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MEETING REPORT

Gastrointestinal Acute Radiation Syndrome: Mechanisms, Models, Markers, and Medical Countermeasures

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There have been a number of reported human exposures to high dose radiation, resulting from accidents at nuclear power plants (e.g., Chernobyl), atomic bombings (Hiroshima and Nagasaki), and mishaps in industrial and medical settings. If absorbed radiation doses are high enough, evolution of acute radiation syndromes (ARS) will likely impact both the bone marrow as well as the gastrointestinal (GI) tract. Damage incurred in the latter can lead to nutrient malabsorption, dehydration, electrolyte imbalance, altered microbiome and metabolites, and impaired barrier function, which can lead to septicemia and death. To prepare for a medical response should such an incident arise, the National Institute of Allergy and Infectious Diseases (NIAID) funds basic and translational research to address radiation-induced GI-ARS, which remains a critical and prioritized unmet need. Areas of interest include identification of targets for damage and mitigation, animal model development, and testing of medical countermeasures (MCMs) to address GI complications resulting from radiation exposure. To appropriately model expected human responses, it is helpful to study analogous disease states in the clinic that resemble GI-ARS, to inform on best practices for diagnosis and treatment, and translate them back to inform nonclinical drug efficacy models. For these reasons, the NIAID partnered with two other U.S. government agencies (the Biomedical Advanced Research and Development Authority, and the Food and Drug Administration), to explore models, biomarkers, and diagnostics to improve understanding of the complexities of GI-ARS and investigate promising treatment approaches. A two-day workshop was convened in August 2022 that comprised presentations from academia, industry, healthcare, and government, and highlighted talks from 26 subject matter experts across five scientific sessions. This report provides an overview of information that was presented during the conference, and important discussions surrounding a broad range of topics that are critical for the research, development, licensure, and use of MCMs for GI-ARS. © 2024 by Radiation Research Society

INTRODUCTION

As of the date of this workshop in August 2022,[3] there were four U.S. Food and Drug Administration (FDA)-approved products for hematopoietic (H) acute radiation syndrome (ARS); however, there remains an unmet need for approaches that address gastrointestinal (GI)-ARS and injuries impacting other organ systems. There are also limitations to studying the natural history of GI-ARS, because a clear consensus remains to be reached on how to characterize the nature of the injuries, and what models are most appropriate. In addition, it is important to look to other areas of the human clinical experience in GI diseases, to be better informed on state-of-the-art practices to assess and treat similar injuries. Further, animal model details and radiation exposure protocols vary from site to site in terms of biomarkers, polypharmacy approaches used, bone marrow (BM) shielding, and radiation quality. A solid understanding of regulatory expectations is required to

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[3] FDA licensure of Udenyca® and Stimufend®, the fifth and sixth products approved to treat myelosuppressive acute exposures to ionizing radiation (H-ARS), occurred on November 11, 2022 and September 29, 2023, respectively.

TABLE 1
Workshop Speakers and Areas of Expertise

Name	Affiliation	Area of expertise
Simon Authier, DVM	Charles River Laboratories, Laval	NHP irradiation models, cardiovascular, neurological and immunological assays. GLP toxicology and pharmacology studies
Max Brenner, MD, PhD	Feinstein Institutes for Medical Research	Rheumatology, biochemistry, molecular biology, genetics, immunology
June Brickey, PhD	University of North Carolina, Chapel Hill	Immune regulators, radiation-induced injuries, gene knockout models, neuroinflammation, cancer
Milton Brown, MD, PhD	Trocar Pharma	Translational medicine, radiation oncology, experimental therapeutics, drug discovery
Polly Chang, PhD	SRI Biosciences	Animal models of radiation injuries, product development, toxicology, physiology, radiation biology
Jonathan Cohen, PhD	FDA	Regulatory MCM development, Animal Rule
Nicholas Dainiak, MD	Yale University	Medical management of ARS, cytokines, mitigators
Andrea DiCarlo, PhD	NIAID, NIH	Radiobiology, product development, MCM testing
Mark Donowitz, MD	Johns Hopkins School of Medicine	Gastroenterology, drug therapies for diarrheal disorders, human GI enteroids, intestinal physiology and pathophysiology
Melanie Doyle-Eisele, PhD	Lovelace Biomedical Research Institute	Drug development, pharmacokinetics, animal models of radiation injury, toxicology, GLP studies
John Esker, PhD	BARDA	Radiation MCM development, organic synthesis, material characterization, analytical testing
Joel Greenberger, MD	University of Pittsburgh Medical Center	Radiation mitigation/protection, intestinal and bone marrow stem cells
Chandan Guha, MD, PhD	Albert Einstein College of Medicine	Radiation injury, gastroenterology
Nyun "Calvin" Han, MD	FDA	Regulatory MCM development, Animal Rule
Theodore Hong, MD	Massachusetts General Hospital	GI radiation oncology, normal tissue sparing, proton beam therapy
Carol Iddins, MD	Radiation Emergency Assistance Center/ Training Site (REAC/TS)	Radiation emergency management, radiological security and safety, disaster medicine
Maureen Kane, PhD	University of Maryland	Analytical chemistry, targeted metabolomics, lipidomics, proteomics, mass spectrometry imaging, biomarkers, disease mechanisms
Richard Kolesnick, MD	Memorial Sloan Kettering Cancer Center	Sphingolipid signaling as a stress response, ceramide-induced cell death, radiation oncology, animal models of radiation-induced GI injuries
Vidya Kumar, PhD	Armed Forces Radiobiology Research Institute (AFRRI)	Radiation biology, PBI mouse models, MCM efficacy screens, biochemistry, high throughput screening
Chang-Lung Lee, PhD	Duke University	Acute and long-term side effects of radiation therapy, oncology, H-, GI-ARS, MCM development
Thomas MacVittie, PhD	University of Maryland School of Medicine	Radiation biology, large animal models of H- and GI-ARS, supportive care, MCMs, delayed effects of acute radiation exposure, oncology
Libero Marzella, MD, PhD	FDA	Regulatory MCM development, Animal Rule
Paul Okunieff, MD	University of Florida	Oncology, radiobiology, growth factors, biomarkers
Mario Sampson, PharmD	FDA	Clinical pharmacology, regulatory MCM development, Animal Rule
Gabor Tigyi, MD, PhD	University of Tennessee for Health Sciences	LPA receptor agonists, stem cells, cancer, radiotherapy
Arthur Tinkelenberg, PhD	Ceramedix, Inc.	Ceramide biology, product development
Ravichandra Vemuri	Wake Forest University School of Medicine	Gut microbiome, metabolomics, aging, immune system

apply these models to the development and validation of potential medical countermeasures (MCMs)[4] to address GI-ARS. To this end, the Radiation and Nuclear Countermeasures Program (RNCP), of the National Institute of Allergy and Infectious Diseases (NIAID), part of the National Institutes of Health (NIH) convened a workshop on August 29-30, 2022, with planning and cooperation from the FDA and the Biomedical Advanced Research and Development Authority (BARDA). This meeting brought together 26 leading experts in the field of radiation biology and medicine (Table 1), as well as representatives from U.S. Government funding and regulatory

[4] For the purposes of this report, "drug" and "MCM" refer to either small molecules and/or biologics, but not devices.

agencies to discuss the latest advances in understanding the mechanisms underlying GI-ARS and the development of effective MCMs. The workshop covered a broad range of topics across five scientific sessions, including clinical manifestations and assessment of GI injuries, molecular and cellular mechanisms of radiation-induced GI damage, appropriate animal models for studying GI-ARS, biological responses that could be used to assess radiation exposure level and predict severity of GI-ARS, and emerging products for mitigating the effects of radiation exposure. Where session presentations and data are discussed, the first initial and the last name of the presenter providing the information is shown in parentheses.

This meeting report provides a summary of the presentations, discussions and key insights gained from the workshop. It is not intended to be an exhaustive overview of GI-ARS or all products that have been under consideration to ameliorate its affect. It aims to serve as a resource for researchers and clinicians in radiation biology and medicine, as well as for policymakers and other stakeholders interested in improving medical preparedness and response to radiological emergencies. The report draws on the latest scientific findings and references a range of high-quality sources to provide a comprehensive overview of the current understanding and advancements made to address GI-ARS.

BACKGROUND

GI-ARS is a condition that can arise due to exposure to high doses of ionizing radiation to a large portion of the body, which damages rapidly dividing cells in the GI tract, leading to variety of symptoms that vary in severity according to the dose of radiation received. GI-ARS is a significant concern in scenarios such as nuclear accidents, radiation therapy mishaps, or intentional acts of radiological terrorism. For GI-ARS in humans, there are three defined response phases: prodromal (0–2 days); latent (2–20 days); and manifest illness (21–60 days). The radiation dose required to cause damage to the GI tract can be quite low, but this kind of exposure is typically recoverable. In discussing life-threatening GI-ARS, the dose levels are significantly higher than those that cause H-ARS (Fig. 1A and B). Understanding and developing interventions for GI-ARS involves use of animal models to simulate human responses to radiation exposure. These models help researchers study the pathophysiology of GI-ARS, test potential treatments, and develop guidelines for medical management. Initially, researchers often use rodents to understand the effects of radiation exposure on the GI tract and to test the efficacy of MCMs to improve GI structure, function, and ultimately, overall survival. Animal models also represent a means of uncovering biomarkers that can be used to assess damage and track efficacy of candidate therapeutics. Once the small animal models are worked out, products can then be tested in larger animals, such as minipigs and nonhuman primates (NHPs).

