Chapter IX: Acute Radiation Effects: Organ Specific, Organs Dose, and Species Differences

Section: J Large Animal Models

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Introduction

For the purpose of this chapter, large animal models of efficacy are defined as all non-rodent species. However, this chapter will focus specifically on non-human primate and swine models. Other models utilizing the canine and ferret have been reported, but have not gained widespread acceptance [1].We will focus on the acute (acute radiation syndrome, ARS) and delayed (delayed effects of acute radiation exposure, DEARE) effect of radiation exposure. In this chapter we have provided a non-exhaustive list of references meant only to introduce the student to the available literature.

FDA Animal Rule and Animal Rule Guidance

Use of animal efficacy models is mandated by the Food and Drug Administration's (FDA) Animal Rule, issued in 2002 [2]. The FDA was clear when the rule is applicable: "This final rule provides for approval of certain new drug and biological products based on animal data when adequate and well-controlled efficacy studies in humans cannot be ethically conducted because the studies would involve administering a potentially lethal or permanently disabling toxic substance or organism to healthy human volunteers and field trials are not feasible prior to approval. The FDA defined how such animal efficacy was to be determined: "The effect is demonstrated in more than one animal species expected to react with a response predictive for humans, unless the effect is demonstrated in a single animal species that represents a sufficiently well-characterized animal model (meaning the model has been adequately evaluated for its responsiveness) for predicting the response in humans." [2] The rule took about a decade to effectively implement but recent years have seen improved usage [3].

In October 2015 the FDA issued a related guidance which detailed what was meant by the Animal Rule [4]. The FDA was clear in this non-binding guidance that an important consideration was the appropriateness of the animal model: "The Animal Rule specifies that the choice of species for the adequate and well-controlled efficacy studies must be appropriate with regard to the disease or condition of interest and the investigational drug [5]. There is no requirement for the use of a specific species. For each animal species selected by sponsors, the sponsors should provide scientific justification that the animal species exhibits key characteristics of the human disease or condition when the animal is exposed to the challenge agent [6]. "In addition, the species should be selected based on an understanding of the drug's mechanism of action, such that the drug's effect in the animal species is expected to be predictive of its effect in humans, and to allow extrapolation from the animal data to the selection of an effective dose and regimen for humans" [4].

While it is clear the Animal Rule does not mandate a large animal efficacy model, it is also evident the FDA will be determining adequate demonstration of efficacy on a case-by-case basis: "Generally, the efficacy of the drug should be demonstrated in more than one animal species expected to react with a response predictive for humans. In certain circumstances, studies in more than two species may be necessary to model the relevant aspects of the human disease or condition and response to the investigational drug" [4].

The FDA has issued news announcements regarding 12 products approved under the Animal Rule [7, 8]:

1. March 18, 2016: FDA approves new treatment for inhalation anthrax (Anthim)
2. November 23, 2015: FDA approves vaccine for use after known or suspected anthrax exposure (BioThrax)
3. November 13, 2015: FDA approves new indication for use of Neulasta (pegfilgrastim) to treat adult and pediatric patients at risk of developing myelosuppression after a radiological/nuclear incident
4. May 8, 2015: FDA approves additional antibacterial treatment for plague (Avelox)
5. March 30, 2015: FDA approves Neupogen® for treatment of patients with radiation-induced myelosuppression following a radiological/nuclear incident
6. March 25, 2015: FDA approves treatment for inhalation anthrax (Anthrasil, Anthrax Immune Globulin Intravenous (Human))
7. February 2, 2015: Ciprofloxacin - supplemental NDA approved to add indication for treatment and prophylaxis of plague due to Yersinia pestis in adults and pediatric patients
8. March 22, 2013: FDA approves first Botulism Antitoxin for use in neutralizing all seven known botulinum nerve toxin serotypes
9. December 12, 2012: FDA approves raxibacumab to treat inhalational anthrax
10. April 27, 2012: FDA approves new antibacterial treatment for plague (levofloxacin)
11. December 15, 2006: FDA approves drug to treat cyanide poisoning (Cyanokit)
12. February 5, 2003: FDA approves pyridostigmine bromide as pretreatment against nerve gas

In all cases a large animal model was used in the demonstration of efficacy. We postulate a continued reliance on at least one large animal model for future Animal Rule approvals. An understanding of large animal models is thus relevant to Animal Rule drug and biologic development.

