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WORKSHOP REPORT

Use of Growth Factors and Other Cytokines for Treatment of Injuries During a Radiation Public Health Emergency

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Due to the threat of a radiological or nuclear incident that could impact citizens, the U.S. Department of Health and Human Services tasked the National Institute of Allergy and Infectious Diseases (NIAID) with identifying and funding early- to mid-stage medical countermeasure (MCM) development to treat radiation-induced injuries. Given that the body's natural response to radiation exposure includes production of growth factors and cytokines, and that the only drugs approved by the U.S. Food and Drug Administration to treat acute radiation syndrome are growth factors targeting either the granulocyte (Neupogen® or Neulasta®) or granulocyte and macrophage (Leukine®) hematopoietic cell lineages, there is interest in understanding the role that these factors play in responding to and/or ameliorating radiation damage. Furthermore, in an environment where resources are scarce, such as what might be expected during a radiation public health emergency, availability of growth factor or other treatments may be limited. For these reasons, the NIAID partnered with the Radiation Injury Treatment Network (RITN), whose membership includes medical centers with expertise in the management of bone marrow failure, to explore the use of growth factors and other cytokines as MCMs to mitigate/treat radiation injuries. A workshop was convened that included government, industry and academic subject matter experts, with presentations covering the anticipated concept of operations during a mass casualty incident including triage and treatment, growth factors under development for a radiation indication, and how the practice of medicine can inform other potential approaches, as well as considerations for administration of these products to diverse civilian populations. This report reviews the information presented, and provides an overview of the discussions from a guided breakout session. **© 2019 by Radiation Research Society**

INTRODUCTION

The U.S. Government has tasked several agencies with the mission to research and develop medical countermeasures (MCMs) to treat injuries that could result from exposure to radiation during a mass casualty, public health emergency. Included among these organizations is the National Institute of Allergy and Infectious Diseases (NIAID), within the National Institutes of Health (NIH), which has primary responsibility for the early research and development of promising approaches. Since 2004, the program has funded critical studies to better understand, diagnose and treat radiation injuries. In addition, there are several non-governmental organizations that share the mission to provide critical medical responses to those injured during a nuclear or radiological incident, including the Radiation Injury Treatment Network (RITN), whose membership includes medical centers with expertise in the management of bone marrow failure (*1*). RITN has a role prior to, during and after a radiation disaster, through development of treatment guidelines and planning of tabletop exercises (*2*), provision of comprehensive care for victims and collection of patient data for retrospective analysis (*3*).

Given the endogenous role of growth factors and cytokines in responding to various injuries and mediating repair, study of these molecules as potential radiation mitigators was central to much of the early research and development of MCMs. The timing of the endogenous increase is key to why administration of these kinds of factors postirradiation is effective in mitigating radiation injury. Although many biologics are naturally increased after irradiation, it takes time for them to reach an efficacious level. Administration of these factors

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during the time frame prior to peak endogenous expression provides stopgap protection, as the body's own responses are increasing. As of December 2018, the U.S. Food and Drug Administration has approved only three drugs to treat the acute radiation syndrome (ARS). All of these are growth factors, which target either the granulocyte (Neupogen® or Neulasta®) or granulocyte and macrophage (Leukine®) hematopoietic cell lineages. Advances in cytokines to date have been leveraged by the pre-existing developments in bone marrow transplantation, wherein a homogeneous radiation exposure is produced. Rather less is known, and therefore progress has been slower, for multiple organ toxicity, combined injuries and inhomogeneous doses that will accompany the vast majority of victims of a nuclear event.

To explore the use of growth factors and cytokines to treat radiation injuries, the Radiation and Nuclear Countermeasures Program (RNCP), within the NIAID, NIH, together with colleagues from the RITN, convened a workshop in Rockville, MD on August 30, 2018. The purpose of the meeting was to explore growth factor and cytokine MCM development by federal, academic and industry partners and to discuss strategies for MCM deployment and use. The overall workshop goal was to convene stakeholders to discuss current trends in clinical practice utilizing growth factors and other cytokines (either those already licensed or currently in clinical trials) as well as preclinical research approaches under consideration and their applicability to a mass casualty response. The invited participants included U.S. Government planning and funding agencies, healthcare providers, hospital-based emergency management staff and pharmacists interested in disaster planning, as well as industry and academic researchers engaged in preclinical development of candidate products. The agenda included presentations to better understand the latest science on preclinical development and clinical use of these kinds of treatments. Through talks and a guided discussion session, participants shared information on the government infrastructure for administration of products during a mass casualty incident, updates on treatment approaches under development, and how physicians approach the use of growth factors and cytokines in other clinical areas. An overview of these talks and discussion are presented below.

BACKGROUND

Ionizing radiation deposits energy in all the biomolecules of the cell (DNA, proteins and lipids), and certain cells; and therefore, organs of the body are more radiosensitive. Although different tissues exhibit varying degrees of radiosensitivity, radiation-induced multi-organ dysfunction is a hallmark of injuries and death resulting from exposure (*4*). Because cytokine and growth factor responses are also ubiquitous within the body, these biologics represented an early area of focus for researchers looking for mitigators and treatments for radiation-induced damage.

The casualties of a radiation public health emergency incident could include tens or hundreds of thousands of persons exposed. Victims might not present with symptoms of radiation toxicity, even if exposed to high doses of radiation; therefore, these individuals will need to be assessed to determine if they have been exposed to potentially-lethal doses of radiation, and thus, would require treatment with growth factors or cytokines. In a scarce-resources environment, it will not be possible to provide these medical countermeasures to everyone; therefore, estimates of radiation exposure (biodosimetry) are critical to identify those individuals would benefit most from administration of growth factors and other drugs. Gold standards to determine absorbed radiation dose have historically involved DNA damage (cytogenetics) (*5*) or lymphocyte depletion kinetics (*6*). However, a number of new technologies are also under development to distinguish between groups of potentially-exposed individuals, including, but not limited to, proteomic, genomic, transcriptomic and metabolomic approaches (*7*). Based on these assessments, better-informed decisions could be made as to who would derive the most benefit from administration of available growth factors or cytokines.

For the purposes of this meeting report, growth factors and cytokines are defined as small proteins important in many biological activities such as cell signaling, fighting infections and inducing immune responses. Also included in this group are interleukins, chemokines and interferons. Hormones are often considered separately in the literature, with cytokines sometimes referred to as the ''hormones of the immune system'' (*8*). Growth factors and cytokines are typically produced by immune cells, endothelial cells, fibroblasts and other stromal cells, and act through receptors to mediate activities including inflammation, tissue repair and remodeling. Nearly all cells in the body also serve as responders to these molecules. These cytokines, and the responses that they evoke, can be both positive, when properly controlled, or negative. For example, they might be involved in protecting against disease, but their overproduction, often in the form of a ''cytokine storm'' could cause further pathologies or interfere with other life-saving treatments such as bone marrow transplants (*9*). Because cytokines have been widely studied for treatment of many abnormal homeostatic states, including lung (*10*) and cardiac fibrosis (*11*), trauma, sepsis (*12*) and cancer (*13*), they have been considered for radiation injury treatment. In addition, there have been several published studies in which growth factors and cytokines are examined, both alone and in combination, in animal models of radiation injury (*14–21*). The clinical availability of these kinds of therapies and wealth of clinical data allow for repurposing, which can often accelerate the licensure for a radiation indication. In addition to growth factors, cytokines and hormones, other products that simulate these responses (e.g., small molecule mimetics) were also considered, as they are also being developed as radiation MCMs. Some of these approaches are outlined in Table 2.

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TABLE 1
Workshop Speakers/Moderators and Areas of Expertise[a]

Name	Affiliation	Areas of expertise
Jennifer Aldrich, MA	RITN, Minneapolis, MN	Hospital administration during disasters or emergencies
Cullen Case, Jr., EMPA	RITN, Minneapolis, MN	Disaster preparedness, response, continuity planning and operations
Nelson Chao, MD	Duke University, Durham, NC	Bone marrow transplant, immunology, hematopoiesis, radiobiology
John Chute, MD	UCLA, Los Angeles, CA	Hematopoietic stem cell biology, immunology, radiation biomarkers
George Cox, PhD	Boulder BioTechnology, Boulder, CO	Protein engineering, pharmacology, product development
Nicholas Dainiak, MD	Yale University, New Haven, CT	Medical management of ARS, cytokines, mitigators
Andrea DiCarlo, PhD	RNCP, DAIT, NIAID, NIH, Rockville, MD	Radiobiology, product development, MCM testing
Ann Jakubowski, PhD-MD	MSKCC, New York, NY	Bone marrow transplant, hematology, oncology
John Koerner, MPH, CIH	ASPR, HHS, Washington, DC	CBRNE[b] science/operations, emergency response
Christopher Lawrence, PhD	Neumedicines, Pasadena, CA	Biotechnology, small molecules/biologics, PK/PD, immunology
Paul Okunieff, MD	University of Florida, Gainesville, FL	Oncology, radiobiology, growth factors, biomarkers
Joseph Park, MD- PhD	Amgen, Thousand Oaks, CA	Drug development, hematology, oncology
Zulmarie Perez Horta, PhD	RNCP, DAIT, NIAID, NIH, Rockville, MD	Immunology, cellular and molecular biology, chemistry
Alix Seif, MD, MPH	Children's Hospital of Philadelphia, Philadelphia, PA	Pediatric oncology/blood and marrow transplantation
William Skinner, MD	USUHS, Bethesda, MD	Radiation oncology, military medicine, radiation protection

[a] Speakers had the opportunity to review this meeting report prior to journal submission.
[b] CBRNE = chemical, biological, radiological, nuclear and explosive.

Historically, growth factors have been used clinically to treat radiation accident victims. Reports of their use include radiation accidents in Tokai-mura, Japan (*22*), Goiania, Brazil (*23*), Fleurus, Belgium (*24*), Dakar, Senegal (*25*) as well as other countries (*26*). Growth factors involved in those treatment approaches included granulocyte-colony stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), erythropoietin (EPO) and thrombopoietin (TPO). Several of these cases are discussed in more detail below. A review by Hofer *et al*. (*27*) also outlines evidence for the benefit of G-CSF use in the treatment of ARS. G-CSF, a glycoprotein discovered and developed in the 1980s, stimulates the bone marrow to produce granulocytes (neutrophils) and stem cells and release them into the peripheral blood stream. In addition, some drugs also have a mechanism of action that involves induction or inhibition of endogenous growth factors to exert their effect; however, these products will not be considered in depth here.