Given the high radiation dose levels necessary to induce GI-ARS, and to develop an animal model that can survive gut damage and show regeneration, it is often necessary to either provide supplementary bone marrow (in the form of a transplant), or to shield part of the marrow to allow for limited hematopoietic stem cell survival. In developing animal models for GI-ARS, one needs to know what type of dose, the exposure geometry of the radiation dose, and the dose rates that should be modeled. During a radiological or nuclear incident, it is anticipated that the radiation dose received by people will be inhomogeneous or non-uniform, since individuals will likely be partially shielded. Therefore, the entire marrow is not likely to be myeloablated, and will retain pockets of active cells (albeit potentially injured). Few people will receive homogeneous total-body irradiation (TBI), and those that do will likely die from other causes (e.g., thermal burns, blunt force trauma, etc.). When animal models were first established for GI-ARS, the goal was to mimic anticipated, real-life exposure conditions, i.e., partial inhomogeneity. This thinking led to the development of the partial-body irradiation (PBI)-BM sparing models and subject-based medical management utilizing a trigger-to-treat model, as would be the anticipated care for patients.

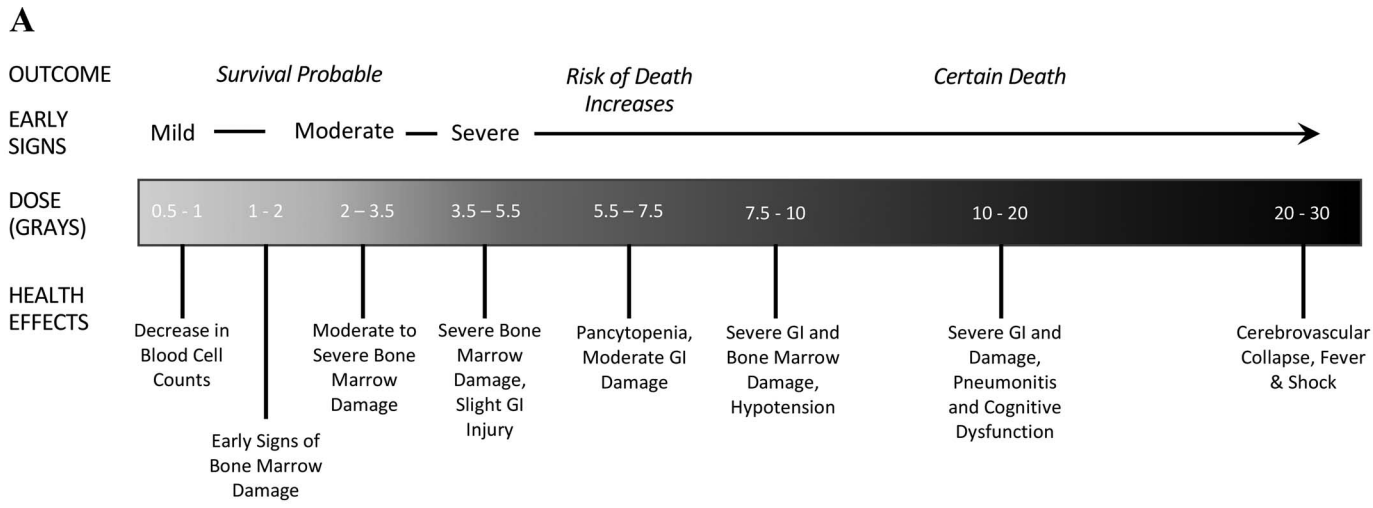
Medical management of GI-ARS involves a multi-pronged approach to mitigate the damage and promote recovery of the gut. Key components of standard medical management include supportive care, for example, to address dehydration and infection; anti-inflammatories to limit tissue damage; and drugs to address symptoms, such as anti-nausea and anti-diarrheal medications. Various products discussed below are under investigation for their potential use as mitigators of GI-ARS. These agents may help improve survival by scavenging free radicals, reducing inflammation, and enhancing tissue repair mechanisms. Although GI-ARS is rare, and therefore limited clinical data are available, it is possible to look at the presentation of GI effects from radiation exposures in the clinic. GI symptoms after radiotherapy of the pelvic region can include pain, rectal bleeding, bloating, changes in bowel habits, diarrhea, and incontinence (*1*). These findings in humans irradiated in the clinic, alongside symptoms and treatments used for other GI disease states, help bridge laboratory animal model findings to anticipated human responses.

Research in the field of GI-ARS continues to evolve, with this meeting report serving only as a snapshot of ongoing efforts to refine animal models, identify new therapeutic targets, and improve the overall understanding of the condition. Research in this area holds the potential to enhance the outcomes for individuals affected by GI-ARS in various emergency and clinical settings.

PRESENTATION OVERVIEWS
SESSION I: CLINICAL MANIFESTATIONS AND ASSESSMENT OF GI INJURIES WITH EMPHASIS ON RADIATION EXPOSURES

Clinical Symptoms and Assessment of Radiation-induced GI Injuries

This presentation began with examples of past patient management after radiological or nuclear incidents, starting with



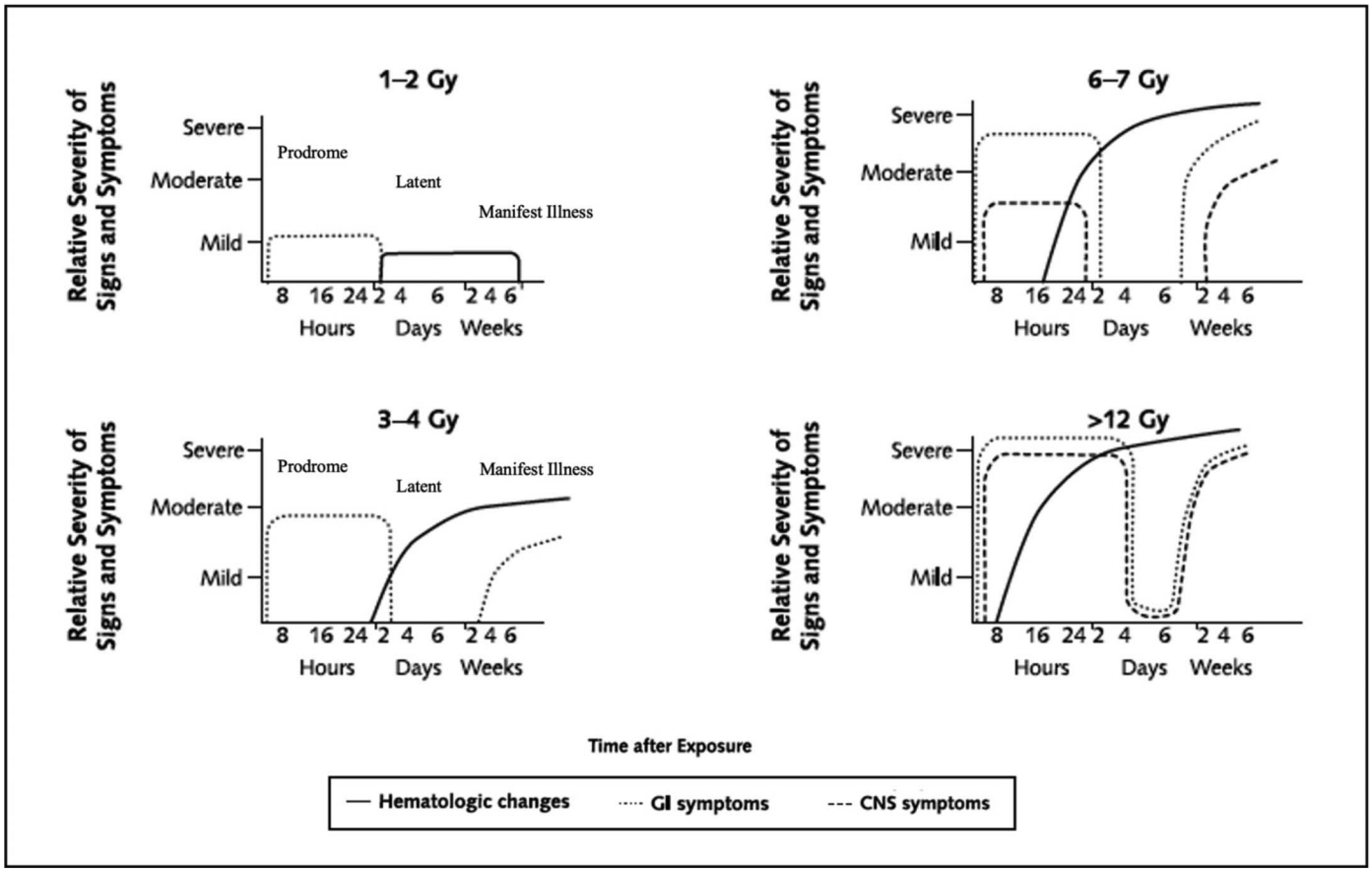


FIG. 1. Panel A: Spectrum of ionizing radiation effects in humans. Effects reflect total absorbed radiation dose in gray (Gy) for a single whole-body exposure without medical intervention. Radiation-induced clinically detectable GI injury may occur at radiation exposures above 3 Gy but are typically recoverable with medical intervention until exposure exceeds ~6 Gy [reproduced with permission from Radiation Research (*102*)]; Panel B: Timing and severity of three ARS sequelae [hematopoietic (H), gastrointestinal (GI), and central nervous system (CNS)] based on radiation dose received [modified from Waselenko et al. (*12*) and used with permission from Ann Intern Med. 2004].

an individual who, after being exposed to total-body irradiation in a 2006 accident in Belgium (estimated $\leq$30 Gy), was treated with a combination of granulocyte-colony stimulating factor (G-CSF), erythropoietin, and stem cell factor (*2*). The primary evaluation of the victim was based on symptoms that occurred within the first 48 h that included vomiting, diarrhea, fever and swelling. It soon became apparent that the dose received by the patient would lead to GI complications

TABLE 2
Gastrointestinal Acute Radiation Syndrome Scoring System[a]

Symptom	Degree 1 (G1)	Degree 2 (G2)	Degree 3 (G3)	Degree 4 (G4)
Diarrhea				
Frequency, stools/dy	2–3	4–6	7–9	≥10
Consistency	Bulky	Loose	Loose	Watery
Bleeding	Occult	Intermittent	Persistent	Persistent with large amount
Abdominal cramps or pain	Minimal/mild	Moderate	Intense	Severe

[a] Adapted and used with permission from British Institute of Radiology. 2001 (*10*).