It should be noted there has been research into replacement technologies for the Animal Rule [9]. Since the Animal Rule emphasizes survival as the critical measure of benefit, “the animal study endpoint is clearly related to the desired benefit in humans, generally the enhancement of survival or prevention of major morbidity”. Animal Rule radiation studies often deal in death – both in treated and control populations. Alternatives to animal testing are attractive. These include in silico, in vitro, and ex vivo (e.g., organ on a chip) [9, 10]. However, the technologies need to be fully developed, qualified, and then accepted by the FDA before sponsors will be able to use them as a basis for drug approval.

Large Animal Models and the Acute Radiation Syndrome (ARS)

Available large animal models include non-human primates, swine, and canines. Other models have been reported, such as ferrets, but have not been widely used [1]. In selecting a large animal model, a sponsor should place emphasis on the FDA admonition: “provide scientific justification that the animal species exhibits key characteristics of the human disease or

condition when the animal is exposed to the challenge agent" [4]. Exposure to a high level of radiation arising from a nuclear detonation (the challenge agent) leads to acute (acute radiation syndrome, ARS), delayed (delayed effects of acute radiation exposure, DEARE) and long term effects [11-13], encompassing all organs and processes in the body. In such a complex biological reaction, one must tailor the animal model to the specific radiation subsyndrome one wishes to address.

Some of the large animal species which have been reported in the literature are listed in Table 1. Rhesus macaques have been used extensively for hematopoietic, lung, and gastro-intestinal studies (see, for example, references 14-19).

Table 1. Large Animal Species and Typical Use

Species	Typical Use
Rhesus Macaque Non Human Primate	Hematopoietic, Gastro-intestinal, Lung
Cynomolgus Macaque Non-Human Primate	Hematopoietic
Beagle Dog	Hematopoietic
Gottingen Minipig	Hematopoietic, Gastro-intestinal, Skin; Lung; vascular injury or coagulopathy
Sinclair Miniature Swine	Skin (cutaneous radiation injury)
Yucatan Miniature Swine	Skin (cutaneous radiation injury)
Hanford Miniature Swine	Skin (cutaneous radiation injury)
Micro-Yucatan Minipig	Skin (cutaneous radiation injury)
Yorkshire Swine	Skin (cutaneous radiation injury)

The FDA is most familiar with the Rhesus radiation injury model and efficacy data in the Rhesus supported approval of Neupogen and Neulasta for treatment of patients with radiation-induced myelosuppression [20]. Although Cynomolgus monkeys have not been widely reported, they offer the practical advantages of lower cost and greater availability. These advantages accrue because Cynos are widely used in large molecule toxicology and safety pharmacology studies [21]. The major disadvantage of Cynos is the lack of a comprehensive scientific literature which demonstrates their radiation injury relevance to humans. Although it is anticipated they will respond in a manner similar to Rhesus, this has not been shown.

Table 2. Comparison of Large Animal Attributes

Model	Typical Weight (Male; Kg)	Approximate Acquisition Cost ($)	Current Domestic Breeding	Availability	Radiation Sensitivity, LD50, TBI
Rhesus	7	2750	No	Constrained	7
Cynomolgus	5	1500	No	Good	Not reported
Göttingen	15	1300	Yes	Single Supplier	2
Yucatan	30	1300	Yes	Excellent	Not reported

Sinclair	20	1300	Yes	Excellent	Not reported
Micro Yucatan	20	1300	Yes	Excellent	Not reported
Yorkshire	40	1300	Yes	Excellent	Not Reported
Hanford	40	1300	Yes	Excellent	Not reported
Beagle	12	750	Yes	Excellent	2-4

The popularity of the minipig in pharmacology, and pharmacokinetic and toxicology safety evaluation studies has increased rapidly in recent years [22]. Pigs are exceptionally well suited for skin studies, as porcine and human skin share critical macro- and microscopic features [23]. Both have a relatively sparse hair coat, and comparable vasculature, lipid composition, biophysical properties, epidermal turnover kinetics, collagen and elastic fibers. Due to these similarities, the pig is an accepted standard model for studies on wound healing, burn lesions and reconstructive surgeries. Furthermore, the minipig has become a standard animal model in dermal toxicity studies submitted to the FDA [24].

