RADIATION RESEARCH **198**, 508–513 (2022)
0033-7587/22 $15.00

DOI: 10.1667/RADE-22-00009.1

COMMENTARY

Commentary on Animal Care in Radiation Medical Countermeasures Studies

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INTRODUCTION

The National Institute of Allergy and Infectious Diseases (NIAID) Radiation Nuclear Countermeasures Program (RNCP) at the National Institutes of Health (NIH), and Biomedical Advanced Research and Development Authority (BARDA) within the Department of Health and Human Services (HHS) have supported medical countermeasures (MCM) and biodosimetry advancement spanning all levels of radiation research, advanced product development and regulatory strategies to support eventual licensure/approval/clearance of MCMs and devices by the U.S. Food and Drug Administration (FDA).

The FDA Animal Rule (*1*) provides guidance for development of MCMs, requiring that animal models simulate the human response to radiological/nuclear exposures and the health sequalae since it is unethical to conduct radiation experiments in humans. Animal models must reflect different and complementary aspects of the clinical scenario, with study endpoints that clearly relate to the desired benefit in humans for MCM efficacy studies. Given the complexity of radiation injury, it is anticipated that a single animal model will not be sufficient to address the continuum of health effects following exposures. Further, to compare efficacy of MCMs across multiple institutes, harmonization of variables that are amenable to standardization is required.

Four MCMs have been FDA approved since 2015 to treat hematopoietic acute radiation syndrome (H-ARS), however MCMs are still needed for several acute and delayed radiation subsyndromes (skin, gastrointestinal, lung, kidney). To ensure continued forward progress, on August 24–25, 2020, NIAID and BARDA hosted a workshop to discuss animal care protocols currently utilized in the field of radiation research, to identify and address gaps in knowledge, discuss solutions to facilitate evaluation of MCM efficacy and to attempt to harmonize variables that are amenable to standardization (full meeting report available online at https://doi.org/10.1667/RADE-21-00211.1). The importance of this area was highlighted by Dr. Francis Collins (Director, NIH)[2] in a recent report on enhancing rigor, transparency, and translatability in animal research. One of the recommendations by the advisory committee was specific to animal care: ''NIH should encourage and support work to better understand, monitor, record, and report important extrinsic factors related to animal care that may impact research results.''[3]

The consensus of the workshop was that there are many variables (barrier conditions, strain, age, sex time of day of radiation, restraint, ear tag/punch, use of acidified water vs not, etc.) existing among the various research institutes which makes harmonization difficult in the immediate future. Researchers are strongly encouraged to stress that these variables be clearly reported in manuscripts so that differences across studies and possible confounders can be better understood by the research community when reading these research reports to help build consensus among the models.

SESSION I: MEDICAL MANAGEMENT FOR ANIMAL MODELS

Session I of the workshop addressed the natural history of several animal models, and how they relate to the human condition, including a consideration of animal housing, infection control, concomitant medications, hydration, diet, clinical condition, laboratory assessments, euthanasia criteria, and study design.

Total- and Partial-Body Irradiation in Rodents (Mice and Rats)

Natural History of the Models. The murine total-body irradiation (TBI) model is commonly used to test MCM

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[2] https://www.nih.gov/about-nih/who-we-are/nih-director/statements/statement-enhancing-rigor-transparency-translatability-animal–research.

[3] ps://acd.od.nih.gov/documents/presentations/06112021_RR-AR%20Report.pdf.

efficacy for H-ARS. TBI elicits H-ARS that result in prolonged immunosuppression, impaired function of hematopoietic stem cells, and depending on the radiation dose, mortality that is observed within 30 days postirradiation. Mice that survive TBI often display onset of multi-organ, delayed effects of acute radiation exposure (DEARE) (*2*). The TBI model has substantial literature published in young adult models of C57BL/6 (*3*), and Jackson Diversity Outbred (JDO) mouse strains (*4*), C57BL/6J pediatric (*2*), and geriatric models; whereas rats used for radiation studies include WAG/RijCmcr, Sprague-Dawley, and some Wistar strains.

The partial-body irradiation (PBI) model is very similar to the TBI model, except that 2.5–5% of the bone marrow is protected from exposure, sparing some hematopoietic tissue from ablation. This model is well suited for evaluating the natural history of gastrointestinal- ARS (GI-ARS), characterized by dramatic weight loss, loose stools/diarrhea and death results within 7–10 days postirradiation. PBI models also are useful for studying multi-organ injury, since the H-ARS insult is not severe and many animals survive and progress to DEARE, including delayed hematopoietic, lung, kidney, or cardiac injuries.

