitigate Radiation Injury

Beyond natural history studies, research has also demonstrated: Improved survival in a H-ARS mouse model using combinations of hematopoietic GFs and angiotensin-converting enzyme (ACE) inhibitors; 2. Enhanced mitigation of radiation-induced injuries by combining ACE inhibitors with other countermeasures in a rat model; and 3. Improved survival in mice treated with drugs combined with pegylated-G-CSF therapy after irradiation alone or combined with wound trauma. To date, most data have been generated only in rodent models, which emphasizes the preliminary nature of the poly-pharmacy approach in radiation research.

A potential poly-pharmacy approach for the treatment of H-ARS using three novel products currently under development at Bolder Biotechnology, Inc. was considered (G. Cox). These are: 1. BBT-015, a PEG-G-CSF analog (neutrophil stimulator); 2. BBT-007, a PEG-GM-CSF analog (white blood cell stimulator); and 3. BBT-059, a

[6] https://bit.ly/2U8T35H.
[7] https://bit.ly/3vhUBXD.

PEG-IL-11 analog (a platelet stimulator). Given that each novel GF has a different mechanism of action and stimulates distinct intracellular pathways, it was hypothesized that observed improvements in survival with single-drug regimens could be additive if given together. In addition, each GF has a long-acting *in vivo* half-life, which reduces the need for repeated daily dosing, compared to the abbreviated (three once-daily doses in mice) (*35*) or conventional filgrastim therapy after irradiation (16 once-daily doses in mice) (*36*).

To determine the best treatment regimen, these pegylated hematopoietic growth factors (PEG-HGFs) were tested individually and together as a poly-pharmacy (PEG-HGF triple combo) approach. The efficacy of individual PEG-HGFs were tested in male and female 10–12-week-old C57BL/6 mice that received γ-ray irradiation from a Cesium-137 source. The mice were injected with a single dose of BBT-015, BBT-007 or BBT-059, respectively, 24 h after TBI ($LD_{50/30}$ or $LD_{70/30}$) and survival was monitored for 30 days after TBI. The PEG-HGFs improved survival by 50% (BBT-015), 42.5% (BBT-007) or 50% (BBT-059) compared to vehicle controls. In addition, significant increases in white blood cells, neutrophils, red blood cells and platelets were also noted. Only a single dose was needed in each case; an increase in the number of doses of each PEG-HGF was not superior over a single dose in improving overall survival (*37*).

The additive effects of combining the different GFs were also examined at a higher radiation dose ($LD_{90/30}$, 9.27 Gy): optimum survival was observed with the PEG-HGF triple combo, providing 80% survival compared to 7.5% in vehicle. Moreover, the PEG-HGF triple combo proved to be just as effective when administered at one tenth of a standard dose. This lowered dose may have significant implications for increased safety margins and drug costs. Rather surprisingly, although the PEG-HGF triple combo increased survival compared to the single PEG-HGFs, the hematopoietic recovery remained comparable. In fact, on day 5 postirradiation, the group receiving the PEG-HGF triple combo had the lowest neutrophil and lymphocyte counts, but the hematopoietic progenitors were significantly higher during that time, potentially leading to a better survival outcome.

Another poly-pharmacy approach, combining PEG-HGF triple combo with lisinopril to treat both H-ARS and DEARE injuries, was also presented (M. Medhora). ACE inhibitors (captopril, enalapril, ramipril, fosinopril and lisinopril), administered alone, were able to address multiple sequelae for both H-ARS and for DEARE (e.g., lung and kidney injury) when given to rats beginning 7 days postirradiation (*38*), and enalapril mitigated radiation pneumonitis even if started at day 35 postirradiation (*39*). Although ACE inhibitors are U.S. FDA-approved to treat hypertension and cardiac disease by blocking the synthesis of peptide hormone angiotensin II, they have also been shown to be useful for other disease conditions. Lisinopril, which has a good safety record for use in humans and in animal models, has a therapeutic effect in lung cancer patients, reducing the incidence of radiation pneumonitis (*40, 41*). With such a favorable profile, lisinopril as a potential MCM was explored using a partial-body irradiation (PBI, ''leg-out'') rat model (*42*). In this model, a higher, yet still survivable dose can be achieved, enabling the development of DEARE-lung complications, and allowing the testing of MCMs to mitigate these late injuries (e.g., lung, kidney) (*42–44*). By day 114 postirradiation, 88% of lisinopril-treated female rats were alive, while all irradiated, non-treated female rats were euthanized for cause (*43*). The PBI leg-out model was also adapted to test lisinopril in pediatric and geriatric rat populations (*43*). Interestingly, juvenile rats in both sexes appeared to be more sensitive to radiation-induced lung injury, while geriatric rats, though more sensitive to hematopoietic injury, were more resistant to the late effects of the radiation. Treatment with lisinopril significantly delayed mortality in both cases; however, without lisinopril each irradiated population, with the exception of geriatric male rats, eventually succumbed to the effects of kidney injury by day 150 postirradiation. Lisinopril was further tested in the presence of Neupogen, to examine any interactions it may have with the U.S. FDA-approved treatment; this combination showed no negative effect or added improvement (*42*).