Williams et al noted [1]: "The pig is widely used as a large animal model to study the skin effects of radiation exposure. The FDA often encourages experiments in the porcine model in support of the approval of an IND package for dermatological agents". Use of swine in full body (hematopoietic, h-ARS) and organ-specific studies (e.g., gastrointestinal, GI-ARS) have been reported [25-32]. In an inter-laboratory comparison the mini-pig has been shown to be a reproducible model [33].

Subsyndrome Challenges

A reasonably well-accepted Rhesus model of hematopoietic ARS has been developed [1, 34-35] under a full supportive care regimen. Additionally, an h-ARS model in Gottingen mini-pigs under no, or minimal supportive care has appeared in the literature [30]. Both Rhesus and minpig models are described for GI-ARS [30, 36], including a bone marrow sparing model in Rhesus developed by MacVittie [17]. However, competing models are under development, with differing levels of bone marrow sparing [37-40]. It is always important to emphasize the appropriateness of the model as it translates to the human and to an actual incident. It is not clear if a single model for a radiation subsyndrome will ever become the definitive or "gold standard" model because medical countermeasures (MCMs) usually work in different ways (mechanism of action), which implies different probes or models. This concept is emphasized when biomarker are considered.

The armamentarium of available large animal radiation models becomes more sparse as one moves beyond hematopoietic, gastrointestinal, lung and skin. Models for the central nervous system and other internal organs are particularly lacking. Well-characterized models for thrombocytopenia, vascular leakage and coagulopathy are also missing. One may safely say that numerous challenges in large animal model development remain.

Delayed versus Acute Effects: Animal Rule Implications

The animal rule requires demonstration of "enhancement of survival or prevention of major morbidity" [2]. Satisfying this requirement is straightforward, if not easy, for subsyndromes such as hematopoietic, gastrointestinal, and lung where lethality studies can be conveniently designed. For other subsyndromes such as cutaneous radiation injury the situation is murkier. Death is not a likely outcome and major morbidity is not well-defined. As always, sponsors should engage the FDA in discussions regarding the appropriateness of an animal model.

Lethality studies, by themselves, are not particularly informative. Secondary measures of efficacy are desirable. Secondary measures have the added benefit of contributing to one's understanding of the MCM's mechanism of action. The Animal Rule requires a "reasonably well understood pathophysiological mechanism of the toxicity of the substance [i.e., ionizing radiation exposure] and its prevention or substantial reduction by the product" [2]. Thus the challenge is to understand the radiobiology of high level radiation injury at a molecular level and then to demonstrate how the MCM candidate interrupts and repairs the relevant biochemical cascade. Since the relevant large animal radiation models are typically limited to non-human primates and swine, it is important to develop a detailed understanding of the physiology of those two species. Translating to the human is critical for Animal Rule approval. In certain cases, species specificity has prevented a straightforward link between animals and man [41].

Supportive care and large animal models

Supportive care for animals in a radiation study has varied considerably. We will briefly consider three levels of supportive care: none, standard, and full.

Some practitioners have developed study protocols without the provision of supportive care [see, for example, 42]. The impetus behind this practice seems to be the notion that in a mass casualty incident there will initially be a lack of medical supplies. As such, an ARS MCM must by itself maintain health or affect some degree of recovery. Thus, in testing an MCM candidate, the most relevant scenario is one in which the drug must act alone. In addition, demonstrating that an MCM candidate can provide benefit without the need for ancillary treatments is a most compelling argument in favor of the MCM's value. A key consideration in developing a study protocol which doesn't provide supportive care is ensuring that the animals do not suffer. We will address euthanasia criteria in a separate section of this chapter.