(Fig. 1 and Table 2). To effectively treat these patients, clinical recommendations include prophylaxis to mitigate the effects of neutropenia (fluoroquinolones, antivirals, antifungals). In instances of febrile neutropenia, a broad-spectrum prophylactic is also indicated, and prophylactic antifungal treatments should also be considered (C. Guha).

Mast cell infiltration in GI-ARS has been linked to chronic fibrosis, is commonly observed in patients who experienced radiation proctitis, and has also been noted in a mouse model after irradiation (20 Gy) (C. Guha). In laboratory animal models used to study regenerative immunology of the intestines, radiation delivered as TBI or abdominal irradiation has been studied for effects on intestinal stem cell death and repair by immune cells. In these studies, molecular pathways involving amphiregulin-epidermal growth factor (EGF), R-spondin-Wnt signaling (*3*), and regulatory T-cell responses have been investigated. Administration of R-spondin led to a decrease in mast cell infiltrates (C. Guha). Administration of mesenchymal stromal cells (MSCs) to animals exposed to TBI or abdominal irradiation (*4*) improved survival of the animals after high-dose irradiation (*5*). Administration of a Toll-like receptor (TLR) 9 agonist led to similar outcomes (*6*). In other work, in mice exposed to PBI, pegylated thrombopoietin mimetic peptide (PEG-TPOm) showed significant improvement in survival when dosing pre- or postirradiation (C. Guha). The PEG-TPOm product mitigates GI-ARS through improvements in intestinal barrier function, villus length, reduced bacterial translocation, and gut permeability. It is hoped that these research efforts will continue to advance candidate approaches toward FDA licensure.

Historical and Clinical Aspects of GI-ARS

To further illustrate possible scenarios and evaluate lessons learned from human radiation exposures during radiological or nuclear incidents (e.g., nuclear criticalities, nuclear power plant releases, and industrial accidents), a brief overview of notable criticality accidents involving GI complications since 1945 were presented (C. Iddins). The Radiation Emergency Assistance Center and Training Site's (REAC/TS) wealth of available data enabled these case studies (*7*). A 1946 criticality accident that involved the "demon core" plutonium sphere at Los Alamos, NM, resulted in an estimated TBI dose of 21 Gy (*8*). The 35-year-old male victim experienced vomiting within the first hour and severe hand edema soon thereafter. As his leukocyte counts dropped, penicillin and blood products were administered; however, he developed a high fever on day 6 along with GI complications and death occurred nine days postirradiation (*7*). GI autopsy findings included loss of esophageal epithelium and sloughing of the mucosa, which was most pronounced in the jejunum and ileum. Gastric and intestinal epithelial damage was observed throughout, including ulcers, swelling and congestion, hemorrhage, bacterial translocation, and shrinkage of the villi. These GI characteristics were only a part of the observed systemic pathologies, which also included the skin, lungs, heart, kidneys, BM, and the immune system. In a 2008 criticality accident in China, workers were exposed to high doses of gamma-ray radiation. Within minutes, the individual closest to the source began vomiting and experienced a high fever; his estimated dose was >14 Gy (*9*). G-CSF and antimicrobials were administered and the patient underwent GI tract decontamination; however, he still showed severe GI-associated symptoms such as bloody diarrhea, emesis, and abdominal pain. Surgery was attempted to address the GI damage; however, the patient died at 62 days postirradiation. Postmortem GI findings included degeneration, necrosis, and loss of mucosal epithelium. Lessons learned from these case studies include suggestions for GI-ARS critical care, most of which are standards of care for any clinical trauma. Primary therapies consist of management of fluid and electrolyte levels, bleeding control (e.g., blood product transfusions and use of approved H-ARS drugs), and infection management. It is also imperative to manage GI-specific complications, such as protection of the mucosal lining (e.g., stress ulcer prophylaxis), ensure proper nutrition, and consider endoscopic exams and surgical procedures as needed.

Development and Application of the METREPOL Classification System for GI-ARS

In order to characterize the severity of injuries in patients that are assessed clinically, a classification system called METREPOL (MEdical TREatment ProtocOLs) for radiation accident victims was developed and heralded as a symptoms-based approach to manage medical treatments (*10*). Subject matter experts were involved in the creation of the scoring rubrics for GI (G), hematologic (H), neurovascular (N), and cutaneous (C) injuries to assess damage postirradiation and predict late outcomes (N. Dainiak), while acknowledging the multi-organ nature of radiation injuries (*2*, *11*). The GI tract is in a constant state of cell turnover, which correlates with radiation sensitivity. Additionally, stem cells in the small intestine

TABLE 3
Hematopoietic Acute Radiation Syndrome Scoring System*

Blood counts/symptoms	Degree 1 (H1)	Degree 2 (H2)	Degree 3 (H3)	Degree 4 (H4)
ALC[a]	More than or equal to 1.5×10^9/L	1–1.5×10^9/L	0.5–1×10^9/L	Less than 0.5×10^9/L
ANC[b]	More than or equal to 2.0×10^9/L	1–2.0×10^9/L	0.5–1×10^9/L	Less than 0.5×10^9/L
Platelet counts	More than or equal to 100×10^9/L	50–100×10^9/L	20–50×10^9/L	Less than 20×10^9/L
Hb[c]	Normal Hb	Less than 10% decrease in Hb	10–20% decrease in Hb	More than 20% decrease in Hb
Infection	Localized and no requirement of antibiotics	Localized and requirement of only local antibiotics	Systemic and requirement of oral antibiotics only	Sepsis may set in and requirement of IV[d] antibiotics

* Adapted and used with permission from British Institute of Radiology. 2001 (*10*).
[a] Absolute lymphocyte count.
[b] Absolute neutrophil count.
[c] Hemoglobin.
[d] Intravenous.

(with the highest turnover rate) are more radiosensitive than those in the colon or stomach (*10*). Since clinical signs evolve over time (Fig. 1B), it is important to continue monitoring METREPOL patient scoring, to assess progress and establish a likely prognosis. There are four degrees of severity evaluation for GI-ARS in the METREPOL system. These are based on stool number (per day), stool consistency (e.g., diarrhea), GI bleeding, and cramps/pain (*10*, *12*). Generally, the higher the number, the more severe and immediate the manifest illness is, and the more grave the prognosis (Table 2).

GI-ARS pathophysiology is known to involve the interplay of pathogens, abnormal permeability of the epithelium and blood vessels, generation of fibrotic tissues, and immune system dysfunction (*13*). Because of the multi-organ nature of radiation injuries mentioned earlier, it is important to also consider the hematopoietic (H) METREPOL scoring system in GI-ARS cases, as radiation damage to the BM is dose-dependent (Fig. 1 and Table 3). Polypharmacy is essential to treat both H- and GI-ARS damage, so combinations of drugs are anticipated, with overlaps between recommended therapeutics to address bone marrow and GI injuries (*14*). The World Health Organization (WHO) has issued recommendations for clinical management of GI-ARS (*15*). The metrics assign fluoroquinolones a weak WHO recommendation for GI-ARS, whereas parenteral antibiotics, ondansetron, and anti-diarrheal drugs are preferred. For patients at low risk, oral and possibly intravenous antibiotics would be appropriate; however, for high-risk victims, hospitalization and administration of parenteral antibiotics should be considered, with the potential to add vancomycin or linezolid, aminoglycoside, or metronidazole for certain GI symptoms. By classifying severity of injury to the GI tract, it is possible to predict clinical manifestations and actively minimize GI damage using antibiotics (based on Infectious Diseases Society of America guidelines), cytokines, anti-emetics and anti-diarrheal drugs, fluids, and electrolyte replacement.[5]

[5] https://rb.gy/uhrydy

Clinical Manifestations of Radiation-induced GI Toxicity

Radiation oncologists employ various approaches to minimize the harmful effects of radiation on the bowel. These include reducing the radiation dose through external shielding, decreasing planning target volume, placing spacers to increase distance between organs at risk, and using radioprotectants (T. Hong). Effective radiation therapy management of tumors located in the abdominal region requires the ability to administer high doses of radiation, especially when surgical intervention is not possible. To achieve this goal, various techniques such as stereotactic body radiation therapy (SBRT), high dose/hypo-fractionated irradiation, and intraoperative irradiation are employed, with the objective of reducing radiation exposure to adjacent tissues and organs. Because there are many innovative methods being advanced in the clinic to improve tumor targeting and minimize normal tissue injuries during irradiation, it is crucial to continue to track clinical developments to determine if they might be applicable to addressing GI-ARS.