The U.S. Food and Drug Administration's (FDA) Animal Rule (*28*) has been used for approval of growth factors for the treatment of radiation injury. These approvals for ARS include filgrastim (Neupogen, FDA approved March 2015; Amgen®, Thousand Oaks, CA),[2] pegfilgrastim (Neulasta, FDA approved November 2015; Amgen)[3] and sargramostim (Leukine, FDA approved March 2018; Partner Therapeutics, Lexington, MA).[4] These products are indicated to increase survival in patients acutely exposed to myelosuppressive doses of radiation (hematopoietic subsyndrome of ARS). Given these approvals, there is no longer a requirement for an Emergency Use of Authorization (EUA) for their use during a radiation incident, if used as advised on the drug label for this indication (*29*). For example, guidelines for the use of Neupogen include administration as soon as possible after a confirmed or suspected exposure to radiation doses above 2 Gy, with continued dosing until the absolute neutrophil count (ANC) exceeds 10,000/ml.[5] The Radiological Events Medical Management (REMM) website, which contains information for first responders, healthcare providers and patients, advises that these drugs are to be dosed as follows:[6]

1. Neupogen: once daily, subcutaneous (SC) injection (10 μg/kg/day) for adult and pediatric patients; continued until ANC remains greater than 1,000/mm^3 for 3 consecutive complete blood counts or exceeds 10,000/mm^3 after a radiation-induced nadir.
2. Leukine: once daily, SC injection (7–12 μg/kg/day) for adult and pediatric patients; same as Neupogen, continued until ANC remains constant at 1,000/mm^3 or exceeds the 10,000/mm^3 threshold.
3. Neulasta: two doses, SC injections, 6 mg each (weight based for <45 kg), administered one week apart.

Other myeloid growth factor biosimilars, FDA approved for the treatment of neutropenia associated with myelosuppressive anticancer therapy but not for the specific indication of acute exposure to myelosuppressive doses of radiation (and would require an EUA) include: tbo-filgrastim (Granix®, Teva; approved 2012)[7] filgrastim-sndz (Zarxio®, Sandoz; approved 2015)[8] and filgrastim-aafi (Nivestym®, Pfizer; approved 2018).[9] In addition to growth

[2] https://bit.ly/2ZJO9KH.
[3] https://bit.ly/2U8OwdE
[4] https://bit.ly/2XYai6h.
[5] https://bit.ly/2OWDKpK.
[6] https://bit.ly/2P28xl9.
[7] https://bit.ly/2I8r7aI.
[8] https://bit.ly/2uXqrfQ.
[9] https://bit.ly/2I6Dq7r.

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TABLE 2
Growth Factor and Cytokine Studies with Past or Current NIAID Support

Growth factor	Indication[a]	Reference[b]
Neupogen (G-CSF, filgrastim)[c]	H-ARS (neutropenia)	(*126*)
Neulasta (PEG-G-CSF, pegfilgrastim)[c]	H-ARS (neutropenia)	(*127*)
Nplate[c] (TPO receptor agonist, romiplostim)	H-ARS (thrombocytopenia)	(*71*)
BBT-059 (PEG-IL-11)[c]	H-ARS	(*80*)
BBT-007 (PEG-GM-CSF)[c]	H-ARS	(*80*)
BBT-015 (PEG-G-CSF)[c]	H-ARS	(*80*)
FGF-P[c]	GI-ARS	(*65*)
Bivalent-G-CSF	H-ARS	(*128*)
EGF[c]	H-ARS	(*55*)
CDX-301 (FMS-like tyrosine kinase-3 ligand, Flt3L)	H-ARS	(*15*)
Human growth hormone (HGH)	H-ARS	(*129*)
Insulin-like growth factor 1 (IGF-1)	CRI	(*130*)
Interleukin-7 (IL-7)	H-ARS	(*131*)
Vascular endothelial growth factor (VEGF)	H-ARS	(*132*)
Platelet-derived growth factor (PDGF)	H-ARS	(*133*)
Keratinocyte growth factor (KGF, Palifermin)	H-ARS, GI-ARS, lung-DEARE	(*121*)
Stem cell factor (SCF)	H-ARS	(*15*)
Neumega (IL-11, Oprelvekin)	H-ARS, GI-ARS	(*134*)
Epogen [epoetin alfa (erythropoietin)]	H-ARS	(*123*)
Basic fibroblast growth factor (bFGF or FGF2)[c]	H-ARS	(*63*)
R-spondin 1 (WNT signaling)	GI-ARS	(*135*)
Mozobil™ (Plerixafor; stem cell mobilization factor)[d]	H-ARS, skin	(*136*)
PLX-R18 (placenta-derived cell therapy)[d]	H-ARS	(*137*)
PEG-TPOm[d] (pegylated thrombopoietin mimetic peptide)	H-ARS (thrombocytopenia)	(*138*)
PBI-1402 (hematopoietic growth stimulant)[d]	H-ARS	(*139*)
dmPGE (dimethyl prostaglandin E analog)[d]	H-ARS	(*140*)
Forteo® (parathyroid hormone)[d]	H-ARS	(*141*)
5-Androstenediol (5-AED, estrogen steroid hormone)[d]	H-ARS	(*142*)

[a] Hematopoietic (H), gastrointestinal (GI), delayed effect of acute radiation exposure (DEARE), cutaneous radiation injury (CRI).
[b] Reference may not reflect NIAID funding.
[c] Presentation at the meeting.
[d] Product that complements/mimics a growth factor or cytokine.

factors targeting the neutrophil and macrophage lineages, there are other approaches under study for possible use as MCMs to treat other forms of radiation injuries. Some of these products, which have received past and current funding from the RNCP, NIAID are listed in Table 2. Much of the efficacy testing of these products in animal models of radiation injury has already been published. Although this list is by no means complete, information obtained from these studies can be leveraged to better understand the safety of growth factors and cytokines and how these could be used clinically for a radiation injury. A discussion on available preclinical data for some products, as well as the clinical use of these therapies for radiation-induced injuries can be found in the ''Meeting Program Overview,'' below.

MEETING PROGRAM OVERVIEW[10]

The one-day meeting was structured with scientific sessions comprised of: 1. an overview of planning for the use of growth factors and cytokines in a radiation emergency; 2. pre-licensure research; and 3. clinical use and practice of medicine. All were followed by short discussion periods. During the three sessions, presenters and meeting participants considered issues concerning the feasibility of using these approaches in a mass casualty setting, and the benefits and drawbacks of their use. As a final activity, participants were divided into discussion groups for in-depth consideration of aspects that the meeting organizers felt were important for the use of growth factor and cytokine approaches during a radiation public health emergency, including prioritization of efforts and consensus recommendations. These discussion topics are listed in Table 3, and an overview of the recommendations are located in the Discussion section, below.

Session 1: Planning Overview

To determine if the use of growth factors and cytokines during and after a radiological or nuclear incident would be advantageous, it is important to understand both the expected concept of operations for their use, as well as the status on their potential use to treat different radiation injuries. To that end, U.S. Government program officials, as well as experts from the RITN were asked to set the stage for the use of these MCMs during a radiation public health emergency.

[10] Where pre-publication data are discussed, the first initial and last name of the presenter who provided the information is shown in parentheses.

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TABLE 3
Topics For Consideration During the Discussion Session

Guided discussion topics
Preclinical model considerations
Animal model and strain selection (different efficacy)
Testing human vs. rodent/NHP growth factor equivalents
Understanding mechanism of action
Supportive care for models (G-/GM-CSF as standard of care)
Dose rates – very low or very high – how do growth factors perform in these scenarios?
Efficacy of growth factors with mixed field irradiation exposures
Heterogeneity of exposure. TBI/shielded/marrow sparing and ''leakage'' of radiation under shield
Science of cytokines
Low-dose vs. high-dose radiation exposures
Thresholds for bone marrow injury, after what radiation exposure are treatments more effective?
Efficacy of growth factors in radiation combined injuries
Doses administered (high-dose vs. low-dose use of growth factors; less drug/dose or fewer doses?)
Timing of administration
Many factors are induced by radiation; supplement endogenous production at an earlier time point to reduce cell stress?
Optimal clinical use
Are some growth factors more efficacious than others?
Possible negative late impacts of early growth factor use (e.g., late lung effects)
Some factors may be contraindicated
What other growth factors in clinical use can be repurposed?
Availability of clinical data
Confounders (chemotherapy, fractionated radiation exposure)
Operational considerations
Use in special populations
Impact of supportive care on growth factor efficacy
Triage guidelines: scarce resources decisions, ethics involved
Off-label use
Stockpiling constraints
Timing and availability
Production capacity and timelines
Storage or vendor managed inventory
Regulatory considerations
Importance of primary clinical indication
Easier to repurpose licensed/approved products?
Challenges for animal rule approval
Poly-pharmacy considerations
Combination strategies to enhance effect of growth factors
G-/GM-CSF as standard-of-care, are approaches additive? Do they interfere?
On the horizon
Novel factors under study: What are the next ''low hanging fruits''?
Other factors to address specific organ systems (e.g., GI, lung, renal, cardiac, vascular, central nervous system, etc.)
Enhancing growth factors [e.g., extending half-life (pegylation) and/or improving potency]

The meeting began with background on the government's planned approach for an improvised nuclear device (IND) disaster, and how it differs from other catastrophes. The discussion began with the need to plan for palliative care, the potential for additional injuries after the initial incident if people do not shelter in place and finally, the importance of triage to maximize the medical benefit, i.e., doing the most good for the most people (J. Koerner). This approach will likely be a paradigm shift for the vast majority of medical providers and is apropos when discussing the use of cytokines after a radiological mass casualty incident, since there will not be sufficient doses of many drugs immediately after the disaster (*30, 31*). In this situation, some people will not receive medication because they are expectant (not anticipated to survive) or because they do not require interventions for recovery. Medical providers will not have the luxury of a multitude of pharmaceuticals available for each patient, as they often do day-to-day in their practices. This scarce resource environment will lead to some difficult decisions on the part of healthcare providers.

The potential extent of the damage from a 10-kiloton (kT) IND, detonated in downtown Washington, D.C., would include a dangerous fallout zone that could extend for upwards of 30 miles downwind. If the general populace follows guidance from public officials and shelters in place, and when directed, seeks assistance for radiological screening and decontamination, the number of casualties can be greatly decreased. Once this process is initiated, it will follow the Radiation TRiage-TReatment-TRansport system (RTR), as described by Hrdina (*32*) and others (*33*). The RTR system provides order in what is expected to be a chaotic situation, by identifying locations where local responders can begin to filter the masses and provide help to the most people. This approach will be essential, given the scarce resources that will be needed for an overwhelming surge of casualties. Eventually, the National Disaster Medical System[11] (NDMS) will transport these casualties to distant cities, where a higher level of care and more resources will be available. These locations could be stadiums, hospitals or schools, and are already identified for every city in the U.S., in the Department of Health and Human Services (HHS) GeoHealth information mapping system.[12] These sites can be ready at a moment's notice for use in disaster response.