Many practitioners prefer a supportive care approach in which standard antibiotics, antidiarrheals, analgesics, and antiemetics are administered [1, 47]. The logic in this case is that these medicines are readily available both in health facilities and pharmacies throughout the country and also in the Strategic National Stockpile (a series of medical warehouses administered by the Centers for Disease Control [43, 44]. In an optimal incident scenario, both

MCMs and standard supportive care supplies will be available to first responders as they treat victims.

Finally, some MCM developers have used a full supportive care regimen [15-19]. In this case blood products are also administered. In full supportive care studies, treatment is generally administered on an as-needed basis. Such a protocol is reflective of the care one would expect to be given to the human victims of an incident, once logistical aspects of the medical response have caught up with the demand [45]. One problem with a full supportive care protocol is that the treatment varies from animal to animal, depending on medical need. A variable protocol can be a confounder when one is trying to demonstrate improvement of a new MCM candidate and may require a higher number of animals on study to control for variability. In addition, the start of full supportive care such as blood transfusions in an animal study may not accurately represent when such treatments would be available in an actual incident. There is some literature indicating that MCMs which are efficacious with full supportive care may not provide the same benefit in the absence of such supportive care [14, 15, 42].

Regardless of the level of supportive care, we recommend a fixed protocol. That is, whatever level of supportive care provided (e.g., analgesics, antibiotics, antiemetics and antidiarrheals, blood products) should be administered to all animals regardless of indication at the same time in the same amount. If supportive care is to be provided based on observation, then once a single subject requires treatment then all study subjects receive the same treatment at the same time. We contend that it is most important to remove known variables in a study protocol. We propose that potentially deleterious effects of unnecessary supportive care are minimal compared to the confounder of variable treatment. Such treatment can lead to questions such as: did the MCM provide benefit or did supportive care? Would the MCM work in the absence of supportive care or with less supportive care? Large animal studies tend to employ a minimal number of animals (see the section on statistics in this chapter) and statistical proof of benefit can often depend on the outcome of only a few study subjects. In such a tenuous circumstance we believe that it is imperative to be as rigorous as possible.

Euthanasia Criteria

There is conflict between adequately powering a study and the cost in doing so. In almost all cases, the number of animals used in a study will be the minimum necessary to deliver a credible outcome [46]. Thus, it is important to ensure that each animal, whether a survivor or a non-survivor, exhibits an outcome free from unintended human interference. However, there is an important and immense obligation on the part of the study institution to ensure that study subjects do not suffer needlessly. The situation is difficult since in many radiation studies, the majority of animals will approach a nadir from which recovery appears unlikely. Nevertheless, in a well designed radiation study, the targeted percentage of survivors (in a control group) can be achieved.

One key to removing inappropriate human intervention as an confounder in radiation lethality studies is to render all euthanasia criteria as objective as possible. Some measures of health,

such as weight loss and body temperature are easily determined. Other criteria, such as distress (pain), are less well-defined. In addition to cross-training all study personnel (veterinary staff) who will be rendering euthanasia decisions, we recommend a step-wise approach to pain management. We base this recommendation on the concept that differences in pain are easier to assess than absolute pain levels. Under this approach, if an animal is observed to be in distress we recommend administration of an analgesic and observation again within a few hours. If no improvement is evident a second, higher dose of the analgesic is given. Again, after an appropriate interval, the animal is observed. If there is still no change in the "soft signs" of distress then the animal may be euthanized per written (e.g., standard operation procedure) criteria.

Dose Optimization

Optimization of the administered candidate MCM dose entails several considerations. These include the dose level, the initiation, frequency and length of dosing, and the route of administration. Adequately addressing all these topics can require several studies, at a considerable cost. Many of these parameters can be determined in a small animal model and then applied to a large animal model using allometric extrapolations [47-49]. This approach is less expensive and time consuming. However, it is advisable to perform at least some confirmatory studies in the large animal model itself.