Several elements of animal care have been identified that can affect survival endpoints in both TBI and PBI models. For example, vendor and barrier differences have been shown to impact survival, with lifespan differences noted in C57BL/6 mice bred and raised in different facilities (*5*), which was attributed to environmental factors impacting mouse phenotype. Maximum barrier housing conditions that employ sterilized, individual ventilated caging and drinking water, and clean-room procedures where workers wear personal protection equipment, are correlated with a lower incidence of the swollen muzzle syndrome deaths (*6*) that can dramatically impact study results.

Selecting healthy animals prior to the start of the study is crucial for biomedical experiments; therefore, animals with conditions such as malocclusion, barbering, dermatitis, or malignancies should be excluded. Variations in radiation sensitivity due to age, weight, and sex are reported in efficacy (*2, 7*). For this reason, and to account for other environmental variables, it is important to periodically reestablish institutional radiation dose-response curves for both TBI and PBI studies, using controlled weight and age ranges, and both sexes. Additionally, identification methods such as tattooing or ear-punch must be carried out early prior to the start of the study to allow for recovery time, as some methods can result in a combined injury and make animals more sensitive to radiation exposure.

Irradiation Setup. The irradiation geometry and setup, and the length of time in jigs during irradiation is another experimental consideration, since body temperature and stress hormones change with handling and restraint, affecting radiosensitivity. To minimize variability in outcomes due to the circadian rhythm, rodents should only be irradiated during a 2-h time window each day, typically in the AM (*8*). Specific to the PBI model, mice/rats should be anesthetized during irradiation, with the lower part of one hind limb shielded.

Husbandry and Handling. Husbandry aspects such as bedding, and enrichment also impact study results. Rodents should regularly receive food, clean bedding with enrichment (e.g., nesting materials), and water ad libitum. Additionally, the use of acidified (pH 2.0–3.0) vs. non-acidified water has been shown to impact the radiosensitivity of mice (*9*). Housing should also be standardized, with vented racks, barrier cages, and animals housed in social groups. Temperature, humidity, air changes, and the light/dark cycle of their environment should be controlled (*10*). Handling, dosing, and weighing should be conducted outside of cages in laminar flow workstations to prevent infection/contamination. Additionally, rodents are ideally acclimated for two weeks prior to study start. MCM dosing volume, administration route/site and frequency, as well as any handling due to blood sampling, will raise stress levels and may impact mortality.

Supportive Care. Analgesia and antibiotic use can have a significant impact on study outcomes and confound MCM efficacy results. Hydration is also important, as sick animals drink less, and animals with diarrhea are more prone to dehydration. Fluid supplementation can increase survival but handling and dosing for supplementation in sick animals can also reduce survival. Therefore, a balance must be struck when determining duration, volume, and frequency of fluid supplementation. Providing wetted chow or gel packs are a less stressful means of supplementing hydration in mice receiving GI-ARS doses of radiation, or for rodents that experience after TBI tooth loss. However, it is not recommended to change the diet during the study, so these changes should be made at the onset of the experiment.

Euthanasia Criteria. Animal health should be monitored on a pre-established schedule and set health criteria, according to the Institutional Animal Care and Use Committee (IACUC) guidelines. Staff making euthanasia decisions should be familiar with these requirements before the start of the study. Deaths due to H-ARS usually occur between 2–3 weeks after TBI, with the first death occurring around day 7–9, and most deaths or major morbidity occurring by day 24. Hence mice are observed twice daily during these critical times (times of major morbidity or mortality), then tapered off to daily monitoring. Mice are considered moribund for H-ARS if they have $>20\%$ weight loss, and exhibit at least one other sign such as hypothermia, hunched posture, rough coat, abnormal respiration, reduced peer interaction, or listlessness. For the PBI model, welfare checks are best done once per day, increasing to 2–4 times per day during the critical period which is contingent on the endpoint studied (lung, kidney, heart), the radiation dose, supportive care, and other factors.