As the patients move through the facilities in the RTR system, there will be a stark difference between the goals of early and late triage. Initially, triage will prioritize those with trauma who are salvageable with available resources. This level of care will differ from location to location and change with time as well. A person may be treated on day 1, whereas the next day a decline in available resources can result in a second person with identical injuries to be determined expectant and receive palliative care. The opposite effect will occur for those with radiation injury; those with salvageable radiation injuries (i.e., low Gy exposures) can wait a few days before receiving the focus of medical attention; however, once their blood counts begin to drop, they will need specialized supportive care to recover. Fortunately, by this time, they can be evacuated to a distant city for this care.

[11] https://bit.ly/2xOfRs5.
[12] https://bit.ly/2IlFmIt.

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To combine these disparate situations into one cohesive picture that can help to guide radiation emergency response, Koerner (*34*) and Coleman (*35*) have proposed a definition of a national concept of operations (CONOPS) as: 1. articulating the goals and decisions for medical response to a nuclear detonation, while 2. describing the systems, entities, resources, geography and timing necessary to achieve the stated goals or decisions. This definition includes three key factors that will determine the ability to care for the patients:

1. Brings together required capabilities: shelter/evacuation, triage, dosimetry, medical management, medical countermeasures, treatment, coordination and communication;
2. Considers the impacts of mitigating or enhancing factors: time, geography, scarcity of resources, infrastructure status, medically relevant timing, behavioral health, the intersections and dependencies among factors; and
3. Evaluates the overall impact on executing the required capabilities and the broader response.

In closing, it was suggested that participants consider a few questions to guide their work and ultimately improve national preparedness. These questions included the following: What is the purpose of the growth factor: is prophylaxis or treatment under consideration? How much of the product must be used? When can it be administered? Where can it be administered and by whom? How can we enhance the availability of the drug, quantity, geographically and timeline of availability? These questions may serve to direct drug development efforts towards improved efficiency. For example, if the drug will not ultimately be effective given the constraints outlined above, then available resources should be applied to another solution.

RITN perspective and Growth Factors Working Group update. The RITN[13] is a group of hospitals with cancer treatment specialty, preparing to care for ARS patients from a mass casualty radiological incident (*36*). The scenario for which the RITN prepares is the detonation of an IND. Many communities have conducted preparedness activities for this scenario; however, the RITN approaches it from a different perspective. This is, in effect, a tale of two cities: the disaster-affected community versus the distant-receiving community. Most communities have focused on the affected community for obvious reasons. When a nuclear detonation is described, people envision a mushroom cloud and how it will devastate their city, state or region. For this reason, much effort has been placed on the local response. However, the RITN is focused on the distant community that will receive the medical surge of patients requiring care (C. Case). All cities are likely to be affected by the medical surge as patients are moved across the country through the NDMS. Not unlike the effect of the evacuees from Hurricane Katrina, refugees from a region devastated by a radiation incident will likely spread widely across the nation.[14]

Formed in 2006 with only 13 hospitals, the RITN now consists of a combined 84 cancer centers, blood donor centers and cord blood banks. The National Marrow Donor Program (NMDP) operates the RITN with leadership from American Society of Blood and Marrow Transplantation and funding through a grant from the Office of Naval Research. Each year, participating hospitals are required to complete tasks ranging from emergency communications tests and creating standard operation procedures, to training and performance of table-top exercises. The RITN has created medical orders for adult and pediatric patients and referral guidelines for non-specialized hospitals. Furthermore, the RITN can track availability of beds and critical cytokines at participating hospitals. These data are fed into State Emergency Operations Center of the Assistant Secretary for Preparedness and Response (ASPR), HHS, via the online ASPR GeoHealth mapping platform. For more information about the RITN, overview videos and downloads can be accessed through the RITN YouTube Channel[15] and the REMM app, which provides important information to first responders, treating physicians and the general public concerning what to do during a radiation public health emergency.[16]

Based on the HHS Scarce Resources Project, the framework for the decision-making and triage process for an individual with ARS will depend on the following: their medical condition and prognosis; the degree of resource imbalance at the place and time that decisions are made; the ability of healthcare providers to anticipate supply and demand changes; and victim re-evaluation as the resources situation changes (A. Jakubowski). It will also be important for providers to have an understanding of survivability of various categories of injury. Current planning for patient care after a marrow toxic injury, mass casualty incident is based on a 10-kT IND detonation in the U.S. It is estimated that over the first 24 h, fallout will be responsible for 180,000 fatalities, 202,000 non-fatal casualties (side note: there are on average only 40,000 hospital beds in U.S.), and >4 Gy exposure (*37*). There will also be limited survival within ~ 1 mile of the detonation from overpressure (blast), thermal damage and prompt radiation. Not only does the planning scenario give estimates on casualties, it also breaks those casualties down further into trauma or combined injuries versus radiation-only injuries. While 90% of casualties would be anticipated to have trauma or radiation combined injuries and to receive treatment at the nearest facility equipped to handle those types of patients, 10% will likely have radiation-only ARS injuries and could be sent to

[13] www.ritn.net.
[14] https://nyti.ms/2FHY2ik.
[15] https://www.youtube.com/channel/UCkd45X1DlPqeRru5lph6Og.
[16] https://www.remm.nlm.gov/downloadmremm.htm.

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RITN centers for definitive medical care (*1*). For this reason, combined injuries represent a high-priority research area. The actual numbers estimated in the IND planning scenario are 63,000 radiation-only patients. Of those, 1% (or 630) would require a bone marrow transplant, 29% (or 18,270) would need intensive inpatient supportive care, and the final 70% (or 44,100) would be best treated as outpatients (daily monitoring of blood counts).

When triaging patients from this type of incident, one of the most important factors is the estimate of radiation exposure. Elements used to determine a patient's radiation exposure comprise location (where was the patient relative to the blast), duration (how long was the patient in the location) and shielding (any barriers between the patient and the blast, such as a wall or being in a basement). Other points the triage/treating clinician would consider are the patient's symptoms (e.g., onset of vomiting) and blood count. Together, this information can give a treating clinician an indication of the patient's risk of ARS and the best treatment plan (e.g., myeloid growth factor, antimicrobials, hospitalization or palliative care). Traumatic or burn injuries as well as combined injuries would be best served under surgical intervention and/or medical or palliative care.

The Strategic National Stockpile (SNS), which transitioned within HHS from oversight by the Centers for Disease Control to the ASPR on October 1, 2018,[17] plays a vital role in the national planning strategy for an IND scenario. The SNS stores resources to counter biological, radiological and chemical threats, as well as many diseases of concern during a public health emergency. Antibiotics, myeloid growth factors, chemical antidotes, anti-toxins, life-support medications, intravenous (IV) administration materials, airway maintenance and medical/surgical supplies are part of the stockpile that could be specifically utilized for response to an IND crisis. Although most of the details on supplies, including information on the availability of growth factors, are not made public, it is known that Neupogen® was the first growth factor to be included (with the largest inventory) followed by smaller amounts of Leukine. In 2016, the Biomedical Advanced Research and Development Authority (BARDA) purchased Neulasta and Leukine in two $37 million agreements (*38*). Although these products are in the SNS, there are issues concerning the time for deployment from the SNS to various destinations and a manufacturer's capabilities to rapidly scale-up production to increase supplies. For these and other reasons, growth factors will most likely be a very limited resource in an actual incident.

Estimates on cases of radiation injury only, as well as trauma and combined injury were discussed. Most victims transported to RITN centers would be expected to fit into the ''radiation injury only'' group with minimal to no traumatic or burn injuries. Triage for victims within the ''radiation injury only'' group are most likely to be affected by resource availability. The triaging process separates victims into those who should receive immediate or delayed care, those who require minimal interventions and those who should receive expectant (i.e., palliative only) management. Under crisis standards, those who received >6 Gy radiation exposure to the whole body or to a significant portion of the body are triaged into delayed or expectant categories, as defined by the HHS Scarce Resources Project.[18]

When discussing whether cytokines should be administered to a patient with additional injuries in combination with radiation exposure, recommendations differ based on patient status (see Tables discussed here[19]). For example, classification of care includes radiation-only exposure or minimal trauma versus combined injury (moderate or severe injury with radiation >2 Gy), with recommendation given as ''indicated'', ''indicated only if supply widely available'', or ''not indicated''. Unfortunately, in a resource-constrained mass casualty environment, most patients falling into the combined injury categories would be ''not indicated''. However, those in the radiation-only or minimal trauma would be grouped in the ''indicated'' or ''indicated only if supply widely available'' categories. Under conditions where myeloid cytokine resources are ''fair'' or ''poor'', crisis standards will be necessary. There may be patients with trauma or special populations (e.g., pediatric, geriatric, or those with co-morbid conditions) who received between 1–2 Gy radiation and would benefit from myeloid cytokines.

To better understand the use of these approaches, the RITN formed the Cytokine Working Group with a committee consisting of representatives from RITN, multiple U.S. government agencies, experts in hematology and transplant for adult and pediatric patients, and representatives from agency partnerships dealing with burns and trauma. The group set the following goals:

1. Create more specific guidelines for radiation mass casualties, under current day conditions, that formally address management of combined injuries in particular, with respect to growth factor support.
2. Formalize collaborations between the RITN and burn and trauma organizations.
3. Develop a toolkit using existing materials that support the cytokine triage guidelines.

This working group is also addressing the questions that present with any mass casualty incident, such as ethical issues surrounding who should receive cytokines, and who will make those decisions, as well as what the supplies on hand will be and if fewer resources can be utilized.

[17] June 6, 2018, Hearing on Pandemic and All Hazards Preparedness Act, House Energy and Commerce Committee, Health Subcommittee.

[18] http://bit.ly/2YYEKOK.

[19] Radiation Injury Treatment Network. Acute radiation syndrome treatment guidelines. 2010. (http://www.RITN.net)

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The RNCP, NIAID growth factors and cytokines portfolio. To date, NIAID's RNCP has provided significant funding to support research and development of many growth factor and cytokine approaches to address radiation injuries (A. DiCarlo). Of greatest importance are RNCP-supported studies that led to the licensure of Neupogen and Neulasta in 2015. Several years later, licensure of GM-CSF was also achieved, based on work funded by BARDA. It is significant that these products are efficacious when given at least 24 h (or 48 h for GM-CSF) after radiation exposure in validated animal models of radiation injury. Many of the products that received funding from the NIAID are highlighted in this meeting report. This research has been conducted under several NIAID grants, contracts and cooperative agreements, including the University of Maryland School of Medicine and SRI International, past and current contractors for NIAID's advanced product development efforts, respectively. Approaches that have been studied by the NIAID include interventions with interleukins, TPO and EPO, as well as specific growth factors that target damage pathways for different cellular lineages affected by radiation. Table 2 provides a full list of growth factor and cytokine-related products that have been studied. Table 2 also lists products that, although not true growth factors and cytokines, are hormones or small molecules that mimic the activity of these biologics in the body. These approaches include drugs such as TPO receptor agonists. Generally speaking, a product is considered efficacious if it leads to improved survival of at least 30% over vehicle-treated controls. It is important to note that not all of the cytokines and growth factors listed show strong efficacy when administered 24 h or later after irradiation. From a civilian standpoint, these findings are a concern. However, to the military, many of these approaches still represent viable treatments. The military community has different requirements, which allow for drugs that can be given prior to or shortly after radiation exposure (discussed in more detail below).