Usually the route of MCM administration can be determined early on in the drug development process. The route of administration is often mandated by the drug formulation. As a practical matter, if the MCM is to be used at or near the incident site immediately after the event, then an easily performed route is preferred. Such routes include intramuscular, subcutaneous, nasal, sublingual, and oral. Dose level (amount of drug administered) can be estimated from small animal work but it is recommended that at least three dose levels be subject to additional testing in a large animal model. The range of dose levels should encompass at least an order of magnitude.

Initiation of candidate MCM dosing, frequency of dosing, and length (duration) of dosing are intimately intertwined and studies to determine optimal values must be carefully designed. We recommend a design of experiments (DOE) approach to this multi-factorial problem [50-52]. A judicious approach in applying DOE principals is required. The DOE formalism arose in the engineering realm in which animal issues were not involved. As always in animal research, one must carefully balance statistical validity and animal use limitations.

An additional parameter to be evaluated is the radiation dose level. In this chapter we will not address issues such as the quality of the radiation (e.g., linear accelerator vs. radioisotope source) or radiation dose rate. However, the total radiation dose administered is a parameter which requires investigation. All MCMs will have a characteristic range of exposures over which their use will be of benefit. This therapeutic range will vary by MCM and by the ARS subsyndrome. It is important to understand this parameter as it will inform real-world use after an incident. For total body irradiations (e.g. hematopoietic ARS) we recommend at least three

radiation doses, equally spaced in the linear portion of the S-shaped lethality curve. That is, LD30, LD50, and LD70. Good data at these three points will allow calculation of the dose modification factor (DMF), which is a commonly used measure of treatment merit [53]. Once again, the interplay between statistical validity, budget, and animal use will influence the study design.

Statistics

Powering of animal efficacy studies is a controversial topic, however evaluation of a candidate MCM under a well powered study design is critical to appropriately demonstrating efficacy. Depending on the level of survival or symptom improvement expected this may require 40 or more animals in each study group [47, 50]. When one adds in non-pivotal studies which investigate the effects of (1) radiation dose, (2) amount of drug administered, (3) administration schedule and (4) supportive care regimen then several hundred animals may be required. The expense of such studies often leads to compromises in study design. To the extent possible it is desirable to use a homogeneous cohort. Controlling for variables such as differences in weight, age, health, history and genetic makeup can support the use of fewer animals. As mentioned elsewhere a fixed protocol which provides the same supportive care to all animals also helps to reduce variability, improving the chances of a definitive study outcome.

Additional Testing

Phase IV (post marketing) commitments are to be expected for radiation MCMs [54]. It is anticipated these studies will often employ large animal models. Such studies will certainly include drug-drug interactions. For example, since Neupogen is now stocked in the SNS, it is reasonable to presume that any follow-on radiation MCMs will need to be tested in a subject cohort similarly treated with Neupogen.

Special populations (at risk individuals) have been a top concern of the Office of the Assistant Secretary for Preparedness and Response [55]. Indeed, the Secretary may give priority to development of qualified MCMs that are likely to be safe and effective with respect to children, pregnant women, elderly, and other at-risk individuals [56]. Thus, one should anticipate having to eventually deal with the problem of creating and using a pediatric large animal model.

Natural History

As indicated previously, we will not address in detail the long-term effects of acute radiation exposure. Extensive work has been done to understand the long-term effects of lower levels of radiation exposure in beagle dogs [57]. These early studies were focused on potential radiation exposure due to fall out after a nuclear detonation, rather than that due to an acute exposure. For example, continuous gamma ray exposure from a cobalt-60 source at Argonne National Laboratory in 1968 used a maximum dose rate of 26cGy/day [57, page 172]. Currently, most NHP acute radiation studies are conducted with a dose rate of 60-80 cGy/minute. That is, most current radiation work is aimed at understanding the effect of a dose from prompt gamma

emission. Thus, a full understanding of the natural history of an acute radiation exposure of several Gray due to gamma rays in a large animal model has not been performed [58]. Such a study represents a major opportunity and a major challenge, particularly since such a study in non-human primates could conceivably last three decades. In order to adequately perform such a study an expensive, lengthy commitment by the US Government would be required.