Learning from GI Conditions that Mimic Radiation Damage to the GI Tract

The extent and lethality of GI injury during radiotherapy in the clinic depends on radiation type, field size, fractionation, and radiation dose (*16*, *17*). The first morphologic change in the intestine after irradiation is mitotic reduction of cells in the crypts. This reduced cell proliferation results in decreased outflow of progenitor cells from the crypts to replace senescent epithelial cells shed from the mucosal surface into the gut lumen (*18*). Epithelial cells in the human small intestine and colon have turnover rates of 1.5 days and 4.2 days, respectively, and are the most rapidly proliferating cells in the body (*19*). During maturation, cells proliferate rapidly, dividing every 12 to 18 h (*20*). It has been estimated that an entire crypt-villus structure could be repaired in six to eight days postirradiation by even a single stem cell that survives exposure to radiation (*21*). As was observed in several case histories discussed above, if the patient survives the acute phase, GI-ARS can progress to a chronic phase, with

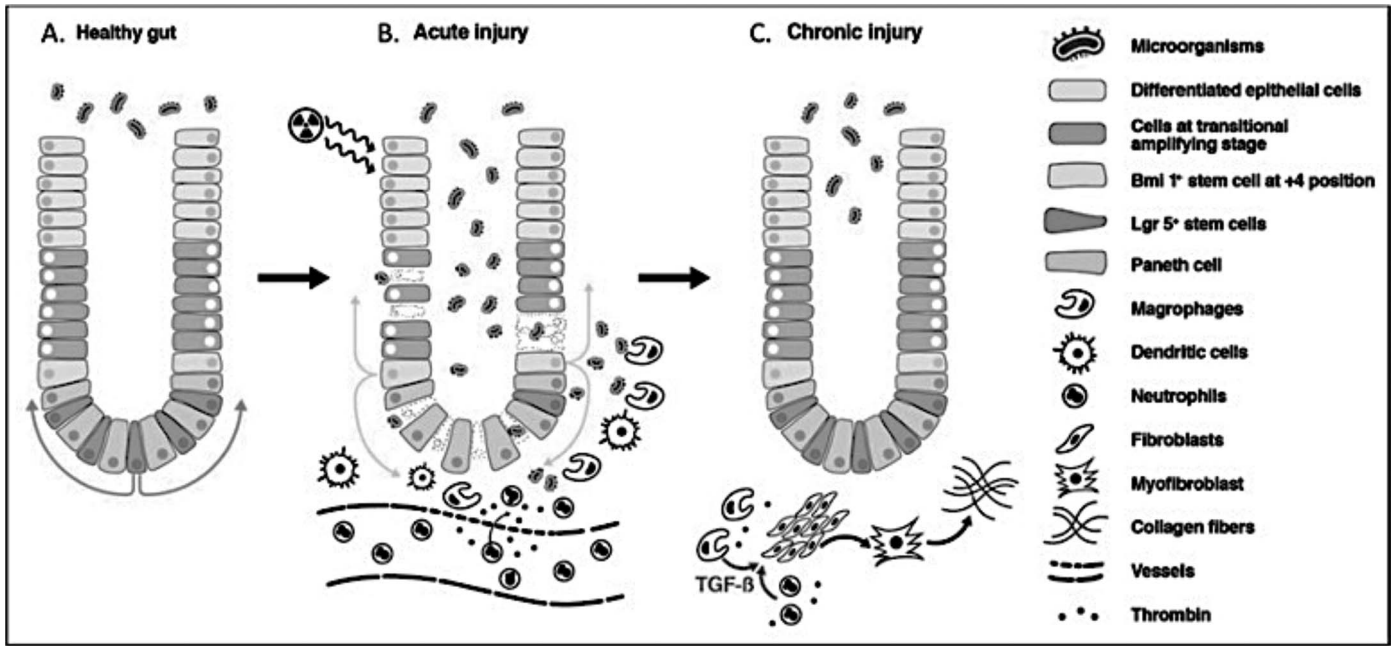


FIG. 2. Schematic representation of radiation-induced intestinal injury (*103*). Used with permission from Nutrients (MDPI).

development of ulcers, fistulas, and adhesions. In cases of recoverable exposure, the mucosa generally undergoes regeneration, and symptoms usually subside. However, this process can take up to six months to complete (*12*) and intermittent bleeding can also occur (*22*). In patients undergoing radiation therapy, the time course of radiation enteropathy is generally considered early (acute) when it occurs within three months of irradiation or delayed (chronic) when it occurs later than three months after irradiation (*23*). Chronic radiation enteritis can occur even up to decades after a patient's life-saving radiation treatment. Clinical consequences of radiation exposure on the GI system are dose-dependent, and other factors can increase the risk of enteropathy (*24*). GI irradiation damage results from a complex interplay of epithelial injury and alterations in the enteric immune, nervous, and vascular systems.

Evidence suggests that stem cells in the GI crypts are composed of cells of different activity and possibly functional states (Fig. 2) (*25*). Lgr5+ stem cells are mitotically active, divide constantly, and migrate toward the mucosal surface, maturing into all the epithelial cells of the GI tract (enterocytes, goblet cells, enteroendocrine cells, Tuft cells and Paneth cells). These cells are highly sensitive to radiation and prone to undergoing apoptosis (*26*), whereas Bmi1+ stem cells are quiescent and more radioresistant (*26*). They, along with many other cell types, are thought to repopulate the intestinal epithelium in the event of tissue damage resulting from infection, inflammation, or radiation injury (*27*). If the entire stem cell population is lost within crypts over a sufficiently large surface area, a prolonged repair process ensues and potentially results in intestinal failure. Acute GI radiation manifestations can develop into more chronic symptoms and pathologic changes with persistent cytokine activation in the submucosa and fibrosis of mesenchymal tissue (*28*).

SESSION II: ANIMAL MODELS OF GI RADIATION INJURY

Multi-Organ Injury Radiation Model with a Focus on GI Injury in Mice

A GI-ARS mouse model (C57BL/6) using PBI/BM2.5 (hind leg-out with 2.5% BM sparing) and irradiation by a linear accelerator (LINAC, Elekta Infinity) (V. Kumar) was presented. This model was developed to screen MCMs for their ability to improve survival, with a threshold of success of 30% or more over vehicle controls (*29*). In this model, ~18% of the total radiation dose is measured in the shielded area due to scattering and the penumbra. Up to eight anesthetized mice can be irradiated simultaneously, with in-run dosimetry probes to validate radiation dose, and GI histopathology, BM cellularity, barrier function, and biomarkers assessed. Total cellularity was quantified in exposed versus spared legs, demonstrating less damage in the spared tissue. Histopathology of the jejunum after PBI demonstrated aberrant crypts and villi, as well as reduced crypt number, width, and length. Mucosal injury scoring demonstrated greater damage in irradiated animals (*30*, *31*). Bacterial translocation was significantly increased, while citrulline levels decreased early after irradiation. Female and male mice were studied, and peak mortality for both sexes was similar during days 6–10 postirradiation (*32*). This model appears to accurately mimic GI-ARS responses that are anticipated in humans, and is also capable of demonstrating mitigator efficacy, having already been used successfully to determine efficacy of several MCMs (details in Session V below).

Characterization of GI Radiation Injury in Small Animal Models

SRI International (Menlo Park, CA) has established and characterized GI radiation injury in mouse (C57BL/6) and rat

(Wistar) PBI models. In addition, substantial work has been carried out to address the effect of beam quality in mouse irradiation studies (P. Chang). Beam quality of orthovoltage X-ray irradiators is a crucial variable that can have a large impact on biological responses. Recent publications (*33*, *34*) demonstrating that the photoelectric effect and Compton scattering vary with beam quality prompted SRI to investigate the effects of softened versus hardened beam quality in their PBI mouse studies. A radiation dose-response relationship (DRR) study was conducted in a PBI/BM2.5 mouse model to assess the effect of beam quality on radiation response. Female mice were more sensitive to radiation when assessed for survival at 30 days postirradiation with aluminum filters (soft beam). When mice irradiated with the softened beam were compared to those irradiated with the hardened beam (Thoraeus filter), no differences in the dose range were observed between the combined sexes; however, males were more resistant to radiation with the hardened beam, following the sex differences in sensitivity trend observed in the softened beam data. Therefore, hardening the beam affected sex-dependent radiation-induced mortality but did not appear to affect acute radiation effects observed in GI histology or BM assessment. SRI also developed a Wistar rat PBI model (*35*) and compared it to the PBI WAG/RijCmcr (WAG) rat model, developed previously at the Medical College of Wisconsin (*36*). The SRI rats were treated similarly to those studied earlier, except that antibiotics were not provided (*36*). In the SRI Wistar model, dose-dependent biphasic weight loss was observed, with the drops in body weight correlating to GI-ARS and lung injury, respectively. Females appeared to be more resistant compared to males in 30-day survival studies. Future work will focus on identifying and applying predictive biomarkers for GI injury, such as citrulline.

Characterization of a GI-ARS Model in Sinclair Minipigs

A Sinclair minipig GI-ARS model has been developed at Lovelace Biomedical Research Institute (LBRI). The Sinclair strain was chosen due to the results of a strain comparative H-ARS study (*37*). For irradiation, a PBI LINAC beam was collimated to produce ~50% shielding. Animals were monitored through clinical observations, pathology, metabolomics, and circulating biomarkers. Radiation injury models are influenced by several factors, including animal distribution, housing strategy, hierarchy of animal socialization, anesthesia regimens, blood collection, quarantine periods, and feeding. LBRI's research focused on determining the impact of feeding and treatment on survival and body weight changes after high PBI doses (10, 12 and 14 Gy). Animals exposed to high doses showed a metabolic decline, evidenced by body weight decreases that partially recovered over time (M. Doyle-Eisele). Citrulline was found to decrease after irradiation, and GI histopathology showed a marked decrease in crypts and villi at early time points, with total mucosal area significantly reduced. Additional work with the model is underway to better understand species-specific mechanisms of radiation-induced injury to the GI tract.

Characterizing a NHP GI-ARS Model: Considerations of Species Differences

Two PBI rhesus NHP models were presented where 5% of the bone marrow was spared. One model involved shielding of the tibia, ankles, and feet, and the second model used shielding of the oral cavity to protect the mucosa during gavage administration of oral MCMs (S. Authier). A prominent finding in surviving irradiated animals was severe weight loss after receiving 9.5 Gy and 10 Gy, with a gradual improvement in body weight. Diarrhea was also observed early after irradiation. Appetite and activity levels were decreased; however, the magnitude of these effects was not sex or radiation dose dependent. Overall, female NHPs were more sensitive to radiation. In the PBI/BM5 leg-shielded model, plasma albumin levels decrease over several weeks and do not recover completely to baseline levels, suggesting acute liver damage concurrent to the acute GI injury. For the PBI/BM5 oral shielding model, less severe decreases in hematological factors and buccal ulcerations were noted, when compared to TBI. The spared stem cell populations contribute to recovery, making this a promising model for development of oral MCMs. Knowledge gaps remain, sex differences are under-characterized and demonstrating mechanism of action (MOA) and drug efficacy to support approval remain challenging. There are also differences in GI physiology between rodents, NHPs, and humans that present challenges in translating findings.