Global World Health Organization (WHO) consensus statement on use of cytokine therapies for ARS. In 2009, the WHO established a consultancy group, with the goal of providing an evidence-based recommendation for clinical management of hematopoietic ARS (*39*). The process used by the consultancy was the Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) System, developed by Holger Schünemann (*40*). This process is considered to be rigorous, and it has been adopted by the WHO for all recommendations. Organized by Colonel Viktor Meineke (previously at the Bundeswehr Institute of Radiobiology), Zhanat Carr (WHO), and Nicholas Dainiak (Yale University), among the consultancy group were 37 participants from 13 countries on four continents, who were asked to review the various ways to manage hematopoietic ARS in a high-volume scenario involving the hospitalization of 100–200 victims. The review included English language articles on eight reported radiological incidents with ARS. This review was supplemented by a review of published data on cytokine use in humans without ARS, well designed and powered animal studies and prior consensus group publications, as well as by a narrative review of non-hematopoietic treatment strategies that were not discussed during the presentation (N. Dainiak).

In following the GRADE system, it was determined that to be included in the final analysis, reports had to: 1. demonstrate bone marrow failure; 2. mention what cytokines were actually used; and 3. state that there was or was not an effect from the treatment on the hematopoietic system. Ultimately, studies meeting these inclusion criteria were reported from: Goiana, Brazil (*41*); Tokai-mura, Japan (*42*); Henan Province, China (*43*); Istanbul, Turkey (*44*); and Gilan, Iran (*45*). Based on the quality of the data, these reports were determined to be moderately strong and critically important, with 20 patients identified in observational studies. Of the 20 patients, 18 were treated with growth factors, and 14 of those 18 treated patients survived beyond one year. Among patients found to have received >5 Gy of radiation, 1 of 3 patients survived, whereas among the 15 patients that received <5 Gy, 14 survived. Taken together, these data justified a strong recommendation to administer G-CSF or GM-CSF when the measured ANC is less than 0.50×10^9 cells/L. A weaker recommendation was made for the administration of erythropoiesis-stimulating agents when prolonged anemia is present, with the intent of avoiding the need for red blood cell infusions. Furthermore, administration of hematopoietic stem cells after failure of 2–3 weeks of cytokine treatment to induce recovery from marrow aplasia in the absence of non-hematopoietic organ failure was recommended but the findings did not strongly support their use.

Acute radiation syndrome analysis and recommendations of the consultancy were presented to a combined meeting of the FDA's Medical Imaging and Drug Advisory Committee (MIDAC) and Oncology Drug Advisory Committee (ODAC) that convened in May 2013. These data and recommendations were evaluated by the FDA advisory committees, together with an assessment of RNCP-funded findings of efficacy in a nonhuman primate (NHP) model (*46*), a statistical analysis on the effectiveness of G-CSF,[20] work presented by pharmaceutical companies and U.S. Government input, as the committees considered the ''safety and efficacy of currently approved leukocyte growth factors as potential treatments for radiation-induced myelosuppression associated with a radiological/nuclear incident''.[21] By a 17–1 vote, the combined FDA committees agreed that these factors were ''reasonably likely to produce clinical benefits in humans exposed to myelosuppressive doses of radiation''.

[20] https://stanford.io/2IluVoo/.
[21] http://bit.ly/2G2q8p6.

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In addition to those studies that were used to inform the recommendation of the WHO consultancy, other studies were published after the consultancy meeting took place. These latter studies included reports of two cases meeting WHO inclusion criteria from radiological accidents that occurred in Fleurus, Belgium[20] and Taiyuan, Shanxi Province, China (*47*). The results of cytokine management in these cases would not have changed the initial recommendations of the consultancy (*48*). Identification and review of 26 additional cases, found through the literature review, resulted in the hypothesis that the dose range of 5–6 Gy was critical to prolonged survival (*26*), and that research should be pursued on the efficacy of agents to treat individuals who are exposed to doses in the 5–6 Gy range, particularly in exposed persons who would be otherwise expected to have a high probability of survival, if provided an appropriate intervention. After the WHO's 15th REMPAN meeting in the summer of 2017, a second WHO consultancy on medical management of ARS was proposed; this should include a consensus on infectious disease care, the creation of an electronic platform for the management of ARS and introduction of expert opinion on emerging cytokines and other complementary therapies (*49, 50*).

Optimizing use of G-CSF in military operations: Scenario-based modeling. The approval of growth factors such as G-CSF and GM-CSF for radiation indications has expanded their potential military use; however, the treatment window is narrow with optimal efficacy demonstrated when treatment is administered within 24–48 h after exposure. There is a consensus recommendation for administration within 24 h of exposure (*39*), leading to significant timing challenges to providing treatment within that time frame. Initiating treatment within the recommended time frame would not be feasible, operationally or logistically, for large numbers of patients in a nuclear detonation, as many patients who would benefit from cytokine administration will not have entered the healthcare system within 24 h (*51*). However, the military requirements and concept of operations for an MCM differ from those for civilian use, which could allow for greater utilization of existing growth factor and cytokine approaches (W. Skinner).

The U.S. military represents a highly-structured and well-resourced organization with a robust ability to train and implement complex medical treatment paradigms in austere environments. Despite these unparalleled strengths, scarcity of resources and challenges in medical treatment facility capacity remain limitations in a response to a nuclear incident. These resource and capacity constraints, combined with diagnostic uncertainty, will impact the timely treatment for patients who would benefit from cytokines even in the most favorable scenarios. In a mass casualty incident with significant numbers of patients that overwhelm the ability to identify, diagnose and treat, delays in both diagnosis and treatment from the point of injury are expected. It would be logistically difficult, if not impossible, to provide point-of-care therapy with cytokines to all potential medical treatment facilities, and it would be numerically impossible to evacuate all potential patients through levels of increasing care within a narrow time window to provide cytokines within 24 h. To examine options for these operational realities and constraints, scenario-based modeling was used to provide more insight on options for optimizing the realistic use of G-CSF in military-based operations.

To better understand how modeling could inform the medical decision-making process, several scenarios were created, utilizing a combined pharmacokinetic (PK) and pharmacodynamic (PD) biomathematical model for the dynamics of granulopoiesis after radiation exposure (*52*). The model evaluated initiation of G-CSF treatment at days 1, 3 and 5 after a total-body free-in-air (FIA) radiation exposure of 2, 4.1 or 6 Gy. The primary end point evaluated was days of neutrophil counts under a critical level of 1,500/mm^3. Duration of treatment was modeled with 3 and 7 days of daily G-CSF given after initiation of treatment. The results of the model showed that a 2 Gy FIA exposure results in no time under critical threshold for neutropenia, and G-CSF administration would not benefit this population with regard to neutropenia duration. With 6 Gy FIA exposure, there was little difference between initiation of G-CSF on day 1, 3 or 5. Additionally, there was little difference with duration of treatment with 3 or 7 days of daily administration with regard to duration of neutropenia under the critical threshold. The duration with no G-CSF was 14 days. G-CSF given at day 1, 3 and 5 showed consistent findings of little difference in neutropenia with a nadir duration of 13 days with three daily doses and 12 days with seven daily doses regardless of the day of initiation. The most significant advantage in G-CSF administration was in the 4.1 Gy FIA dose, where administration of seven daily doses of G-CSF reduced the duration under critical level to 3 days regardless of initiation on day 1, 3 or 5. The duration without G-CSF was 8 days, and 3 days of G-CSF administration reduced the nadir duration to 7 days when given on day 1, and to 6 days when initiated on day 3 or 5. In the scenario modeled, the administration of seven daily doses of G-CSF within 5 days to the cohort that received 4.1 Gy FIA yielded the greatest benefit of decreasing the days of neutropenia with no significant decrement in a delay outside of the recommended cytokine guidance on timing of administration.

The use of scenario-based modeling demonstrated several important planning points, most notably providing more flexibility in delaying initiation of treatment. In addition, the potential to use IND modeling to geospatially map areas of exposure to FIA doses linked to patients may improve the ability to provide data predicting treatment outcome to cytokines. Patient populations in these areas would represent the worst-case exposures since real-world exposures would likely involve some measure of physical shielding resulting in partial body, non-uniform exposures

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(*53*). The ability to optimally deliver resources to areas where patients would have the greatest benefit in a resource-constrained environment has the potential to increase the efficacy of triage and treatment protocols, and to enable surge of these medications to more strategic medical treatment facilities based on providing the most good to the most patients. Data on duration of treatment and time of initiation are also important in providing emergency planners and first responders with information on how to best respond and assisting in making difficult scarce-resources treatment decisions. This information can also assist researchers and emergency planners in considering the use of cytokines and other treatments in an evolving clinical treatment scenario that involves a time delay of first responder, recovery of patients, entry into a medical treatment facility and consideration of the challenges of the initial treatments given by the first receivers in medical treatment facilities. The ability to provide best supportive care in a mass casualty that involves a significant number of trauma and thermal injuries further complicates the ability to provide optimal care. The military scenario reflects the ability to use expert medical decision makers within a well-established command and control structure in a chaotic environment, to receive and provide real-time inputs that can alter treatment recommendations. Current treatment recommendations are based on narrow, controlled administration parameters that limit the ability to optimize treatment based on operational limitations for a given scenario. The use of scenario-based modeling can assist in expanding research goals, identifying opportunities to improve outcomes, and to help determine optimal treatment protocols and concepts of operations under the constraints and limitations that will be confronted in a real-world scenario.

Session 2: Pre-Licensure Research

In Session 2 presentations, updates were provided on several MCMs that are under study to treat the sub-syndromes associated with acute radiation exposure. These include products approved for radiation and other indications, as well as factors still in early stages of research. In addition, given the availability of growth factors approved for radiation injury, there were discussions on the need to include an ARS-approved growth factor as a treatment arm, alone and in combination with any product under study, to determine how the products might interact with a treatment that could be considered standard of care.