Other Factors to Consider. As with any animal model, there are often differences between the mouse model and the human condition. An important distinction is the

difference in lethal dose result in 50% mortality (LD-50) for H-ARS- $LD_{50//30}$ for mice ranges from 6 to 9 Gy, where the $LD_{50/60}$ for humans is estimated to be 4 Gy. Similarly, the LD-50 for GI-ARS, and lung-DEARE is much higher in the mouse/rat strains compared to humans. Age equivalence is also difficult to estimate, as mice are estimated to age faster than humans (*11*). Another difference specific to MCM research is the species-specific effect of G-CSF on recovery of blood count parameters during H-ARS- in humans, G-CSF promotes the recovery of neutrophils only, while in mice, it promotes the recovery of neutrophils, platelets, and erythrocytic lineages (*12*).

In humans, DEARE after GI-ARS is characterized by intermittent episodes of nausea, vomiting, diarrhea, and constipation. Mice do not develop these episodes of vomiting, diarrhea, and constipation, but they do lose weight and develop intestinal fibrosis. At the radiation doses used to elicit GI-ARS/DEARE, mice do not develop oral mucositis, but do experience loose teeth/tooth loss, necessitating the use of wetted chow or gel packs early on to maintain adequate nutrition. Differences in radiosensitivity, immune-tolerance, and gut permeability have been noted across mouse strains and strain variants. For lung-DEARE, different strains of mice present with differing pathologies and severity of lung injury (*13*), and sex differences have been reported for the incidence of pneumonitis in the WAG/RijCmcr rat lung-DEARE. Finally, inbred mice used in radiation research with standardized age, weight, and housing conditions do not necessarily represent diversity seen in the human population.

Topic 2: Total- and Partial-Body Irradiation in Large Animals (Minipig, Rabbit, NHP)

Natural History of the Models. The overall goal of preclinical models is to link human responses to radiation exposure with those of irradiated animals. Several large animal models developed to study irradiation include NHPs (specifically rhesus macaques), Göttingen minipigs (MPs), and New Zealand White rabbits. As with rodent models, TBI animal models are used for H-ARS studies, and the irradiated animal presents with bone marrow myelosuppression and myeloablation that is highly dependent upon the animal model, radiation dose, receipt of supportive care, and other factors. The natural history of the various models is akin to what would be expected in humans; with clinical signs of radiation exposure in animals including emesis (not seen in rodents), diarrhea, ulcerations of the oral mucosa, as well as decreased activity, appetite and body weight.

The PBI model with 5% bone marrow sparing more closely approximates a ''real world'' scenario, where subjects are likely to be exposed to inhomogeneous, partial-body irradiations rather than a uniform TBI. Since the PBI models are currently being developed, much is still to be learned, specifically in areas of sex and age differences for ARS or DEARE, impact of different radiation qualities, natural history in months and years after exposure to prompt, high dose rate, non-uniform, unilateral, exposure, and later effects of organ specific MCMs.

Irradiation Setup. For TBIs, NHPs can be irradiated when conscious or unconscious. In the conscious TBI setup, NHPs are restrained in position, whereas in the unconscious setup, NHPs are sedated and placed in a supine position. For PBIs, NHPs are first sedated and then exposed such that 5% of the femur marrow is spared, allowing for dose- and time-dependent survival through ARS to delayed injuries. Animals are made comfortable by playing soft music during irradiation studies and are monitored continually over camera to decrease stress for the animals and staff. For the MP TBI setup, animals are sedated and restrained in a sling. Similarly, rabbits are also sedated and placed in slings prior to TBI.

Husbandry and Handling. NHPs, MPs, and rabbits are typically provided purified water via automatic sippers, and certified food, with commercial biscuits given to NHPs and commercial chow provided to MPs and rabbits. Irradiated large animals are housed singly to facilitate monitoring of individual outcome (feces, diarrhea, or emesis) and individualized medical management, and to prevent contamination or infection between animals. NHPs are housed in ''squeeze back'' cages with perches, MPs in cages with side doors, and rabbits in cages that allow visual and scent cues with limited physical contact. Additional species-specific enrichment involves edible enrichment and socialization, as well as puzzles and movies for NHPs, or rooting behavior toys for MPs and rabbits.