Prolonged GI Injury in the Rhesus Macaque: Concomitant Multi-Organ Injury and Medical Management

Data from natural history studies of prolonged GI injury in rhesus macaques was discussed (*38–40*). This NHP model encompasses the progression of multi-organ injury from H- and GI-ARS to delayed effects of acute radiation exposure (DEARE) after high-dose TBI or PBI (*41*, *42*). The NHP PBI model was developed to resemble a real-world radiation exposure, and to evaluate interactions between multiple organ systems where a small fraction of the bone marrow is spared (*43*), but still receives a small exposure (~50 cGy). A PBI/BM-sparing protocol resulted in a considerable right-shift to the respective DRR for GI- and H-ARS (i.e., from 11.33 Gy to 11.97 Gy for GI-ARS) (T. MacVittie). An important pathophysiological finding in NHPs is the prolonged GI syndrome, characterized by incomplete recovery of the GI tract in animals that receive a radiation dose of 10–11 Gy (PBI/BM5), despite only 5–15% mortality (*42*, *44*, *45*). Jejunal injury manifests as loss of villi, mucosa, and submucosa, incomplete regeneration over several months, and persistent disorganization observed up to 6 months out. Citrulline is a useful GI biomarker (*46–48*), as levels decline after irradiation in NHPs, with a slow rise to above pre-irradiation levels (*49*). GI functions affected by irradiation include reduced glucose absorption, and increased mucosal permeability reflecting prolonged GI dysfunction, as observed by diarrhea, edema, and dehydration (*50*).

Characterization of Late-Term Effects of GI Injury and the Gut Microbiome in Radiation Resilience in NHPs

A NIAID-supported, irradiated NHP survivor colony (>200 animals) serves as an important resource to examine the gut microbiome and severity of late-term effects of GI injury in NHPs. Housing both irradiated animals (1.1 Gy to 8.5 Gy TBI, and some PBI) and age-matched, unirradiated controls, late effects of acute radiation are studied through multidisciplinary methods (R. Vemuri). In addition to GI dysfunction, the NHP survivor cohort also manifests other radiation-induced comorbidities (e.g., type 2 diabetes, heart disease, immune dysfunction, chronic inflammation, cancer). In irradiated NHPs, the percentage of days with diarrhea over their lifetime increases with increasing radiation dose, reflecting long-term GI injuries after TBI. Published literature suggests that gut microbiota can affect response to irradiation (*51–53*); therefore, the microbiomes of long-term NHP survivors in the colony were examined to identify differences in microbial signature that correlate with radioprotection and increased survival. NHPs with higher survival rates and lower postirradiation morbidity appear to have a distinct *Lachnospiraceae*-enriched intestinal microbiome (also observed in rodents), which contributes to their recovery from GI injury. Fecal microbiome signatures from NHP survivors and non-survivors suggest that the radiation survivors have more complex, diversified microbiomes, whereas non-survivor fecal material had microbiomes with less complexity. There was an enhancement of beneficial gram-positive bacteria in survivors, compared to an enrichment of opportunistic gram-negative bacteria in non-survivors (R. Vemuri). With regards to the virome, diversity was significant, with a greater abundance of Caudovirales and Enterobacteria phage in non-survivors and enrichment for Lactobacillus phage in survivors. These findings suggest the gut microbiome is an important mediator of radiation injury and recovery, even years after radiation exposure.

SESSION III: MECHANISMS AND BIOMARKERS OF GI-ARS

Identification and Development of Biomarkers for Radiation-induced Injury of the GI System in Animal Models

A biomarker is a biological endpoint that is objectively measured and can serve as an indicator of injury or response to a therapeutic intervention and could include clinical endpoints, histological, functional, and imaging analyses, clinical observations, and survival (*54*). The NHP PBI/BM5 translational model, which permits concurrent analysis of short- and long-term injury to organ systems and survival in a time- and radiation dose-dependent manner (*41*, *42*, *45*, *55*) has been studied for its radiation-induced biomarker responses that appear to be indicative of GI damage. The use of unbiased metabolomics, lipidomics, and proteomics to identify biomarker candidates from plasma samples collected in GI-ARS studies shows concordance across different animal species (M. Kane). In studies in rhesus macaques using different radiation protocols, timed euthanasia allowed for sampling to assess correlations between histopathology findings and tissue and plasma biomarker levels. Similar assessments of the natural history of organ damage and biomarkers were performed in a mouse TBI model, with timed necropsies and tissue/plasma collections. In irradiated NHP studies, jejunal and plasma samples showed overlapping metabolites (*49*, *56*). Similar results were obtained in the mouse GI-ARS model (*57*). Citrulline showed clear changes and tissue-plasma correlation (*58*, *59*), with radiation dose- and time-dependency observed (*49*, *56*, *57*). Longitudinal plasma citrulline data were compared across normal adult humans and NHPs, and were found to be similar (*60*, *61*), and no confounding effects on plasma citrulline were observed for Neupogen® and Neulasta® injections tested in the PBI/BM5 NHP model (M. Kane). These findings are important, as translational models and validated biomarkers will be essential for development of a GI-ARS MCM under the FDA Animal Rule (*62*).

Radiation-induced Microvascular Injury Determines Small Intestinal Stem Cell Fate

Studies were conducted in a mouse model of GI-ARS, with survival, GI and bone marrow histopathology, and crypts evaluated (*63*). Endothelial apoptotic cells in irradiated crypts and reduced BM cellularity correlated with reduced survival at ≥14 Gy vs. 12 Gy (R. Kolesnick). Cell membrane ceramides (lipids found in the bi-layer) are involved in endothelial cell death signaling after exposure to a range of diverse stressors, including radiation. Exposure damages endothelial cell membranes, engaging a pathway that results in apoptosis (*64*), and the prevalence of this pathway in these cells makes them highly vulnerable to ceramide-induced apoptosis (*65*). A full-length, anti-ceramide monoclonal antibody that binds ceramide and prevents subsequent signaling was developed and found to reduce endothelial apoptosis and increase survival in irradiated mice (*66*). More recently, an anti-ceramide, single-chain variable fragment (scFv) was designed that is equally effective in mitigating GI-ARS (*67*). In a TBI mouse model, the scFv product mitigated ongoing apoptosis and increased overall survival (*67*). Taken together, these data suggest that the anti-ceramide scFv may be an effective MCM for radiation-induced GI-ARS that acts by reducing radiation-induced endothelial apoptosis.

Innate Immune System Responses to GI Injury after Acute, High-dose Irradiation

Danger signals released in response to trauma-induced cell death or pathogen exposure activate innate immune system responses via receptor interaction with damage-associated (DAMP) or pathogen-associated (PAMP) molecular patterns. In turn, these interactions increase immune cell infiltration and tissue inflammation, and compromise tissue barrier functions (J. Brickey). Studies of a subpopulation of "elite-survivor" mice that survived high-dose irradiation were devised to include a combination of fecal engraftment and dirty cage sharing. This work demonstrated that these animals have a distinct gut microbiota that develops after radiation exposure

and provides radioprotection (*68*). Further, an unbiased microbiome analysis identified certain taxa as the most enriched bacteria in elite-survivors. Reconstitution with *Lachnospiraceae* offered GI protection. Metabolic analyses of feces from elite-survivors compared to controls identified an increase in small chain fatty acids (SCFAs) in elite-survivors, and treatment with SCFAs induced resistance to irradiation. Clinical relevance of these findings was supported by sequencing of the gut microbiome of patients with leukemia undergoing TBI. Patients with elevated *Lachnospiraceae* and *Enterococcaceae* showed reduced GI adverse reactions. An untargeted metabolomics study revealed a number of metabolites that were affected by radiation, including some that were selectively increased in elite-survivors. Together, these data support that distinct microbial populations and SCFAs may help protect both animal and human subjects against GI-ARS. Given the importance of the innate immune system in GI homeostasis and injury, the role of TLRs in GI-ARS was also explored (J. Brickey). In TBI-exposed mice (*69*) and NHPs, treatment with the TRL2 agonist fibroblast–stimulating lipopeptide (FSL)-1, 24 h postirradiation mitigated H-ARS. Similarly, in a GI-ARS PBI mouse study, FSL-1 delayed and/or reduced GI symptoms and improved survival and GI barrier function (J. Brickey). The next steps will investigate the mechanism of TLR activation in mitigating GI-ARS, and evaluation of other pathways involving innate cell pattern recognition receptors and immune sensors that respond to microbial infection.

Development of Novel Mitigators against GI-ARS by Targeting the Hippo Signaling Pathway

Characterization of GI stem cell populations at homeostasis and in response to injury has been conducted (*70*). Normal homeostatic turnover of the intestinal epithelium is driven by crypt base columnar cells. These cell types are diminished after irradiation and other GI insults; nevertheless, the intestinal epithelium is still able to recover in most cases; suggesting additional crypt populations that may direct regeneration of intestinal epithelium. These findings have led to the search for these reserve stem cells (RSCs) (C-L. Lee). Several studies (*71–73*) suggest that intestinal crypt regeneration in irradiated mice originates from recent crypt cell progeny of Lgr5+ cells by dedifferentiation, and not by recruitment of RSCs. Single-cell RNA sequencing of crypt cells identified a distinct, damage-induced quiescent cell type, termed a "revival stem cell (revSC)". These cells are extremely rare under homoeostatic conditions, but after injury, give rise via a temporal hierarchy to all the major intestinal cell types. The Hippo pathway has been shown to regulate cell proliferation, survival, differentiation, and tissue homeostasis, as well as play a role in tumor suppression (*74*). These observations suggested the hypothesis that pharmacological inhibitors of Hippo pathway elements may mitigate GI-ARS by promoting transcriptional "reprogramming" in intestinal epithelial cells. To test this hypothesis, a 3D organoid system that mimics GI morphology was developed. Using this irradiated system, revSC-mediated crypt regeneration was shown to be conserved, and small molecule inhibitors of the Hippo signaling pathway promoted growth of the organoids (C-L. Lee). These results implicate Hippo pathway inhibitors as potential MCMs against GI-ARS.