Epidermal growth factor as a radiation medical countermeasure. Many diverse cell types, including those located in the duodenum and some tumors, secrete epidermal growth factor (EGF), a member of the EGF family of proteins. After binding to the EGF receptor (its canonical ligand), the transmembrane tyrosine receptor dimerizes and is auto-phosphorylated, leading to activation of several pathways (*54*). These pathways can exert many changes inside the cell, including survival, proliferation, oncogenesis, angiogenesis, anti-apoptosis (also apoptosis), cell cycle progression, gene expression and malignant transformation. Prior studies on genetically-modified BAK/BAX mice possessing a radioprotective phenotype (100% survival), showed secretion of EGF into the supernatant of the bone marrow (~10-fold change in levels) after irradiation (7.5 Gy) along with other compounds such as IL-17, insulin-like growth factor-binding protein-2 and amphiregulin (*55*). Other molecules such as IL-5, KC (CXCL1), IL-17 and GM-CSF were found to be downregulated. Prior to these observations, EGF was not recognized to be a growth factor for hematopoietic stem cells (HSCs) (J. Chute). Bone marrow-derived HSCs express the EGF receptor in response to radiation, and EGF, in turn, has been shown to promote HSC regeneration *in vivo*. In a repopulation study, wild-type C57BL/6 mice were irradiated at the 7.0 Gy $LD_{50/30}$ (lethal dose expected to yield 50% survival at 30 days), and subsequently administered EGF (or normal saline vehicle) SC daily for 7 days beginning 24 h postirradiation. Their bone marrow was then harvested and injected into an animal receiving 9.5 Gy irradiation. At the time of harvesting, greater bone marrow cellularity was observed in the EGF-treated femurs than the vehicle-treated controls (J. Chute). Animals that received bone marrow from EGF-treated donors also had significant increases in Kit (+), Sca-1 (+) and Lin (–) (KSL) cells, colony-forming cells, and colony-forming unit-spleen and day-12 cells. Stem progenitor numbers were also found to be elevated at day 10 postirradiation.

In a separate experiment, EGF increased survival when administered 24 h after 7 Gy irradiation (given daily for 7 days). The $LD_{50/30}$ seen in the saline-treated group was reduced to an $LD_{10/30}$ in the EGF-treated study group (*55*). Addition of G-CSF (2 μg/day) to EGF (10 ug/day) treatment led to a further increase in survival (20% in controls; 67% in EGF; and 86% in EGF with G-CSF). In addition, given prior to irradiation, erlotinib, an EGF receptor antagonist, reduced survival, demonstrating that EGF receptor signaling is sufficient and necessary to mitigate radiation damage. EGF has also been shown to repress activity of the pro-apoptotic protein PUMA in HSCs after irradiation (suppressing apoptosis), and also improved DNA repair in these cells *in vivo* (as assessed by comet assay) in HSCs *in vitro*. EGF receptor signaling is needed for the regeneration of HSCs after irradiation and for DNA repair (J. Chute). In other work, an *in vitro* study indicated that Dkk1 (a Wnt inhibitor made by osteoblasts and osteoprogenitors) regulates secretion of other niche factors. Bone marrow endothelial cells were cultured in the presence of Dkk1, which results in a 5,000-fold increase in EGF levels. After 5 Gy irradiation, EGF levels were higher in Dkk1-treated animals (especially at day 10 and 15 postirradiation), with treated animals also showing increases in white blood cells, neutrophils and KSL cells in the femur (*56*). In summary, EGF released by bone marrow endothelial cells that line

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blood vessels binds to EGF receptors on HSCs. Dkk1 released by osteoprogenitor cells in the bone matrix also interacts with the HSCs and acts on endothelial cells to make EGF. This cross talk in the bone marrow niche is critical to the mechanism of action of EGF as a radiation mitigator.

Fibroblast growth factor (FGF) for radiation-induced gastrointestinal injury. Clinically, fibroblast growth factors have shown many beneficial effects, including healing of thermal burns and ischemic wounds in humans, and promotion of gastrointestinal (GI) tract healing of inflammatory bowel disease. FGF2 and FGF7 (also known as keratinocyte growth factor, or KGF) have been used safely in over 1,500 patients for treatment indications, e.g., burns (*57*), ischemia (*58*), diabetes (*59*) and bone marrow transplants (*60*). Endogenous FGF is found in many tissues in the body, and its levels can vary dramatically. For example, in bone marrow transplant patients, FGF levels can go down to almost zero after irradiation and remain low for several weeks (*61*). FGF and related compounds have been studied for over 20 years (*62*); however, cost restricts the use of human recombinant FGF2 (hrFGF2). FGF-P, a 17 amino acid peptide fragment of FGF2, was developed as an alternative (*63*). The FGF-P molecule has a simple synthesis process, is many times less expensive than native FGF and binds well in the activation site of the FGF receptor-1 (FGFR1).

In some ways, FGF-P is superior to other forms of FGF, since its steric features allow it to work in strains of mice that do not show binding to either hrFGF1 or hrFGF2 (*64*). In addition, FGF-derived growth factors, but not most other angiogenic factors [e.g., vascular epidermal growth factor (VEGF)], have also been shown to protect the GI crypts against radiation injury in the duodenum (*65, 66*). Published studies with hrFGF2 showed benefit of treatment in hematopoietic ARS, when the factor was given 24 h after total-body irradiation (TBI) (*64*). FGF-P administered SC has also been tested in a partial (5%)-shielded, GI-ARS mouse model, where, at most drug doses tested, it led to improved survival in male NIH Swiss mice irradiated up to 20.5 Gy. The best regimen was found to be injections for 3 days beginning at 24 h postirradiation (P. Okunieff). This may be because the nadir of the crypt count in an irradiated mouse small intestine is 3.5 days, whereas the human nadir is expected to be approximately 7 days (transit time from crypt to shedding at the tip of the villus). In the gut, FGF-P preserved small intestinal stem cells and mature microvilli [leucine-rich repeat-containing G-protein coupled receptor 5 (LGR5) progenitor cells are increased and brush border enzymes are retained) and maintained mitochondrial biogenesis and cytochrome function.

In addition, platelet counts were found to be significantly higher in FGF-P treated animals, and the platelets were better aggregators compared to vehicle-treated animals (*67*). FGF-P also improved stool formation and reduced bleeding (leading to a lower hemoccult score) in the GI tract (10.5 Gy sub-TBI) assessed at day 4 postirradiation. Finally, a reduction in bacterial translocation was noted in treated animals, assessed by plasma endotoxin units in C57BL/6 and BALB/c mice (P. Okunieff). FGF-P effects have also been studied in a mouse model of TBI with a cutaneous β burn (surface burn cause by β particles). In those experiments, topical administration of FGF-P prevented ulceration, as assessed at day 16 postirradiation, compared to saline-treated controls (*68*). Future studies include learning more about the pharmacology of the compound, looking at efficacy in older male and female animals, determination of the maximum tolerated dose (MTD) of FGF-P and identification of surrogate markers and receptor binding. There is also research underway to explore potential uses of FGF-P for other indications (e.g., ischemic wound healing, nerve regeneration, cosmetics, etc.), and meetings with the FDA concerning Animal Rule licensure of the product are planned.

Evaluating romiplostim (Nplate®) as a medical countermeasure for hematopoietic ARS. After licensure of Neupogen and Neulasta, Amgen turned their attention to other drugs in their portfolio that might show efficacy in models of radiation injury. Presented at the meeting was an update on recent work evaluating romiplostim (Nplate) for the treatment of radiation-induced thrombocytopenia (J. Park). Nplate, a fusion protein containing a peptide region designed to bind to the TPO receptor (c-Mpl) and an Fc carrier domain to increase the half-life of the circulating protein, has been approved for more than 10 years for the treatment of thrombocytopenia in patients with chronic immune thrombocytopenia (ITP). This second-generation TPO receptor agonist acts through four peptide-containing domains that do not contain homology to endogenous TPO, yet bind the TPO receptor to stimulate megakaryocyte-mediated production of platelets. It is generally believed that thrombocytopenia is an important contributor to the morbidity and mortality of hematopoietic ARS, leading to risk of bleeding (*69*). Prior published studies have also suggested that the depth and duration of thrombocytopenia can serve as a predictor of mortality in animal models, thus implying that a strategy to treat this condition may increase survival of patients exposed to myelosuppressive doses of radiation (*70*). Studies in mice demonstrated that when Nplate was administered for 1, 3 or 5 days post-lethal TBI, 100% of the irradiated mice survived (*71*). Furthermore, Nplate demonstrated hematological and survival benefits when combined with other growth factors such as G-CSF and EPO (*72*).

Recent studies conducted through NIAID/RNCP's advanced product development contract at SRI International showed that a single dose of Nplate led to a 40% improvement in survival of irradiated mice at an $LD_{70/30}$ TBI model (*73*). Treatment with Nplate reduced the platelet nadir, hastened the recovery of platelets in the animals and increased platelet volume, suggesting that Nplate acts to produce new platelets in the bone marrow as well as to push

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them out in the circulation. When compared to Neulasta, an existing known intervention for ARS, survival curves for a single dose of Nplate overlapped with those from a single dose of Neulasta or the combination. These promising mouse data led investigators to proceed with a different model system and pursue PK and PD studies in NHPs with the goal of understanding the PK/PD in the NHP model and determining the dose for eventual pivotal efficacy studies (*74*). Two different doses and treatment regimens were studied, alone or in combination with Neulasta. With a radiation dose of $LD_{30/45}$, all treatment options improved the platelet nadir. Interestingly, Nplate treatment, when co-administered with Neulasta, resulted in a further improvement of neutrophils in addition to the platelet response, suggesting a synergistic effect in NHPs when Nplate is provided concomitant with Neulasta (Fig. 1). Efficacy studies are in progress under the RNCP contract with SRI International to pursue Animal Rule approval for the thrombocytopenia component of hematopoietic ARS.

HemaMax™ (rHuIL-12) for pancytopenia: Clinical phase 3 and pre-EUA. HemaMax, recombinant human interleukin-12 (rHuIL-12), has been shown to be a mitigator of acute radiation injury in mice and NHPs. Currently, it is under development to provide treatment for hematopoietic ARS injury when administered immediately after radiation exposure, and in the absence of supportive care. Clinical phase III studies are planned in addition to an ongoing submission for pre-EUA consideration to allow for U.S. government stockpiling (C. Lawrence). The pleiotropic effects of IL-12, originally known as natural killer (NK) cell stimulatory factor, on innate and adaptive immune cells have been extensively studied. More recently however, its ability to stimulate hematopoiesis has been identified, thereby increasing lymphoid and myeloid progenitor cell generation (*75*). Both lymphoid and myeloid lineages give rise to dendritic cells (*76*), which are key sensors of the environment, responding to the antigenic determinants from pathogens such as viruses, bacteria and cancer cells with the production of IL-12. IL-12 immediately deploys the innate immune system, particularly through NK cell mobilization and activation, mobilizes the adaptive immune system to generate antigen specific pro-inflammatory responses, including generation of immune memory, and induces the bone marrow to generate new immune cells to replenish those already mobilized. Elimination of the threats to the body is thus controlled and directed by IL-12.