Apparatuses such as chair restraints (NHPs), slings and chairs (MP), and bunny snuggles (rabbits) are used to secure animals before and after irradiation, for blood draws and assessment of other functions. The risk benefit of each restraint must be carefully studied, for instance, slings for NHP are more secure but limit access for blood draws, while some restraints result in irritation to the animals. Rabbits are restrained with rabbit snuggle restraints that cover the eyes to decrease stress while allowing access to the ears for blood draws or treatments.[4]

Supportive Care. Medical management will be specific to each model, but the products and care provided to large animal models should mimic the human, and these criteria should be defined by IACUC prior to the start of the study. Prophylactic care (support given before symptoms appear) may include antibiotics, analgesics, antiemetics, food, and fluid supplementation. Trigger-to-treat supportive care is provided when symptoms manifest, per pre-set criteria, and can include additional antibiotics (systemic and topical), analgesics, parenteral fluids, nutritional support, wound support, and blood products. NHP nutritional support can include crushed biscuits with banana and/or fruit/vegetable buffet. Pigs may receive Ensure® (Abbott Nutrition), fruit and vegetable buffets, hay, or grass, and rabbits may also be

[4] https://www.lomir.com/snuggle/snuggle-rabbit/.

provided Ensure. Full supportive care models include blood or blood products administration for pre-determined triggers.

In the PBI model, medical management of NHPs is based on triggers-to-treat and stop. For GI- and H-ARS, clinical signs, and blood parameters such as absolute neutrophil (ANC) and platelet counts, as well as diarrhea and hydration, are used to inform trigger endpoints. For lung and kidney DEARE, clinical signs such as non-sedated respiratory rate, SpO2, and arterial blood gases can be evaluated, computed tomography (CT) scans can be done periodically, and parameters like blood urea nitrogen and other molecular biomarkers or -omics can also be assessed.

For H-ARS, NHPs receive antibiotics for febrile neutropenia, and whole blood transfusions for triggers related to hematocrit and platelet counts. During the DEARE period, NHPs are given dexamethasone if the non-sedated respiration rate is $\geq$80 breaths per minute. Other supportive care based on trigger-to-treat in the PBI/BM-sparing model includes antipyretics, fluids, antidiarrheals, antiemetics, nutritional support, analgesics, and diuretics. With large animals, endpoints can be monitored throughout the in-life portion of the study, such as body weight, temperature, clinical chemistry, hematology, and coagulation. Additionally, for NHP studies, serum bacteriology may be assessed to help assess euthanasia criteria, initiation of supportive care, and monitoring of the animal. The use of infrared cameras for nocturnal monitoring, or surgically inserted telemetry devices can facilitate remote and continuous data monitoring in NHPs without incurring handling stress.

Euthanasia Criteria. Well-developed, predetermined euthanasia criteria specific to each animal model are key to any well-constructed study. Main criteria used across both TBI and PBI models include severe respiratory distress with increased and/or labored respiration, anorexia, or decreased appetence over multiple days with no interest in food treats, sustained and/or severe weight loss, recumbency or unresponsiveness, gross blood loss, or hemorrhage that cannot be controlled, seizure activity, or severe dehydration with hypo- or hyperthermia, and severe pain.

Other Factors to Consider. Staff consistency and communication minimizes bias and variables; maintaining the same technical staff throughout a study allows the animals to become accustomed to handling and interacting with those staff, decreasing animal stress levels. Activities should be scheduled for the same time every day such that animals are used to the routine and stress is decreased. The frequency of endpoint assessments, both invasive and non-invasive, should be determined before the study start, with established frequency and amount of sample collections to minimize stress to the animals.

SESSION I: DISCUSSION

The discussion centered around harmonization of animal protocols that would allow for reproducible data across multiple institutes and reduce the unnecessary utilization of animal subjects, while bridging to human data. At the onset, it was acknowledged that while harmonization was a goal, it is very difficult to achieve harmonization of all elements across different institutes. Focused harmonization on specific elements such as radiation dosimetry, statistical analysis, and animal-use protocols for each species and model (TBI/PBI), can improve reproducibility and robustness of data. Given that individual IACUC decisions can have a significant impact on study design, good communication between investigators and veterinarians, and implementation of standard operating protocols (SOPs) will improve quality of the studies. Discovery stage research in rodents may allow for more difference in harmonization, but advanced studies conducted under Good Laboratory Practices (GLP) in large mammals require well-controlled, well characterized animal models. However, at any stage of research, it is important to publish these research and model development studies with detailed methods (e.g., animal handling, radiation pie/jig setup, dosimetry, etc.) to benefit the whole research community. Pivotal studies in a well-characterized animal model aligned with the criteria of the FDA's Animal Rule can inform about the MCM efficacy and help bridge the gaps from an animal model to the human condition. Finally, funding agencies must also be part of the conversation to align mission priorities with the animal models and MCM studies.