SESSION IV: REGULATORY CONSIDERATIONS FOR GI-ARS PRODUCT DEVELOPMENT

FDA Regulations and What We've learned from TBI as it Relates to GI-ARS

The FDA develops regulations based on laws set forth by the Food, Drug, and Cosmetic Act (FD&C Act 21 U.S.C. 301). An Emergency Use Authorization (EUA) permits the FDA to facilitate availability and use of MCMs during public health emergencies, provided certain criteria are met and in the absence of adequate approved and available alternatives (*75*). Human efficacy trials of MCM products for GI-ARS are neither ethical nor feasible; therefore, drug developers are encouraged to conduct product development under the FDA Animal Rule, which allows for the approval of drugs and biologics based on adequate and well-controlled efficacy studies in animals that establish that the product is reasonably likely to provide clinical benefit when administered to humans (*62*). Challenges posed by GI-ARS MCM development include the lack of a mechanistically similar human radiation-induced condition, varied responses of animals to irradiation, and limited therapeutic repurposing candidates (C. Han). Clinical experience with half-body, palliative irradiation and TBI for BM transplant may provide valuable insights. Unfractionated radiation therapy in this clinical context has been administered up to 12 Gy, but such large single doses were associated with significant risk of injury to normal tissue such as development of radiation pneumonitis (*76*).

Product Development Under the Animal Rule for GI-ARS: Nonclinical Regulatory Considerations

Animal Rule efficacy findings are based on adequate and well-controlled animal studies (*62*, *77*), and should generally be demonstrated in more than one animal species expected to exhibit a response predictive of that in humans. However, a single animal species may suffice if it represents a well-characterized animal model for predicting the desired benefit in humans. Studies that encompass radiation natural history are needed to select animal models and to confirm that they represent the key manifestations and time course of GI-ARS in humans (J. Cohen). Efficacy and pharmacokinetic (PK) studies, with and without radiation exposure, are performed in the chosen animal model to test a range of radiation and drug doses. Safety studies evaluating dose escalation and PK assays are also conducted in humans using investigational drug doses that are supported by nonclinical data (*78*). Survival assessed at a clinically meaningful time interval after radiation exposure is a standard primary efficacy endpoint. BM sparing models are recommended to permit evaluation of survival, GI injury progression, and recovery. The FDA is also open to considering other clinically meaningful endpoints, which could

include GI function (e.g., malabsorption, weight loss, vomiting, barrier), or structure (e.g., histopathology of viable crypts, apoptotic cells, and villus height). Large animal models should mimic elements of the supportive care human patients would receive, such as antibiotics and analgesics. Pharmacodynamic (PD) measurements in exploratory efficacy studies are also needed to support dose translation. The FDA's Division of Imaging and Radiation Medicine (DIRM), Center for Drug Evaluation and Research (CDER) provides advice on (i) leveraging data from clinically similar diseases or conditions that might inform the GI-ARS development plan; (ii) addressing technical challenges associated with GI-ARS studies; (iii) developing a clear understanding of MOA of the study drug and PK/PD parameters; and (iv) reaching an agreement on study design.

Clinical Pharmacology Considerations for Products Developed for GI-ARS

PK/PD data from animal studies are used to inform dose selection in humans. Key data for the investigational product are (i) PK in animals and humans; (ii) PD and efficacy in irradiated animals (and humans, if possible); (iii) the target of the study drug; (iv) human experience in other related indications; and (v) the product's safety profile. Two approaches (PD- and PK-based) are applicable for selection of a human dose for a novel GI-ARS MCM. The PD approach is based on identification of endpoint measurements associated with efficacy in animal models. The human dose is determined based on the dose that results in the desired biomarker levels in the animal model (*62*). So far, no biomarkers have been qualified for GI-ARS (M. Sampson). The PK approach is based on a comparison of drug exposure in humans to animals receiving the fully effective MCM dose. The human dose is derived from a comparison of relevant exposure parameters between humans and animals. Standard clinical pharmacology studies for investigational drugs are also applicable under the Animal Rule.

Points to Consider for Efficient Development of MCMs for GI-ARS

There are clinical conditions from which developers and regulators may glean insights into the fundamental mechanisms of organ injury and dysfunction caused by lethal radiation exposure, such as abdominal and pelvic radiation for cancer therapy (L. Marzella). No specific therapy has been approved for the enhancement of recovery from this injury. A clear understanding of the MOA of organ injury and dysfunction targeted by the MCM candidate is key for animal efficacy studies, a difficult task in GI-ARS injury due to development of multi-organ injuries. The rhesus macaque has long been recognized as the standard NHP model for H-ARS and other radiation syndromes; however, the global shortage has prompted consideration of a cynomolgus macaque model. Porcine models are also under development, and rodents are particularly useful for initial proof-of-concept, PK, and dose finding studies. Survival is strong evidence of clinically meaningful activity, and for other efficacy endpoints, the FDA recommends those that characterize the prevention or recovery of organ injury or dysfunction. Such endpoints are used to support the primary endpoint and can also serve as a trigger for initiation of supportive care.

SESSION V: MCMS FOR GI-ARS - LESSONS LEARNED AND SHARED

Overview of the NIAID GI-ARS Portfolio

The RNCP supports basic research and early nonclinical work, as well as advanced development (A. DiCarlo). Animal models for GI injury under investigation in the RNCP portfolio are primarily focused on PBI models, and general classes of GI-ARS products under study are cytokines/cytokine blockers, immunomodulators, cellular therapies, steroids/hormones, anti-apoptotics, anti-inflammatories, anti-microbials, antioxidants, growth factors, and products targeting the microbiome and the vascular endothelium. The products highlighted below, along with the others mentioned in this report are summarized in Table 4. The RNCP will continue to support laboratory animal model refinement for GI-ARS, mechanistic studies to identify targets for MCM development, and work to accelerate promising approaches toward regulatory approval and possible use during a radiation public health emergency.

CX-01, A First-in-Class, Ceramide-Rich-Platform Disrupting Antibody for Treatment of GI-ARS

The CX-01 product is a humanized monoclonal antibody fragment specific for ceramide, which can selectively target cell surface ceramide and block downstream injury and death signaling (discussed above). Anti-ceramide antibodies could potentially have broad application, as ceramide signaling also drives renal disease (*79*) and lung injury (*80*). For this product, technical challenges include developing a robust bioanalytical in vitro potency assay, formulation development, and safety and toxicology assessments, especially since ceramide signaling does not occur in the absence of injury (*81*). The CX-01 compound is in nonclinical development and the regulatory path as a GI-ARS MCM will be in accordance with FDA Animal Rule Guidance (*62*). A Type B, Pre-Investigational New Drug Application (Pre-IND) meeting was held in 2021 with the FDA (A. Tinkelenberg). Commercial indications using the same product formulation are highly desirable since sustainability in a commercial market is necessary to have these products available for emergency use and for familiarity by medical practitioners. Therefore, the company is considering parallel commercial development for CX-01 to treat diabetic eye disease and other indications. It is crucial that developers invest up front in potency assays and raise equity capital early.

GI-MCMs Emulating an Endogenous Radioprotective Pathway

Lysophosphatidic acid (LPA) is an endogenous membrane growth factor, which activates a cellular receptor and initiates

TABLE 4
Novel Products to Address GI-ARS Discussed During the Workshop

Product name/approach	Description/mechanism	Refs.
FSL-1	TLR-2 agonist	(*69*)
YK-4-250	Angiotensin receptor-targeted antioxidant	Unpublished[a]
Ghrelin	GI hormone with para-, auto-, endocrine roles	(*89–93*)
Genetically engineered *Lactobacillus reuteri*	Probiotic bacteria; engineered to deliver plasmids to release cytokines in GI tract	(*96*, *97*)
PEG-TPOm	Thrombopoietin receptor agonist	Unpublished[b]
CX-01	Anti-ceramide antibody, apoptosis inhibitor	(*67*)
FGF-PT	FGF2 peptide mimetic	Unpublished[c]
RX-100	LPA2-receptor agonist,	(*84*, *85*)
Mesenchymal stromal cells	Tissue scaffold elements that improve marrow responses via paracrine anti-inflammatory signals	(*4*, *5*)
Novel TLR-9 agonist	Anti-apoptotic, stimulates crypt regeneration	(*6*)
Microbiome (e.g., *Lachnospiraceae; fecal transplant; SCFAs)*	Targeted reconstitution of the GI tract with beneficial bacteria or their metabolites to reduce inflammation and improve gut function and survival	(*68*)
Sm. molec. Hippo inhibitor	Hippo pathway inhibitor, regulates cell proliferation	Unpublished

[a] https://connection.asco.org/blogs/potential-and-promise-overview-strategies-explored-treatment-mitigation-covid-19.
[b] https://reporter.nih.gov/project-details/9502912.
[c] https://grantome.com/grant/NIH/U19-AI150574-01-6503.

a process that protects against cell death (*82*). Due to its short half-life, an LPA mimetic (octyl-thiophosphate; Rx100) has been developed (G. Tigyi). Studies have shown that Rx100 has favorable effects on crypt survival via the LPA2 receptor (*83*), and improves gut barrier function when administered postirradiation (*84*). Rx100 also mitigates GI-ARS injury and improves survival in the C57BL/6 mouse and rhesus macaque GI-ARS models. Regulatory progress has included meetings with the FDA on animal models, toxicology plans, and efficacy study designs, with formal pre-IND meetings on the horizon to finalize the number of animals needed in the studies, and manufacturing. The compound is also being explored for use in other indications such as secretory diarrhea, given the finding that Rx100 reduced cholera toxin-induced damage in the mouse gut (*85*) and prevented diarrheal-associated weight loss in a mouse model of bacterial infection (*86*). Next-generation LPA2 receptor-specific nonlipid compounds and/or analogs have now been identified and are under development for GI-ARS, and other indications (*87*).