A NIAID/RNCP-sponsored collaboration also led to the testing of lisinopril in combination with the PEG-HGF triple combo from Bolder Biotechnology, Inc. (Boulder, CO) Since treatment with the PEG-HGF triple combo activates the cytokine cascade during the acute stage of ARS (1–30 days after TBI) and lisinopril reduces the late effects of radiation injuries (90–180 days after TBI), the investigators proposed combining the PEG-HGF triple combo with lisinopril. In each case, the poly-pharmacy approach was tested in both mice and rats at each respective laboratory, where a single subcutaneous dose of the PEG-HGF triple combo was administered 24 h postirradiation followed by lisinopril (60 mg/l in mice or 40 mg/l in rats) in drinking water starting on day 7 after TBI and continued throughout the duration of the study. Using the PBI leg-out rat model, lisinopril combined with the PEG-HGF triple combo improved 150-day survival to 100% compared to lisinopril alone (90%) and to PEG-HGF alone (0%) (*45*). Similarly, using an established H-ARS TBI mouse model (*17*) ($LD_{90/30}$; 9.04 Gy) a significant 30-day survival benefit (100%) was also demonstrated with the combination of lisinopril and the PEG-HGF triple combo compared to lisinopril (40%) and to PEG-HGF (65%) alone.[8] The survival benefit was significant when all mice were

[8] Narayanan FG, Fish BL, Jacobs ER, Cohen EP, Moulder JE, Cox GN, et al. Efficacy of lisinopril for mitigation of late radiation effects in rats when combined with mitigators for hematopoietic toxicity. 61st Annual Meeting of the Radiation Research Society. Weston, FL; Sept 19–22, 2015.

combined, but it is important to note that sex differences were observed. The addition of lisinopril provided an increased survival advantage in females, but no added benefit could be seen in male mice. The addition of lisinopril had a neutral effect equivalent to PEG-HGF triple combo alone in male mice. While interesting, the number of animals was too low to statistically support the sex differences noticed; this should be further explored.

Poly-Pharmacy Approach in a Radiation Combined Injury (RCI) Model

Combined injuries, such as cuts and burns, can exacerbate the consequences of radiation exposure (*46–48*). Researchers at AFRRI have studied the effects of radiation in the presence and absence of wounds in B6D2F1 mice (J. Kiang). The damaging effects of RCI were demonstrated by the finding that the $LD_{50/30}$ from TBI was reduced from 9.65 Gy to 8.95 Gy in the presence of a punch wound trauma. Furthermore, wound healing was delayed beyond 28 days, in contrast to a 14-day healing process in nonirradiated animals. In addition, the damaging effects of RCI were shown in a CD2F1 mouse model of hemorrhage, where the radiation $LD_{50/30}$ was reduced from 9.25 Gy to 8.75 Gy (*48*). In this RCI mouse model, significant increases were noted in γ-H2AX (a marker of DNA damage which precedes cell death), iNOS (marker of protein dysfunction), IL-6 (proinflammatory cytokine) levels, and a significant decrease in citrulline (marker of GI damage) compared to radiation or wound conditions alone.