SESSION II: BASELINE ANIMAL CARE IN RADIATION RESEARCH

To harmonize research and laboratory practices across institutions, similarities, and differences in animal care between species were discussed in this session, with the following topic areas: 1. animal housing and handling, 2. infection control, 3. hydration and diet, and 4. euthanasia criteria.

Panel Discussion Topic 1: Animal housing and handling

The macroenvironment and facility design as outlined in the *National Research Council Guide for the Care and Use of Laboratory Animals,* is consistent among most facilities conducting radiation research (*14*). Sources of variability in animal housing and handling include: 1. macroenvironment and facility design (e.g., air, humidity, temperature, lighting, noise, vibration); 2. staff knowledge, training, and interaction with animals; 3. social housing/interaction; 4. enrichment strategies; 5. choice of bedding; 6. sex and age of animals; and 7. animal transport.

Mice. Husbandry practices at the vendors and different institutes can influence lifespan and radiosensitivity of animals Mice are usually housed at a high density (5–6 mice per cage), but sometimes single housing is required. Enrichment is minimal, consisting of autoclaved tissues or food-grade sample cups for nesting/housing. Bedding differs for H-ARS studies (alpha-cellulose for GLP studies) and non-

H-ARS studies (standard pelleted paper or aspen hardwood sawdust). Stress from frequent handling for multiple dosing or blood sampling increases lethality (*3*). Extended restraint in irradiation pies and transportation can also increase levels of stress cytokines as well as hyperthermia.

Minipigs. MPs are group-housed when quarantined, but male and female MPs are single-housed in separate rooms in large metal cages that allow safe visual, touch, and smell cues between animals during study. The type of cages used determines bedding- raised cages need no bedding, but hay or pine shaving bedding is used when animals are housed on a hard surface. To reduce stress due to transportation, acclimation periods are necessary, or the use of sedation for the transport, and separation of experimental animals from naïve cohorts. Male MPs are more aggressive than females, while females gain weight faster than males; therefore, diet and enrichment strategies must be carefully maintained. Rooting, foraging, and exploration should also be encouraged using appropriate toys.

NHPs. NHPs of both sexes are single housed in the same room, to allow visual and olfactory cues. Facility temperature and relative humidity are kept constant and monitored closely. Lights and noise are controlled to maintain 12-hour cycles of light and dark. Enrichment for NHPs is critical and includes foraging toys, mirrors for visual stimulus, and soft foods treats and a high fiber diet. Sedation is not necessary for NHP transportation, but an acclimation period is needed. For both sexes, NHPs between 3–5 year and weighing 3.5–6 kg at the start of the study are recommended. For longitudinal blood sampling studies, the size of the animals determines the volume of blood that can be sampled, and it should not exceed 7.5–10% of blood volume per week to prevent hypovolemia and anemia.

Panel Discussion Topic 2: Infection Control

Antibiotic selection and schedule are important in controlling infection and influence the outcome of a study. Rodent infection control consists of acidified, autoclaved water, with the use of ciprofloxacin or enrofloxacin in the water as a prophylactic regimen as needed for mice, while rats are typically treated with enrofloxacin in the drinking water. Rabbits are treated with a daily administration of Bactrim, but this is not useful in the treatment of *Pseudomonas* infections, as seen after exposure. MPs are administered with amoxicillin twice daily or gentamicin once daily to control common bacteria. There is a significant survival benefit of trigger-to-treat medical management along with intravenous fluids, prophylactic antibiotics, blood transfusions, anti-diarrheal drugs, analgesics, and nutrition. Antibiotic use and efficacy are further confounded by the supportive care regimen under study (full or minimal support), or whether a subject-based care (trigger-to-treat) or population-based care (antibiotic administered regardless of symptoms) (*15*), and antibiotic resistance. An ANC of $<500/\mu L$, a sign of severe neutropenia, triggers the administration of enrofloxacin. Depending on persistent fever and if antibiotic resistance occurs, other antibiotics can be used (*16*). Another strategy to reduce cross-contamination is by thorough sanitation and changing the cages often- twice weekly for rabbits, and daily for MPs and NHPs. Sanitization of high-touch areas and floors and correct procedures by staff for entry/exit and use of PPE will also reduce contamination.