HemaMax has shown efficacy when administered at 24 h post-TBI in NHPs and 48 h postirradiation in mice (*77*). HemaMax has also shown apparent significant survival over G-CSF after 7 Gy TBI ($LD_{90/60}$), when a single SC dose of 175 ng/kg HemaMax was compared to 18 daily doses of 10 μg/kg G-CSF administered at 24 h postirradiation. This was a good laboratory practice (GLP), randomized, vehicle-controlled and blinded study in a lethally-irradiated NHP model with no supportive care (no fluids, antibiotics and blood products). Survival with vehicle control was 36% (n = 36 NHP), and with G-CSF was 31% (n = 26), whereas HemaMax yielded significant survival of 56% ($P \leq 0.05$, *t* tailed chi-square test, n = 36) and HemaMax with G-CSF yielded 58% (n = 26) survival (*78*). In addition, a significant reduction in severe systemic infections was noted with HemaMax ($P \leq 0.05$) and combination therapy-treated NHP compared to controls and G-CSF-treated NHP. Hemorrhage and gastrointestinal ulcer scores were lowest in HemaMax-treated NHP, and highest in G-CSF-treated NHP compared to vehicle control-treated NHP.

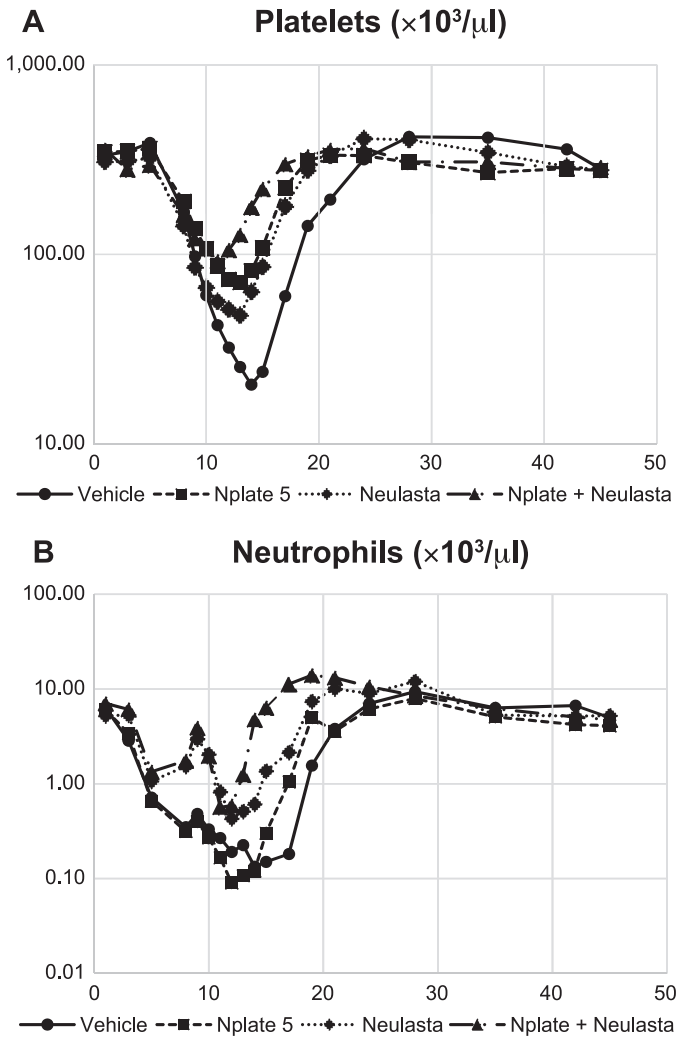


FIG. 1. Romiplostim (Nplate) reduces platelet nadir and hastens recovery of platelets and neutrophils in irradiated NHPs. Panels A and B: Platelet and neutrophil counts, respectively, in NHP administered Nplate (romiplostim), either alone or in combination with Neulasta (pegfilgrastim) are shown. Nplate (5 mg/kg) was given on day 1, and Neulasta was given on days 1 and 8 after TBI at an $LD_{30/45}$ dose (5.50 Gy). Animals were first dosed 24 h after TBI. Modified figure presented [Published and modified, with permission, from: Wong K, Chang PY, Fielden M, Downey A-M, Bunin D, Bakke J, *et al*. Pharmacodynamics of romiplostim alone and in combination with pegfilgrastim on acute radiation-induced thrombocytopenia and nuetropenia in nonhuman primates. Int J Radiat Biol 2019. (In press)

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HemaMax has a highly complex glycosylation structure, with occupied O and N glycosylation sites that result in a half-life of ~41 h (ranging from 7 to 137 h) after a SC administration of 12 μg and detectable circulating interferon (IFN)-gamma levels that range from 12 to 312 h (IFN-gamma is a cytokine effector molecule produced in response to IL-12 administration). In the treatment of cancer patients these glycosylation properties and the need to avoid causing tachyphylaxis necessitate a dose and dosing schedule of 12 μg every 2–4 weeks depending on the standard of care.

To date, first-in-human, phase Ib and phase II safety trials in healthy subjects have been completed. A fixed single dose of 12 μg HemaMax has been evaluated in an integrated analysis of safety that has been presented to the U.S. FDA with a highly acceptable safety profile. There was no difference in adverse events and no immunogenicity noted when the population was broadened to be representative of the U.S. population in regard to gender, age, body mass index, weight or ethnicity (C. Lawrence). In addition, a characteristic HemaMax signature was routinely observed in all subjects immediately after administration with rapid mobilization of major peripheral blood cell types out of the vasculature and the upregulation of hallmark cytokines and chemokines, including IFN-gamma and IP-10 (CXCL10), respectively.

In summary, a single dose of 12 μg HemaMax given SC elicits hematological and immune-related effects with little toxicity. This dosing has been safe and well tolerated in 243 healthy human volunteers and patients treated to date. Compared to Neupogen, Neulasta and Leukine, the single SC administration of HemaMax in the absence of supportive care, with the mobilization of innate and adaptive immune responses and the restoration of all cell types in regenerating bone marrow, suggests an advantage for its immediate deployment and use during a mass casualty incident. HemaMax is sufficient to get victims to supportive care, which could include the named growth factors, since the combination with Neupogen was safe and well tolerated in the NHP study.

BBT-059 analog for hematopoietic ARS. A novel, pegylated interleukin-11 (IL-11, BBT-059) represents another potential treatment approach for hematopoietic ARS. Although recombinant IL-11 (Neumega®) is approved to treat thrombocytopenia in cancer patients, the molecule has a very short half-life and must be injected every day, making its use inconvenient (*79*). To circumvent this problem, BBT-059, a mono-PEGylated IL-11 analog, was created by covalently attaching polyethylene glycol (PEG) to the C-terminus end of the IL-11, resulting in an increased molecular weight, slower clearance from the body and increased potency *in vivo* (G. Cox). In studies performed in rats, compared to Neumega, which peaks in circulation at 1–2 h after injection and is no longer detected by 10 h, BBT-059 is absorbed slower, peaking at 24 h and lasting 2–3 days in the circulation. This extended half-life makes BBT-059 longer-acting and stimulates a greater increase in platelets than IL-11 in nonirradiated rats and mice. Studies performed in mice demonstrated that a single SC injection of BBT-059, administered 24 h post-TBI, increased the 30-day survival of mice by 50–60% (*80, 81*). Further experiments, in which different dosing regimens were evaluated, demonstrated that a single injection is as effective as three injections every other day. Furthermore, survival correlated with a multi-lineage hematopoietic reconstitution leading to an accelerated recovery of platelets, red blood cells and neutrophils (more than IL-11, Neumega). Importantly, the 30-day survival efficacy of PEG IL-11 is comparable to that of PEG-G-CSF and PEG-GM-CSF during moderate TBI exposures ($LD_{50/30}$–$LD_{70/30}$), while PEG IL-11 appears to increase survival more than PEG-G-CSF and PEG-GM-CSF at high TBI doses ($LD_{93/30}$). When verifying the effect of BBT-059 in combination with G-CSF, now considered the standard of care for radiation injuries, treating mice with both PEG-IL-11 and PEG-G-CSF did not adversely affect survival but instead improved survival (*80*).

Session 3: Clinical Use and Practice of Medicine

In Session 3, discussions turned to a consideration of the practice of medicine, and what approved growth factor or cytokine approaches (e.g., in the fields of bone marrow transplant and/or oncology) might represent promising MCMs for radiation injury. Also, in the event of a civilian radiation incident, patients will include pediatric and geriatric victims, as well as individuals with other comorbidities. Given their special requirements, treating these populations with growth factors and cytokines involves advanced planning. To address these concerns, physicians with relevant expertise were asked to comment.

Growth factors/cytokines in transplantation: Considerations for use. Given that the focus of the meeting was primarily on hematopoietic growth factors, an overview of past research on this class of growth factors was provided in the context of treatment-related neutropenia. Transplants performed in the 1980s, before implementation of growth factor use, involved long recovery times from ablative conditioning, even if patients were transplanted with stem cells (N. Chao). After chemotherapy with or without radiotherapy, the incidence and duration of days with infection increases dramatically when the ANC decreases to 100 cells/μl (*82*). Therefore, an ANC of 500 cells/μl (which is the inflection point for increased incidence of infections) is commonly used as the threshold for initiation of antibiotics and supportive care. It has been noted that patients with even 100–200 neutrophils/μl do better than those with zero counts. Infections can occur anywhere in the body, most commonly at portals of entry for bacteria such as the mouth, throat, skin, gut and indwelling catheters. The dose response for G-CSF is quite linear and its MTD was not reached in clinical testing, even at 60 μg/kg.

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GM-CSF, another prominent clinical cytokine, is not used as frequently as G-CSF. This monomeric glycoprotein is secreted by macrophages, T cells, mast cells, natural killer cells, endothelial cells and fibroblasts. GM-CSF stimulates stem cells to produce granulocytes (neutrophils, eosinophils and basophils) and monocytes, and thus, has a broader effect than G-CSF, which stimulates primarily neutrophils. The monocytes produced exit the circulation and migrate into tissues where they can potentially have an effect on the immune/inflammatory cascade and aid in infection control. Data on clinical use of GM-CSF shows that although it has an effect on monocytes and macrophages, in the clinical settings studies, it is marginal. The drug is sometimes used in patients with fungal infections; however, the supportive data are relatively weak (*83*). The original recombinant product was not glycosylated and was produced in *E. coli*; however, that product had more side effects compared to the currently licensed product, which is produced in yeast cells.