Given the major differences noted between the health outcomes of radiation alone compared to combined injury, it is imperative to understand the efficacy of MCMs under both conditions. To accomplish this, Neulasta was tested using the RCI model and the results showed that the GF increased survival after irradiation alone, but not in the presence of a punch wound (*49*). In an effort to find an approach that is efficacious in a combined injury scenario, several poly-pharmacy combinations were tested, including administering Neulasta alongside ALXN4100, a thrombopoietin receptor agonist. This combination showed a 35% and 20% improvement in the 30-day survival outcome after irradiation alone and combined injury, respectively (*50*). Additionally, this poly-pharmacy treatment showed mild mitigation of bone marrow and weight loss, as well as water consumption, but did not accelerate wound healing (*50*). A second approach tested ghrelin, also known as the hunger hormone, that has been shown to mitigate radiation injuries (*51, 52*). In combination with Neulasta, an increase in survival was noted in the irradiation alone group, but no benefit was seen in the RCI group. At the cellular level, the Neulasta and ghrelin poly-pharmacy approach increased eosinophils, leukocytes, platelets and bone marrow cellularity, while protecting the GI tract and reducing brain hemorrhages in radiation alone groups (*53*), and to a smaller extent in the combined injury group (*54*). In another combination study, ciprofloxacin, a fluoroquinolone antibiotic, was tested in combination with Neulasta. The Neulasta-ciprofloxacin poly-pharmacy approach improved survival by 80% in the irradiation alone group but resulted in only 10% survival improvement with combined injury.[9]

Based on the data presented in the workshop, combined injury leads to a more severe medical condition. Other studies have shown that trauma such as dorsal skin burns and wounds can result in additional underlying disturbances to the immune system, which can confound the calculation of radiation dose and the severity of the radiation injury (*55*). In light of this information, it is important to examine MCMs, whether novel or FDA-approved, under different conditions that include combined injury. It is also important to note that a survival advantage has also been shown in mice receiving a subcutaneous incision after irradiation, presumably due to a cytokine cascade caused by the wound (*56*). Given the complexity of RCI, investigators should consider testing combination or poly-pharmacy approaches that target the distinct disease pathologies to find a useful treatment for this scenario.

Regulatory Considerations

As MCMs are being developed under the Animal Rule (*15*), it is important to understand what is required for approval/licensure. The U.S. FDA Center for Drug Evaluation and Research (CDER), Division of Imaging and Radiation Medicine (formerly Division of Medical Imaging Products) recommends meeting with the agency early and often when considering the development of a MCM under the Animal Rule (A. Laniyou) (*15*). Product developers, along with funding partners, should communicate with the review division to reach agreement on the key objectives of any development program, including the design of adequate and well-controlled animal efficacy studies intended to provide substantial evidence of the efficacy of the drug. It is also recommended to begin development with the end goal in mind and consider the conditions under which the product would be used. If it is anticipated that the efficacy of the drug will be optimal if used in a specific way, e.g., when administered daily for three days beginning within 48 h of irradiation, then the animal efficacy study design should reflect that expectation. If a drug is already approved for the same indication (e.g., Neupogen for H-ARS) and the approval was based on the same animal species in which the investigational drug is being evaluated, an approved drug comparator arm should be included, in addition to the investigational drug, placebo, and a combination arm.

Clinical pharmacology factors that may affect human dose selection must also be considered, including (M. Sampson): 1. drug or biologic targets; 2. relevant biomark-

[9] Kiang *et al.* (unpublished data).

ers that correlate with a desired clinical outcome such as reduced mortality or major morbidity; 3. available information about prior use or indications in humans; 4. comparison of effective dose in animals with predicted human dose using relevant exposure parameters (e.g., AUC, Cmax, Cmin, Css); and 5. safety profile (e.g., maximum tolerated dose). When possible, a simulation of PK profiles for specific populations (e.g., pediatrics, geriatrics, etc.) should be done. This is a complex process and selecting a human dose under the Animal Rule may require unique approaches and the use of multiple data elements; therefore, frequent interaction with the FDA is encouraged.

All medical products in the SNS are subject to U.S. FDA regulations to assure the public of the quality, safety and efficacy of the drugs, biologics and medical devices contained therein (Y. Yu). The SNS includes both FDA-approved MCMs as well as unapproved (investigational) MCMs. For these MCMs to be covered under the Public Readiness and Emergency Preparedness (PREP) act, they must be: 1. approved, licensed or cleared by FDA; 2. used under an Investigational New Drug (IND) or Investigational Device Exemption (IDE); or 3. used under an Emergency Use Authorization (EUA), Emergency Use Instructions (EUI), and/or emergency dispensing orders. The PREP act ''. . .authorizes the Secretary of the Department of Health and Human Services to issue a declaration (PREP Act declaration) that provides immunity from liability (except for willful misconduct) for claims of loss caused, arising out of, relating to, or resulting from administration or use of countermeasures to diseases, threats and conditions determined by the Secretary to constitute a present, or credible risk of a future public health emergency to entities and individuals involved in the development, manufacture, testing, distribution, administration, and use of such countermeasures (*57*).'' In addition, PREP authorizes a compensation fund for serious physical injuries or death directly caused by use of covered MCMs.