Panel Discussion Topic 3: Hydration and Diet

Rodents. In TBI and PBI rodent models, where the head is irradiated, wetted chow or gel packs are sometimes introduced on the day of exposure in anticipation of tooth decay and loss several days/weeks post-exposure. The chow can be wet with normal or acidified water, or if the water contains MCM or antibiotics, that water/drug solution is used to wet the chow. In the rat PBI model, animals are provided powdered food on days 35–70, ahead of tooth loss. For extended oral gavage, a measured amount of drug is fed to rats in pudding to avoid daily tube feeding that can aggravate esophageal radiation damage. For hydration, in both TBI and PBI models, rodents are often provided with chlorinated water. If the radiation dose is above 13 Gy, rats are also provided antibiotics in drinking water (*17*). Subcutaneous administration of saline (4% of body weight) during GI-ARS is another alternate to hydration.

Large Animals. Rabbits and MPs normally receive water in a self-watering system. Juice or Pedialyte can also be administered, which provides an additional means to deliver MCMs. Rabbits, MPs, and NHPs are provided with fresh fruits and vegetables, and rabbits are provided with hay post-irradiation to stimulate GI mobility. Further, NHPs receive a high protein diet rich in medium-chain triglycerides oil if body weight loss exceeds 10% of baseline, and citrus fruits are avoided (*8*).

Panel Discussion Topic 4: Euthanasia Criteria

According to American Veterinary Medical Association (AVMA) guidelines,[5] the goal of having euthanasia criteria is to euthanize animals justifiably and humanely, and to not inflict undue pain and distress. The adoption of criteria decreases inter-study variability and reduces observational bias among assessing personnel making euthanasia determinations. A mouse intervention scoring system can help identify mice meeting the pre-set euthanasia criteria (*18*). These criteria can be refined upon to meet the individual model requirement and the radiation sensitivity of the test species.

Panel Discussion Summary

Additional considerations for housing mice are the issues of loss of littermates, nocturnal monitoring and the

[5] https://www.avma.org/resources-tools/avma-policies/avma-guidelines-euthanasia-animals.

coprophagic nature of rodents. Some potential solutions are pooling of mice in the study, or use of infrared cameras for night observations. If mice are randomized in same cages across study arms, the chances of control mice ingesting excreted MCM and thereby skewing survival is high. Communicating the detailed procedures used in animal housing, husbandry, transport, and irradiation conditions is critical to robust and repeatable experiments. The group consensus emphasized publishing and describing detailed aspects of the models and experiments for reproducibility and harmonization purposes.

One outcome of the discussions on infection control was the recognition that definitions of sepsis in animal studies are not harmonized among study sites. Arriving at a common definition is important. Regarding diet and hydration, the panel was split over the use of gel packs vs. wetted chow as the post-irradiation nutrition source for study animals, primarily mice, since data on the benefit of gel pack is equivocal. Rodent chow wetted with medicated water (containing MCM and antibiotics) enable the rodents to acquire nutrition and medication simultaneously, whereas gel packs are regarded as having reduced nutritional content. One issue with wetted chow is the potential for contamination of the food with fecal material. Seasonal variability in the content of bulk chow and nutritional status of the animal (fasting or fed) can impact study outcome (*19*).

Finally, there are significant challenges in developing international or even inter-institutional harmonized euthanasia criteria. IACUC opinions vary across institutions and protocols. Discussion of select criteria with site veterinarians and IACUC members can help provide the information needed for protocol acceptance. Although there are many factors to consider when designing small or large animal irradiation studies, advanced planning and careful thought can result in robust experiments, which when fully detailed in the literature, can lead to repeatable findings and further strengthen shared knowledge in the research community.

ACKNOWLEDGMENTS

The opinions contained herein are the private views of the authors and do not necessarily represent those of the NIAID/NIH, or BARDA. Many thanks to RNCP/NIAID Director, Andrea DiCarlo, and colleagues David Cassatt, Thomas Winters, and Olivia Molinar-Inglis for their critical review of the manuscript.