Mitigation of Acute and Delayed GI Injury with Pluripotent Growth Factors

Fibroblast growth factor (FGF)-2 mimetic peptides have been studied for their potential to be mitigators of GI-ARS and DEARE. The lead candidate is FGF-PT, a 17 amino acid peptide mimetic of FGF2. The primary indication is for GI-ARS, as data have shown a >30% increase in survival in studies using mice and rats (P. Okunieff).[6] In a PBI mouse model, FGF-P (an earlier form of FGF-PT) and FGF2 provided long-term preservation of small bowel stem cells and mature microvilli. Regulatory challenges for this program include selecting a suitable pivotal animal model, cGMP production, and Good Laboratory Practice (GLP) toxicology testing. Commercial indications include cutaneous radiation injury in cancer patients, and ischemic wound healing. In providing guidance to other GI-ARS product developers, a lesson learned was that commercial indications should be pursued in parallel with MCM studies. Such an approach would have accelerated acceptance by the MCM research community, resulting in an earlier and greater impact on human health.

[6] https://patents.google.com/patent/WO2019160910A1/en

Development of Human Ghrelin as a MCM for GI-ARS

Ghrelin as a GI-ARS treatment was explored in mouse, rat, and NHP models. A hunger-inducing growth hormone peptide, ghrelin promotes increased appetite and improves GI function, with systemic effects on metabolism. In irradiated mice and rats, beneficial effects have included enhanced wound healing, hemorrhage prevention, and a decrease in inflammation (*88–92*). In an irradiated NHP model, ghrelin showed only a marginal improvement in survival, resulting in re-evaluation of the product's development (M. Brenner). Changes to the irradiator, dose rate and model BM shielding created large variability and uncertainty in the data. Trial design challenges included underpowered NHP experiments resulting in inconclusive data, and the need to work closely with NHP facilities on treatment and monitoring protocols to ensure all are invested in the outcome. Regulatory hurdles included understanding what concomitant therapies should be provided, as well as the challenge of partnering with pharmaceutical companies with little interest in GI-ARS. Development of another small molecule ghrelin receptor agonist and use of these agonists in veterinary practice also made finding partnerships difficult.

Radiation Mitigation by Administration of Second-generation Probiotics

To investigate the potential of IFN-β, implicated as a mediator of GI regeneration (*93*), as a radiation mitigator, the ability to deliver it in a manner that could be clinically utilized needed to be developed (J. Greenberger). *Lactobacillus reuteri* (LR), a probiotic bacterium, was selected to deliver plasmids that have been genetically engineered to release cytokines in the GI system. Two LR strains were engineered to release IL-22 or IFN-β after cell lysis, with both mouse and human forms of the cytokines tested (*94*, *95*). The LR-IL-22 and LR-IFN-β strains were administered by gavage 24 h postirradiation in several models, with increased survival noted (*96*). A separate study showed that oral administration of the bacterially produced cytokines led to higher survival when compared to administration of the recombinant protein by SC injection. These probiotics are not designed to colonize the gut, and detectable levels of LR-IFN-β bacteria and the LR-derived IFN-β gene were rapidly cleared from fecal matter. As IFN-β is FDA-approved for treatment of multiple sclerosis, this MCM has been prioritized, as it may have a more straightforward regulatory path. A lyophilized form of human LR-IFN-β is being developed, as the delivery of live cells is not a viable approach after a radiological or nuclear incident.

YK-4-250 Mitigates GI-ARS from 14.3 Gy PBI

YK-4-250 is a radiation mitigator comprised of two conjugated clinical phase drugs that target tissues and cell types expressing angiotensin receptors. The product is an antioxidant that has a multipronged effect on the GI tissue by decreasing the inflammatory response, thus reducing GI tissue damage and permeability, and bacteremia, and mucosal damage. Oral administration of YK-4-250 was shown to increase survival when given postirradiation in a PBI mouse model (M. Brown; V. Kumar). Based on the presumptive MOA of the small molecule, the company has looked to develop indications for treatment of ulcerative colitis, colon cancer, and lung fibrosis. Larger-scale drug synthesis is ongoing, and FDA interactions are planned.

DISCUSSION

Although separate panel and participant deliberations followed the speaker presentations for each session, to eliminate redundancies and more clearly organize the conversations, topics that were brought up across all the discussions are addressed together (Table 5).

Adopting Protocols and Therapies from the Clinic to Better Understand GI-ARS Models and Approaches

Clinicians often administer multiple fractions of high-dose TBI as preconditioning for BM transplant using irradiation protocols that do not induce lethal GI-ARS. In TBI used as preconditioning for clinical transplants, many critical radiosensitive organs are protected, and irradiation is focused on BM ablation to allow for transplant engraftment. Radiation oncologists are primarily concerned with chronic GI injury sequelae, which arise due to a combination of stem cell senescence, immune dysfunction, vascular insufficiency, impaired regeneration, and fibrosis, which manifest in some patients. It is of interest to understand the therapeutic approaches used in typical clinical GI presentations, such as inflammatory bowel disease, or gastroenteritis. For example, drugs like keratinocyte growth factor (KGF), indicated for treatment of oral mucositis in patients receiving chemotherapy and radiation therapy, or other growth factors could be considered, although some nonclinical studies in NHPs suggest that KGF might be harmful when used as a radiation mitigator (*97*).

Supportive care refers to medical management that is given to patients in the expectant care category, and the level of support generally depends on resources that are available. There have been radiation exposure incidents, for example, in Tokaimura, Japan where patients lived for >150 days, for whom the life expectancy without supportive care antibiotics and cytokines would have been on the order of a few weeks (*98*). When considering clinical cases of GI-ARS, physicians cannot focus solely on the one sub-syndrome, as GI-ARS occurs concomitant with H-ARS. Therefore, it is important to ensure bone marrow reconstitution and address hematopoietic symptoms alongside any GI therapeutics to ensure patient survival. It is critical that clinicians be involved in all stages of the development of models and treatments for GI-ARS, such that any approaches that are under study will be optimized for success in humans.

Animal Models for GI-ARS

It is crucial that the scientific community continues to investigate and characterize the natural history of animal models of radiation injury and determine whether animal models accurately reproduce clinical conditions expected in humans experiencing GI-ARS (e.g., electrolyte imbalances, dehydration, loss of GI barrier function, and microbial translocation). In addition, it will be important to understand how antimicrobials, growth factors, and rehydration may influence progression of GI-ARS. The potential impact of animal diets on GI-ARS survival, with a particular focus on the microbiome was also discussed. The source and composition of food are important considerations, as many healthy bacteriophages may be present in the feed, which could affect the immunologic response of animals to radiation. Early work to longitudinally evaluate the microbial flora of irradiated NHP survivors suggests that microbiome changes may be associated with hypertension. Future studies with multiple readouts should be used to assess the relationship between the microbiome and observed pathologic changes. Findings in animal models of GI-ARS have also identified differential radiation responses between animals of different sexes (*99*). It is possible that some of these differences could be attributed to weight variances since there are differences in absorbed dose for larger compared to smaller subjects. Therefore, studies should include both male and female subjects, the radiation DRR and natural history of

TABLE 5
Meeting Sessions Discussion Prompts

SESSION I – Clinical Manifestations

- With four licensed products for H-ARS, there still remains an unmet need for approved products to address GI-ARS and injuries impacting other organ systems at high radiation dose levels.
    - What new concepts and/or approaches will need to be considered to develop and expand the field?
    - What common pathophysiologic mechanisms can be modeled from the clinical setting to the animal?
    - What baseline standards of care need to be applied to the animal model (e.g., fluid resuscitation, antibiotic use, etc.)?
    - What state-of-the-art approaches are used in clinical practice to assess and treat damage for other GI injuries/disease states?
    - Are human data available for TBI (e.g., for bone marrow transplant), or PBI (e.g., for total pelvic exposure for ovarian and endometrial cancers or brachytherapy for prostate cancer) that could be considered, in terms of GI-ARS relevant damage to the intestines?

SESSION II – Animal Models

- There are limitations to studying the natural history of GI-ARS, because there is no clear consensus on how to approach the injuries, nor what animal models are most appropriate to recapitulate the human condition.
    - Must be able to show damage and recovery of the GI tract, with bone marrow myelosuppression (survivable)
    - Do metrics exist to define and distinguish GI-ARS from other ARS effects? How intertwined/interdependent are H-and GI-ARS? Are they sufficiently distinct to be assessed independently, or are they a continuum?
    - How does this affect practical implementation of GI-ARS MCM and model development?
    - Are *in vitro*/*in vivo* models and radiation exposure protocols adequate for development and validation of GI-ARS MCMs?
    - Usefulness of rodent models, and their ability to reflect findings that extrapolate well to humans.
    - Different PBI shielding models, e.g., levels of shielding (e.g., 2.5% vs. 5% in mice; 8% in rats; 5% in NHPs
        - Anatomical area shielded (e.g., full leg, two legs from the knees down, including the tail or not, head only, hemi-body, all but abdomen, etc.)
        - Other irradiation protocols (e.g., AIR, TBI+BMT, etc.)
        - Do these models and protocols need to be standardized across sites?
    - Radiation quality could impact outcomes (orthovoltage x-rays vs. higher energy LINAC, gamma sources, etc.).