Using thymidine incorporation studies in mice, it was shown that G-CSF initially causes a demargination of a compartment of neutrophils not in the circulation that rapidly leak out, and which have a low percentage of thymidine-labeled cells (*84*). There is also immediate synthesis of large numbers of new neutrophils: the number of day-14 colony forming units cultured from blood after IV administration of G-CSF at 1–60 μg/kg/day are markedly increased, and the increase was observed with 3 μg/kg given by IV or SC routes. Since the half-life of the IV administered drug is much shorter than when it is given SC, and since SC administration is easier, this led to the use of the drug as a SC injection. Original efficacy was demonstrated by the increased number of neutropenic patients free of fever who received G-CSF versus placebo (*84*). Similarly, the time to neutrophil recovery after chemotherapy was significantly faster than for the control group (*84*). In the transplant population, there was a three-day improvement in median days to reach an ANC >500 when G-CSF was given after stem cell infusion. This treatment resulted in a lower number of in-hospital days but did not affect platelet count recovery or days on IV antibiotics. For GM-CSF use after transplant, the benefit in time to neutrophil recovery to >500 and $>1{,}000$ cells/μl was primarily observed for patients who received GM-CSF after infusion of peripheral blood, with or without bone marrow stem cells and not with bone marrow alone. The benefit was most dramatic for those patients who had received chemotherapy and irradiation versus chemotherapy only (*85*). In conclusion, in the clinic these growth factors do have an effect on shortening neutrophil recovery. Furthermore, there may be an improvement in hematopoietic recovery to a level of <500 neutrophils, which may confer sufficient protection from infection and fevers. In general, these growth factors are very well tolerated even at high doses, which makes them easy to use.

Cytokine considerations for children and other special populations. The many differences between children (19 years and younger) and adults in hematopoiesis can contribute to the toxicity and clinical management of radiation exposure. One of the major differences in pediatric hematopoiesis is where the cells are produced. In the fetus, most hematopoiesis is handled by the yolk sac/liver and this is transitioned to primarily marrow-based production as the child develops (*86*). While adults ($\sim$70 kg) have 1.5 to 2.5 times the total marrow space of a 15 kg child, the active red marrow dedicated to blood cell production is dramatically higher in the child. Infants are capable of substantial extramedullary hematopoiesis and children have much greater stem cell division and productive capacity, with fewer cells in G_0 than adults (*87*). This finding, in part, explains why cord blood transplants can engraft successfully, despite containing fewer total stem cells than peripheral stem cell or marrow collections. Despite the lower number, they have a greater number of stem cells with a higher proliferative capacity.

There are also many differences in pediatric versus adult pharmacokinetics, including the finding that the volume of distribution of hydrophilic (higher) and lipophilic (lower) drugs can be altered due to the increased water-to-fat ratio in children. There is also decreased protein binding with higher free fraction of drugs, and decreased hepatic metabolism and renal excretion in children, which results in decreased hepatic and renal clearance (*88*). The glomerular filtration rate in children increases until it reaches adult level at $\sim$10 years of age. In terms of growth factors for pediatric use, dosing of G-CSF, based on PK studies and efficacy, was determined to be 5 μg/kg (*89, 90*). The GM-CSF PK was similarly studied for both IV and SC administration in children (*91*). A review of the clinical indications for the use of G-CSF in the pediatric population highlights its use in marrow failure states such as severe congenital neutropenia and aplastic anemia (*92, 93*), to permit dose intensification of chemotherapy in patients with sarcomas and Hodgkin's lymphoma (*94, 95*), for mobilization of young stem cell donors (*96*) and to facilitate engraftment and count recovery in the post-stem cell transplant setting (*97*). In addition, no increase in cancer risk from G-CSF injections has been noted in healthy marrow donors (*98*).

In the management of pediatric patients with ARS, it is important to consider that children have a smaller mass with greater force/body surface area, and larger surface area-to-volume ratio. In addition, their skin is thinner and less keratinized, resulting in higher bioavailability of toxins exposed to the dermis (*99*). Other physiological differences in children that would need to be considered in a disaster setting, since they could affect radiation injuries and growth factor dosing and efficacy, include faster baseline respiration and heart rate (resulting in higher minute ventilation and greater exposure to inhaled contaminants) and lower

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blood volumes resulting in earlier dehydration and consequences of blood loss (*100*).

Conversely, children are also better at compensating, such that they are more likely to appear well until just before collapse. Attention needs to be given to these physiologic differences when dosing medication and fluids during resuscitation. Other considerations for children are late effects of radiation exposure, which can be significant and quite different from those in adults, and could include: 1. growth delays; 2. endocrine disorders such as thyroid dysfunction and infertility; 3. developmental delays; and 4. secondary cancers (with a longer lifetime risk) such as breast, thyroid cancer and leukemia.[22] Adding to these complexities are the potential difficulties in determining symptoms in preverbal children, who might be separated from parents/caregivers, which could delay growth factor administration. The recommended use of cytokines in children for uncomplicated exposures of $\geq$2 Gy would be similar to that for adults (filgrastim 5–10 μg/kg/day, pegfilgrastim 0.1 mg/kg, sargramostim 250 μg/m^2/day), with the potential addition of TPO receptor agonists as deemed appropriate by the treating physician (A. Seif). In addition, prophylactic and therapeutic antimicrobials, nutritional and transfusion support, and stem cell transplant in the appropriate setting should be included in the supportive care.

DISCUSSION

After the presentation sessions, participants were divided into several breakout rooms, where a range of subject matter expertise was represented (e.g., researchers, physicians and U.S. government). Each group was asked to discuss the topics shown in Table 3. Subjects to be discussed were broadly defined as follows: pre-clinical model considerations; the science of cytokines; optimal clinical use; operational considerations; regulatory considerations; and concepts on the horizon. Below is the combined summary of the discussions from each breakout group and the consensus session.

Pre-clinical Model Considerations

In animal model development, the importance of choosing the correct model for a cytokine's proposed mechanism of action cannot be overstated. For example, an antibiotic approach may not show efficacy in an animal model where infections are not the main drivers of mortality. A researcher might incorrectly assume a drug is not working, when in fact, the model is not appropriate. Even for different strains of mice or for one particular strain in different facilities, the microbiomes, which could influence drug effect and radiation survival curves, can be different, without even considering the wide range of radiation exposure devices in use. Gender of the animal model selected, as well as whether a facility is ''clean'' or ''dirty'' also matter. For these reasons, before embarking on a large-scale experiment, a pilot study using the selected species and strain is advised. Since it is necessary to test efficacy for a radiation MCM in more than one animal model, alternatives to rodents and NHPs may be considered. In addition to mouse and NHP, both Neupogen and Neulasta have been shown to confer a survival benefit in the Göttingen minipig that is similar to that seen for other species. (*101*).

Also important is a full understanding of the radiation exposure. Because it is highly unlikely that any human exposure resulting from a radiation incident will yield a homogenous dose, and because humans, unlike laboratory animals, have high genetic variability, consideration of outbred strains of animals and partial-body radiation exposures should be considered. Since many growth factor and cytokine approaches require some surviving cells, with which the growth factors interact to yield an effect, these products may work better if some percentage of the bone marrow is spared. Finally, the level of support that will be provided to the irradiated animal can influence whether efficacy can be observed. For example, one study showed that in a medically unsupported, TBI NHP model, G-CSF did not impact survival, unlike HemaMax (*78*). Earlier studies on the product showing efficacy had been done in a fully-supported NHP model (*46*). Administration or lack of support could also impact the efficacy of a product; in an NHP study, the dose modification factor (DMF) of supportive care alone was estimated to be 1.3.[23] Discussants felt that to the extent possible, animal models should include medical management (e.g. fluids, antibiotics, etc.), while acknowledging that providing this care is difficult in rodents. In addition, many survivors of pure radiation may have been exposed to fallout, which has a lower dose rate (under ~1 Gy/h) (*102*). Because different repair pathways might result from low-dose rates, compared to high-dose rates, it may be advisable to determine if growth factors are efficacious in this kind of model. Typical laboratory dose rates are 0.50–1.5 Gy/min. These rates, although generally available with existing instrumentation, are not ideal, in that they do not adequately model either fallout exposures or prompt radiation dose rates.

Science of Cytokines

Since many published studies of growth factors have been performed in TBI models, it is possible that lower doses of a growth factor, or later administration might also be effective if part of the bone marrow was shielded (*103*). The timing of the administration of a growth factor was an issue of

[22] http://bit.ly/2Gc8q3L.

[23] Farese AM, *et al*. Medical management alone increases survival of lethally irradiate nonhuman primates within the hematopoietic syndrome. 54th Annual Meeting of the Radiation Research Society, Boston, MA; 2008.

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much conversation. If the product is given too early, it may be wasted, although the package labeling for Neupogen and Neulasta advise to ''administer... as soon as possible after suspected or confirmed exposure to radiation doses greater than 2 Gy''.[24] It was generally agreed that timing of growth factor administration is important, and certain time windows prior to radiation exposure can be ineffective, beneficial or deleterious due to cell synchrony or change in cell maturational stage. For example, prior administration of growth factors at time points nearer that of irradiation can be protective (*104*). Timing of administration is critical. In a bone marrow transplant setting, growth factors are sometimes not given until 5 days postirradiation (N. Chao), and no clinically relevant differences in outcome have been noted if the drug was started on day 1 or day 5 postirradiation (*105*). Even for use of these products after chemotherapy, it is advised to begin them several days after dosing. The understood mechanism of action for filgrastim or peg-filgrastim clearance is through binding to neutrophils, which are then cleared, taking the drug out of the circulation (*106*). Because there is a documented transient increase in neutrophils after irradiation (*107*), if the drug is administered too early it will bind to the demarginated neutrophils. In the case of Neulasta, this could modify the half-life of the drug from 7 days down to hours via partial consumption of the drug. It was also noted that GM-CSF might represent a preferable treatment option if early growth factor administration is not possible, as NHP data suggest that it is efficacious at 48 h or later postirradiation (*108*). Shelter-in-place guidance might also delay administration of treatment (*109*).