The Project BioShield Act of 2004 authorized the U.S. FDA commissioner to allow the emergency use of unapproved MCMs in CBRNE emergencies. If a serious, life-threatening disease occurs and no U.S. FDA-approved alternatives exist, an EUA can be issued if the totality of the data demonstrates a clinical benefit, where the known risks are outweighed by the benefit of using the MCM. For instance, the CDC worked with the U.S. FDA to file an EUA for the use of Radiogardase® (Prussian blue) in children between 6–23 months of age. Although this is a stop-gap measure, it provides a provisional option for an emergency scenario.

In 2013, the authorities for emergency use were amended under the Pandemic and All-Hazards Preparedness Reauthorization Act (PAHPRA) (*58*). The act eliminated the one-year automatic termination of an EUA and increased the data collection time period to continue after the EUA period. New emergency use authorities were also granted, including mass dispensing, shelf-life extension, a current good manufacturing practice (cGMP) waiver, a Risk Evaluation and Mitigation Strategy waiver, and EUI. One of the most important tools for emergency situations, the EUI issuance authority, was delegated to the Director of the CDC by the HHS Secretary on December 6, 2013. An EUI allows the CDC to create and issue special fact sheets about FDA-approved directives for the use of MCMs before a CBRNE event occurs. These instructions can be disseminated by government stakeholders to health care professionals or MCM recipients. Since an EUI provides event-driven prevention and treatment information during emergencies, it can be an essential tool for delivering special instructions on the use of multiple stockpiled MCMs. Presently, EUIs have been created for Neupogen to include an option to administer 5 mcg/kg in shortage conditions. For Neulasta, an EUI was created for a special dose preparation for children $<$45 kg. Ultimately, this can be a very complex situation, so pharmacists and medical doctors will be essential in understanding and tracking drug-drug interactions for patients.

More importantly, if multiple MCMs are developed for use in a poly-pharmacy approach, the regulatory requirements for the use of the combination should be clear. Any existing supportive data will likely be assessed when considering the use of a poly-pharmacy approach, but most importantly, post-marketing studies will likely be needed as well. The CDC works closely with the U.S. FDA to review existing supportive data for the intended use of MCMs and to ensure that the appropriate regulatory mechanisms are in place to allow for timely deployment and optimal utilization of MCMs. This is especially important when handling investigational MCMs or products that the FDA has approved for the specific indication. A CDC-sponsored, expanded-access IND application is required for stockpiling of investigational MCMs in the SNS during the development pathway and for emergency use. This expanded-use IND allows for post-marketing studies, as well as MCM evaluation during and after an incident (*59*). Some examples include: 1. The approved use of the anthrax vaccine for post-exposure prophylaxis in children given the absence of pediatric data; and 2. The use of tecovirimat, a novel antiviral for treatment of smallpox, as a post-exposure prophylaxis in children weighing at least 13 kg. In general, the expanded-access IND allows the use of products before they are approved by the FDA for that indication and allows for the collection of first-in-human safety data in diseased patients.

While a bit more complex, the same rules apply for poly-pharmacy approaches, but the experimental design should consider and support the end use scenario. Furthermore, clinical pharmacology studies that address drug-drug interactions must be taken into account, especially in the case of poly-pharmacy. In all cases, it is advised that the FDA be contacted early and often to discuss the details of the planned studies.

CONCLUSION

After a radiological incident, chances are high that individuals will suffer a systemic assault worsened by combined injuries from wounds and burns (*60*). A poly-pharmacy MCM approach may help strengthen the mitigation of a variety of injuries by targeting different mechanisms, pathways, and/or organ systems (e.g., vascular damage, cell loss, inflammation, oxidative stress, cell signaling, skin), resulting in an improved overall survival or reduction in major morbidities (*61*). The research presented here supports the hypothesis that certain drug combinations can improve health outcomes, as demonstrated by the combination of the PEG-HGF triple combo plus lisinopril, which results in a 100% survival. This example demonstrates that a combination of MCMs may be more effective in improving survival after lethal-dose irradiation than a single MCM, by potentially acting on multiple biological pathways or on different organ systems. In fact, HGFs were shown to be effective at 10% of the original dose, which can potentially lessen side effects and reduce drug costs. The radiation research, funded over the years by the NIAID RNCP, continues to shed light on the complexities of injuries induced by radiation alone or in a combined injury scenario. The information suggests that a poly-pharmacy approach is advised. Clearly, more research is still needed to determine the best poly-pharmacy approaches and to assess any drug-drug contraindications.

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