SESSION III – Mechanisms and Biomarkers

- Appropriate primary/secondary endpoints for GI-ARS – qualitative vs. quantitative; functional, imaging
    - Survival and mean survival time of decedents; weight loss
    - Best biomarkers (e.g., citrulline, etc.) to assess injuries/trigger treatment and track impact of MCMs
    - Best targets to exploit radiation-induced injuries (e.g., regions of the small intestines/colon; crypt stem cells; villi; endothelium; immune tissues, microbiome)
    - Biomarker qualification – importance of biomarkers to trigger treatments vs. bridge across species for efficacy pharmacodynamics vs. inform on treatment impact

SESSION IV – Regulatory

- How to approach the FDA? Animal Rule information, existing guidance
- FDA input on species/percent shielding/medical management/endpoints
- Considerations for animal models of GI-ARS (animal care, euthanasia criteria)
- Study design and data quality issues (reproducibility, single vs. multiple labs)
- If MCM is approved for another indication, are two efficacy models needed? Other questions surrounding repurposing

SESSION V – MCMs

- Key elements to successfully developing new therapies for GI-ARS
    - Polypharmacy using H-ARS mitigators will be expected/required in the course of GI-ARS model development, and for testing/evaluating GI-ARS MCM efficacy
    - Do GI drugs need to provide a survival benefit in the absence of a heme support beyond bone marrow shielding? What about bone marrow transplant in the model?
    - How do these factors affect practical implementation of GI-ARS MCM testing and model development?

disease should be determined for each sex, and different radiation doses should be used for each sex if needed.

Appropriate euthanasia criteria were considered, since in some GI-ARS murine models that use scoring to determine moribundity, there can be a rapid progression to animal death in the critical period (days 6 to 10). Standard euthanasia criteria include the sum effects on several clinical signs, such as diarrhea, temperature, hunched posture, labored respiration, and weight loss. Weight loss is an important consideration in euthanasia decisions, as it has been correlated with all stages of the biological response to irradiation, but it may be possible to mitigate some weight loss with food supplementation and provision of wetted chow. These efforts, however, may be complicated by radiation-altered signaling of cytokines that upregulate acute phase proteins such as albumin, that can exacerbate fever, leukocytosis, and increase cortisol.

Radiation Considerations

Increasingly, radiation source and beam quality differences in animal model development are being recognized for their role in reproducible radiation science. More facilities are engaging in dosimetry validation and providing detailed reporting of their radiation sources in publications. This advance is important because natural history study data show that orthovoltage X rays differ from radiation sources such as ^{137}Cs and ^{60}Co (*33*, *34*). In the absence of standardized irradiation protocols and reproducible parameters, the observed survival advantage for a MCM may be partly attributed to the beam quality and source rather than the MOA of the drug alone. One must also recognize that survivable doses of radiation exposure noted in animal models do not necessarily translate directly to humans. For example, an $LD_{50/60}$ of $\sim$3.5 Gy has been estimated in humans (*100*), but provision of basic medical management can improve survival dramatically. A 2018 review (*101*) considered human exposure cases that estimated patients were exposed to 5–6 Gy, and almost all of these patients died. NHP work has helped to understand the transition from an animal model to humans, as has extrapolation of best practices from the radiation oncology and BM transplant communities.

Laboratory radiation dose rates established in the 2000s were $\sim$100 cGy/min, selected to mimic radioactive fallout rates, not the dose rate anticipated from a prompt exposure. Generally, dose rates between 10 and 250 cGy/min should exhibit similar levels of damage. More recently, use of flash irradiation ($\geq$40 Gy per s) is gaining acceptance as a possible model for prompt exposure. It is also important to note that animal model dose rates are up to 10 times higher than what is given for marrow-preparative regimens in the clinic (generally 10-15 cGy/min), making translation from animals to humans more complicated. In early BM transplant protocols giving 10 Gy in one fraction, with a dose rate above 15 cGy/min, patients often died of radiation pneumonitis instead of GI-ARS. However, when the dose rate was lowered, the incidence of radiation pneumonitis decreased.

Concerns from the scientific community suggest that the currently favored PBI model employing $\sim$2.5% BM sparing is insufficient to protect the hematopoietic compartment. As the radiation dose increases, stromal effects occur and treatment modalities such as Neulasta, BM sparing and transplant may fail, because the number of healthy stem cells remaining could be insufficient for animal survival. Therefore, an argument was made to include BM transplant in these models, to confirm that animals are not dying from accelerated marrow dysfunction (which could occur on a similar timescale to GI injury), but rather from GI damage. The advantages of utilizing transplants have been well studied and can be used to further assess GI injury, clinical manifestations, and to better understand the mechanism of disease. In theory, for PBI models to work, BM expansion in the protected area should occur, followed by stem cell engraftment and integration, which is key for animal survival postirradiation. From the literature and previous TBI studies, animal model developers came to an agreement that $\sim$5% bone marrow sparing was a good starting point to assess GI-ARS injuries, and other ARS and delayed sequelae.

Considering Real-World Radiation Injuries

In some cases, unless a clinician suspects radiation exposure, it may be difficult to assess a patient presenting with GI-ARS symptoms. An anecdotal example of this was discussed in which a patient presented symptoms consistent with irradiation, yet hospital records indicated there was no exposure. Patient treatment was initiated assuming irradiation, even though there was no record that he was exposed. In addition, there will be patients who present with GI comorbidities who are also irradiated, which can lead to a difficult diagnosis requiring clinical expertise.

Some GI-ARS clinical manifestations can be attributed to underlying ischemia that can be further complicated by radiation injury to neutrophils, resulting in clots that are derived from nets with chromosomal DNA intertwining with fibrin monomers and platelets to cause clots. Therefore, it may be important to include aspects of ischemia treatment as part of medical management. Focusing on products to inhibit the release of these components might identify a therapy that is systemic in terms of its effect since impaired or altered blood flow ultimately leads to multi-organ failure.

Regulatory Considerations

The FDA INTERACT (Initial Targeted Engagement for Regulatory Advice on CBER ProducTs) pathway involves an informal, non-binding meeting between a sponsor and the FDA Center for Biologics Evaluation and Research (CBER) early in product development. Similarly, CDER offers meetings with the Counter-Terrorism and Emergency Coordination Staff. INTERACT and other information meetings provide sponsors an opportunity to receive advice from CBER and CDER staff, respectively, prior to a pre-IND meeting on a wide range of development-related topics.

In the discussion of orally administered drugs, it was recommended to characterize oral absorption in a validated animal

model while relating any differences observed as a function of GI-ARS. Absorption will also depend on the type of drug, intended dosing schedule, and route of administration. A drug intended for repeat dosing would need to demonstrate safety and efficacy in exploratory PK and PD studies in an animal model, to determine the effects of systemic radiation exposure on the absorption of the drug. Especially important in oral dosing is a consideration of the concomitant effects that manifest in GI-ARS, such as vomiting and prolonged diarrhea, which might complicate studies in some animal models. In addition, loss of crypt and villus architecture could lead to malabsorption of the drug. Therefore, it is important to complete PK/PD studies for a drug both with and without irradiation to assess the effects of radiation on metabolism and absorption.

The FDA acknowledges that prolonged survival, while ideal, would not fit all types of therapies and therapeutic candidates. According to the Animal Rule, the FDA may grant marketing approval for drugs in which the animal study endpoint is related to the desired benefit in humans, including the prevention of major morbidity (*62*). Major signs of morbidity for GI-ARS could include weight loss, dehydration, diarrhea, effects on absorption, and other clinical signs that would typically be triggers for euthanasia. For the FDA to consider amelioration of major morbidities as an efficacy endpoint, it is recommended that developers provide a plan and justification for GI-ARS severity scoring and incorporate survival as a safety endpoint.

Product Development

There is a need to identify biomarkers that are not derived from terminal histopathological assessments, and which enable the FDA to evaluate the effect of an MCM on PD markers and not just PK. Especially for GI-ARS, a direct correlation between improvement in animal survival, drug dose assessment, and PD markers are key. Although not all development programs found value in citrulline levels in NHP models, some found steady-state levels of ceramides to be predictive. Drug development efforts may need to be staged with increasing supportive care, including potentially polypharmacy, to be predictive and have a roadmap for the MCM development process. It is well understood that the polypharmacy approach might complicate issues, especially when evaluating GI injury with concomitant multi-organ failure. It is also likely that drugs that benefit the GI tract may mitigate radiation damage to other organs and systems. In a realistic scenario, victims will be administered antimicrobials and other standard care (e.g., anti-emetics and anti-diarrheals); therefore, it is important to verify that these treatments do not affect MCM efficacy, at least in larger animals, and consider that model animals may be administered different antimicrobial regimens than humans would receive.

The discussants also addressed difficulties associated with products that have mechanisms of action for mitigation that are specific only to the GI tract. For instance, is it reasonable to require that GI mitigators also mitigate H-ARS in a model with only a small percentage of BM sparing? Is it permissible that a model might require additional hematopoietic support beyond BM sparing to demonstrate efficacy? One strategy would be to evaluate GI models with the least intensive BM support in the initial development phase. Including maximal hematopoietic support might complicate early studies and would result in a more limited indication for use in a mass casualty incident. Ultimately, drug developers should understand all aspects of their selected radiation animal model, as not all GI-ARS models are the same, especially in terms of the level of exposure within the PBI-shielded anatomic region. Drugs that are designed to mitigate early GI damage should not be penalized, nor be expected to mitigate H-ARS.

CONCLUSION

This trans-agency meeting met its primary goal of providing an open forum for considering multiple aspects of MCM development for GI-ARS. By bringing together academic researchers, companies pursuing advanced drug development, as well as U.S. Government funding and regulatory agency staff, the assembled subject matter experts offered valuable information to the research community on early and late product development, while providing insight into appropriate animal model selection, identification and targeting of radiation-response pathways, and lessons learned. These efforts are expected to lead to a more straightforward and accelerated development pathway for MCMs intended to mitigate GI-ARS.

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