Preclinically, three doses of G-CSF have been shown to yield a survival benefit that is indistinguishable from 14 or more doses in the mouse model (*110*). Therefore, it was suggested that providing fewer doses of the factor could enable treatments to be spread across more casualties. In addition, the comment was made that a 5 μg/kg dose of G-CSF could be used, instead of the 10 μg/kg recommendation made on the labeling, as the lower dose was shown to be effective in preclinical models. Because the biological response of G-CSF is linear to dose (e.g., 10 μg is better than 5 μg), 5 μg still works clinically. In some clinical cases, up to 20 μg/kg/day is used (*111*); however, there are many chemotherapy studies showing efficacy at 5 μg /kg (*112*). It may be the case in receptor binding that once the receptors are saturated, a higher dose will not be effective, but a longer duration of dosing could be important, so Neulasta might achieve that. Similarly, repeat dosing of Nplate did not give greater benefit than was found for a single dose in mice (*73*) and NHPs (*74*). It may be possible that use of more than one growth factor (e.g., addition of a mobilization factor) with filgrastim might reduce the amount of G-CSF needed to provide benefit (*113*); however, this outcome may depend on the dose of radiation received.

[24] https://emergency.cdc.gov/radiation/physicianneupogenfacts.asp.

Optimal Clinical Use

Given the decades of clinical use that pre-dated the approval of G-CSF for ARS, physicians and patients understand that it is non-toxic and has few side effects. In addition, there is very little data to suggest that short-term use has any long-term negative effect. Because its PK profile is known, and it has also been used extensively in all populations (e.g., pediatrics and geriatric patients 65 years or older), it has become the gold standard. Although novel agents under development may offer some advantages over approved drugs for ARS (e.g., they may have improved efficacy or ease of administration), there are still risks involved in their further development, which makes their potential use less certain. It may be the case that these newer agents have superior efficacy to existing products, for example, in a mixed field radiation exposure. Filgrastim is known to work well for photon exposures and fallout scenarios and has been shown to provide a survival benefit after mixed field irradiations (neutron and gamma) (*114*). Mouse studies demonstrated that administration of pegylated G-CSF worked only weakly (yielding a 15% improvement in survival) in a radiation combined injury model (radiation with wounding) (*115*), although patients with multiple injuries in addition to a radiation exposure might be considered expectant, and thus, might not receive growth factor treatments in a scarce-resources scenario. Novel approaches might also have additional multi-lineage hematopoietic and/or non-hematopoietic impact. For example, data presented at the meeting for drugs such as EGF, FGF and HemaMax indicate that they may also confer GI, renal and/or wound healing benefits. Nonetheless, given the earlier stage of development of these kinds of products, more studies are needed on their safety and toxicity.

As for the paradigm of use for drugs like the approved leukocyte growth factors, it is apparent that the level of care provided for the animal model can influence the findings. It is now generally believed that an appropriate MCM efficacy study for any radiation sub-syndrome should include an approved growth factor for hematopoietic ARS as a stand-alone treatment arm, and also in combination with the MCM under study. Operationally, combination drug approaches lead to logistical challenges, and their implementation will likely be different depending on the scale of the incident, and also whether they are implemented in a battlefield, triage center or hospital environment. In addition, the radiation dose received by any individual could inform health care providers on the organ system anticipated to have the most critical damage (e.g., higher doses might lead to GI complications) and could influence the timing of interventions. The participants generally felt

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that U.S. FDA-approved agents would be prioritized for use, and if the situation warranted (e.g., scarce availability), other drugs with lesser-known safety profiles could be utilized.

There were some comments made concerning some growth factors, which might not be indicated for use in certain patients. For example, G-CSF use is contraindicated in patients with sickle cell disease (*116–118*), although the benefit could outweigh the risk in a myelosuppressed individual. There were also some concerns about the availability of products that might be contraindicated, specifically in elderly populations. For example, increasing white blood cell counts in older individuals could lead to cardiovascular complications (*119*); however, given the transient nature of the increases from growth factor treatment and the expected survival advantage to be gained, their use might be justified; also, they have been used safely in elderly patients after chemotherapy for years. Although there is still some controversy in the published literature, there is limited preclinical evidence in rodents that use of G-CSF during the acute phase of radiation injury can lead to worsened lung complications during the delayed phase,[25] while no effect of G-CSF on late kidney radiation injuries was noted in NHPs (*120*). In general, participants felt that given the benefit from the use of growth factors to treat radiation-induced bone marrow suppression, late effects that might result from their early use should not necessarily be a focus, given all the available data for their use in the clinic. Other cytokines of interest that target other organ systems may also have use restrictions. For example, KGF as an MCM for GI-ARS showed either no beneficial effect in irradiated rodents, or increased damage and decreased survival in irradiated NHPs compared to untreated control animals when given after irradiation.[26] However, when given before irradiation, KGF was protective in a C57BL/6 model of radiation injury (*121*). Therefore, this drug might be indicated only for preirradiation use, and guidelines should be developed to explain drugs that should and should not be used off-label. Emergency use instructions (EUI)[27] might be an appropriate and helpful way to inform physicians of what products to use and which ones should be avoided during a radiation incident.

The constraint that most drugs should not be developed as MCMs unless their target product profile suggests efficacy at time points later than 24 h postirradiation was also discussed. There were concerns that the possibility of extended (24 h or longer) shelter-in-place guidance would render many potential drugs ineffective. In addition, it might be possible that the lack of available refrigeration could impact the efficacy of a cytokine product that requires cold storage.

There may be other growth factors already in clinical use that could be repurposed, including Nplate (romiplostim) (*122*) and Epogen® (EPO) (*123*), which could reduce the need for blood transfusions. The questions in terms of repurposing involve whether or not an EUA could be implemented, how quickly companies could scale-up production in a crisis, and if vendor-managed inventory would be feasible (influenced by the size of the vendor and their market). The willingness of companies to work with the government to provide their drug for MCM testing and use is also in question and could be influenced by factors such as the size of the company and their past government experiences. It is possible that clinical data are available for other indications that would have relevance to the consideration of radiation-induced injuries. For example, there is overlap between the management of acute burn and/or blast injuries and radiation exposure. In addition, most clinical radiotherapy exposures involve gamma- or X rays. Although there are known confounders (e.g., radiation is fractionated, and many radiation protocols are coupled with chemotherapy), it should be possible to extrapolate findings from those types of exposures to expected fallout and/or prompt irradiation.

Operational Considerations

Although, as mentioned above, there appears to be an impact of the level of supportive care provided on growth factor efficacy, there is also the potential that the reverse is true; that is, one might require less supportive care if an MCM is used. Triage guidelines must include considerations as to whether the U.S. government will be able to work with vendors on managed inventories. Participants agreed that there needs to be more cooperation between the U.S. government and industry to address scarce-resource situations and expectations for companies. In incidents in which resources are constrained, it is probable that patients with a high degree of combined radiation injuries will be triaged into expectant categories. However, it was suggested that physicians outside of the emergency response networks (e.g., radiation oncologists or emergency room staff in suburban or rural medical centers, or general practitioners) may not be familiar with those triage guidelines, which could lead to confusion as to who should receive potentially life-saving cytokines. It may also be possible that half-doses of growth factors (e.g., 5 μg/kg instead of the recommended 10 μg/kg MCM use), or shorter treatment courses might allow for more patients to be treated. Pre-clinically, these alternate dosing regimens are being tested, in the hope that those results might translate into updated mass casualty recommendations or EUIs. Also, shifting to the use of drugs with less frequent dosing requirements (e.g., Neulasta) could lead to higher patient compliance and drug availabil-

[25] https://mwne.ws/2Z0JraT.

[26] Shea-Donohue T, Fasano A, Zhao A, Notari L, Stiltz J, DeVito J, McFarland E, Fares A, MacVittie T. MS1107: An acute radiation syndrome (ARS) nonhuman primate (NHP) research platform: prolonged gastrointestinal (GI) dysfunction observed in NHPs surviving the acute heme and GI syndromes. 55th Annual Meeting of the Radiation Research Society, Savannah, GA (2009).

[27] http://bit.ly/2uVJ2st.

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ity. However, use of a long-acting version of a growth factor might not be advised in special populations such as pregnant women. Since issues concerning radiation and growth factor use during pregnancy are unknown (and once administered, a long-acting growth factor cannot be taken back), it may be best to also stockpile shorter-half-life cytokine products as well. A final stockpiling constraint that was considered was the limited availability of storage, and the point that agents with more than one possible use would be an advantage. This could be a drug that works systemically, benefiting many organ systems after irradiation, such as the bone marrow, GI tract and lung, in addition to treatment of myelosuppression resulting from chemical exposure, such as what has been observed for G-CSF and sulfur mustard exposure (*124*).

Regulatory Considerations

Recent efforts to repurpose drugs with existing clinical indications is an important step toward minimizing the cost and time involved in licensing a product for use during a radiation public health emergency. However, even for other novel compounds, it is critical that a primary, clinical indication is being sought, as it is unlikely that the government could wholly support development costs, and a good business model would not rely on only one purchaser (*125*). The expectation that complicated polypharmacy approaches may be needed, and the practical impact of an increasingly complex baseline group of therapies that might be required was also discussed. For MCM development, it is assumed that standard medical management would now include either G- or GM-CSF, necessitating larger studies with additional treatment arms (drug of interest, with and without G- and G-CSF alone). To address this and other issues concerning administration of drugs in the context of other products, the NIAID held a polypharmacy meeting in October 2018 (report pending).

On the Horizon

Although there are now three products licensed to treat hematopoietic ARS, there are still other lineages and drugs supporting other mechanisms of action that are of interest. In addition, it may be possible to improve on existing products (e.g., by increasing their half-life, decreasing the effective dose to maximize availability, optimizing formulation and/or route of administration). Participants indicated that several drugs in use for bone marrow transplant patients, as well as TPO receptor agonists and anti-oxidants approved for other indications, represent promising approaches. In addition, studies of growth factors to address radiation damage to other organ systems were underrepresented compared to those impacting hematopoiesis. It was also noted that approaches should be systemic (e.g., multi-organ), and that there were many growth factors that interact with the vasculature that could represent targets of interest. Specifically, although products such as VEGF are not yet in the clinic, they could represent a class of products that would be of interest.

CONCLUSION

As demonstrated through the presentations and discussions held during this meeting, growth factors and cytokines represent an important tool in the MCM armamentarium to address radiation-induced injuries. Given the availability of clinical data for many of these approaches, which is important for repurposing licensed/approved products, and physician familiarity, their continued study is advised, to make them feasible for SNS stockpiling and to optimize their potential use during a radiation public health emergency. In addition, the cytokines that are closest to Animal Rule consideration are optimized for hematopoietic damage and homogenous exposures. Research and development gaps still exist for inhomogeneous exposures, radiation combined injuries and other organ system damage such as GI-ARS and lung-DEARE. The U.S. government will continue to work within its agencies, with non-governmental groups such as the RITN and with academic and industry researchers, to address these gaps, and advance growth factor and cytokine approaches for future use in case of a radiological or nuclear incident.

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