Received: December 22, 2021; accepted: July 18, 2022; published online: August 23, 2022

REFERENCES

1. U.S. Food and Drug Administration. Product development under the Animal Rule - Guidance for Industry. Silver Spring, MD: Center for Drug Evaluation and Research, Center for Biologics Evaluation and Research; 2015.
2. Patterson AM, Sellamuthu R, Plett PA, Sampson CH, Chua HL, Fisher A, et al. Establishing pediatric mouse models of the hematopoietic acute radiation syndrome and the delayed effects of acute radiation exposure. Radiat Res. 2021; 195(4):307-23.
3. Plett PA, Sampson CH, Chua HL, Jackson W, Vemula S, Sellamuthu R, et al. The H-ARS dose response relationship (DRR): Validation and variables. Health Phys. 2015; 109(5):391-8.
4. Patterson AM, Plett PA, Chua HL, Sampson CH, Fisher A, Feng H, et al. Development of a model of the acute and delayed effects of high dose radiation exposure in jackson diversity outbred mice; comparison to inbred C57BL/6 mice. Health Phys. 2020; 119(5):633-46.
5. Turturro A, Witt WW, Lewis S, Hass BS, Lipman RD, Hart RW. Growth curves and survival characteristics of the animals used in the biomarkers of aging program. J Gerontol A Biol Sci Med Sci. 1999; 54(11):B492-501.
6. Garrett J, Sampson CH, Plett PA, Crisler R, Parker J, Venezia R, et al. Characterization and etiology of swollen muzzles in irradiated mice. Radiat Res. 2019; 191(1):31-42.
7. Meadows SK, Dressman HK, Muramoto GG, Himburg H, Salter A, Wei Z, et al. Gene expression signatures of radiation response are specific, durable and accurate in mice and humans. PLoS One. 2008; 3(4):e1912.
8. Plett PA, Sampson CH, Chua HL, Joshi M, Booth C, Gough A, et al. Establishing a murine model of the hematopoietic syndrome of the acute radiation syndrome. Health Phys. 2012; 103(4):343-55.
9. Laissue JA, Bally E, Joel DD, Slatkin DN, Stoner RD. Protection of mice from whole-body gamma radiation by deuteration of drinking water. Radiat Res. 1983; 96(1):59-64.
10. DiCarlo AL, Perez Horta Z, Rios CI, Satyamitra MM, Taliaferro LP, Cassatt DR. Study logistics that can impact medical countermeasure efficacy testing in mouse models of radiation injury. Int J Radiat Biol. 2021; 97:S151-S167.
11. Flurkey K, M. Currer J, Harrison DE. Chapter 20 - Mouse models in aging research. In: Fox JG, Davisson MT, Quimby FW, Barthold SW, Newcomer CE, Smith AL, editors. The Mouse in Biomedical Research (Second Edition). Burlington: Academic Press; 2007. p. 637-72.
12. Satyamitra M, Kumar VP, Biswas S, Cary L, Dickson L, Venkataraman S, et al. Impact of abbreviated filgrastim schedule on survival and hematopoietic recovery after irradiation in four mouse strains with different radiosensitivity. Radiat Res. 2017; 187(6):659-71.
13. Jackson IL, Xu PT, Nguyen G, Down JD, Johnson CS, Katz BP, et al. Characterization of the dose response relationship for lung injury following acute radiation exposure in three well-established murine strains: developing an interspecies bridge to link animal models with human lung. Health Phys. 2014; 106(1):48-55.
14. National Research Council (U.S.). Committee for the Update of the Guide for the Care and Use of Laboratory Animals., Institute for Laboratory Animal Research (U.S.), National Academies Press (U.S.). Guide for the care and use of laboratory animals. 8th ed. Washington, D.C.: National Academies Press; 2011. xxv, 220 p. p.
15. Yu JZ, Lindeblad M, Lyubimov A, Neri F, Smith B, Szilagyi E, et al. Subject-based versus population-based care after radiation exposure. Radiat Res. 2015; 184(1):46-55.
16. Farese AM, Cohen MV, Katz BP, Smith CP, Jackson W, 3rd, Cohen DM, et al. A nonhuman primate model of the hematopoietic acute radiation syndrome plus medical management. Health Phys. 2012; 103(4):367-82.
17. Medhora M, Gao F, Gasperetti T, Narayanan J, Khan AH, Jacobs ER, et al. Delayed effects of acute radiation exposure (DEARE) in juvenile and old rats: Mitigation by lisinopril. Health Phys. 2019; 116(4):529-45.
18. Koch A, Gulani J, King G, Hieber K, Chappell M, Ossetrova N. Establishment of early endpoints in mouse total-body irradiation model. PLOS ONE. 2016; 11(8):e0161079.
19. S YA, Kenchegowda D, Holmes-Hampton GP, Moroni M, S PG. Impairment of IGF-1 signaling and antioxidant response are associated with radiation sensitivity and mortality. Int J Mol Sci. 2021; 22(1):451.