Government Priorities

The Pandemic and All-Hazards Preparedness Act of 2006, which created the Biomedical Advanced Research and Development Authority (BARDA), specified that among the duties of advanced research, development, and procurement of qualified medical countermeasures was the design and development of tests or models, including animal models [59]. In 2011 BARDA established a Nonclinical Development Network and facilitate development and qualification of animal models [60]. The National Institutes of Health (NIH) maintains a similar capability [61] and recently awarded a new five year contract [62]. Thus, there is continued interest at both BARDA and NIH in supporting animal efficacy models related to radiation exposure.

These networks can develop well-characterized animal models which can then be used by MCM developers. Ideally, since such a government-developed model is well understood, the model itself will not be a subject of concern when submissions are made to the FDA. However, as mentioned above, animal models may or may not generalize from one MCM candidate to another. It is important to determine early in the drug development process whether an existing model is applicable for a new MCM candidate. If not, then a sponsor may need to approach the government regarding funding to support a new or revised model. As we have mentioned, the only two widely used models are non-human primates and swine. Importantly, there are still numerous variables related to these two general models which could significantly affect the adequacy and appropriateness of a specific model.

Concept of Operations (CONOPS)

The government has given considerable thought to the response to a nuclear incident [63]. This analysis explicitly acknowledges that many resources will not be available in sufficient amounts during the first hours and days following an incident. MCMs may be used without supportive care and standard supportive care may be given without MCMs. Thus, CONOPS has a bearing on large animal models in that the details of the model will vary depending on where and when the MCM will be employed. Some MCMs may be used at or near the incident site in the first few days after the detonation. Such “field use” [64-66] MCMs will need to have certain characteristics. These include a good therapeutic index (safe to use in individuals who did not actually receive a significant radiation dose), ease of administration (both route and preparation), facile deployment (light with small footprint; stable for an extended period at elevated temperatures such as those of a hot summer day), and affordable (several hundred thousand units may be needed). For field use MCMs, a large animal protocol can accommodate the beginning of drug treatment as soon as 24 hours post-radiation exposure. Field use MCMs will likely be administered to most anyone who meets minimal diagnostics (location when blast

occurred, prodomal symptoms). Thus, efficacy will need to be demonstrated over a wide range of radiation exposures, perhaps one to ten Gray.

In contrast, MCMs which are targeted to administration in a medical facility will need a different set of attributes. The most challenging characteristic will be the ability to demonstrate efficacy starting at four (or so) days post-exposure and continued efficacy if the first dose is not administered for up to 7 to 14 days. This requirement is extremely challenging from a medical perspective, since some subsyndromes of ARS (hematopoietic, gastrointestinal) will begin exhibiting serious, if not life threatening, outcomes in that same time frame [64]. Other requirements are relaxed, compared to field use. For example, the drug may be more difficult to administer (e.g., slow infusion), require cold temperature storage and lengthy preparation before use. The drug may also be more costly since fewer units will be needed. It is presumed that within several days the biodosimetry diagnostics currently under development will come to fruition and that the exposures of most patients will be known [67-69]. This will serve to accurately select patients who are in need of additional treatment. The "definitive care" MCM can also be used in conjunction with ancillaries not available in quantity at the incident site such as blood transfusions. In designing large animal radiation models for definitive care products one will need to demonstrate efficacy with much delayed drug administration. However, a full supportive care plan can and should be part of the protocol design.

Summary

In this chapter we have endeavored to highlight the complexity of developing and using a large animal efficacy model of acute radiation exposure. Because the Animal Rule is a relatively new regulatory pathway there are few well-characterized models. Even the best developed models have limitations and are subject to controversy regarding some of their attributes. A practitioner in this area usually has the dual challenge of satisfying the FDA and the government funding agencies. Nevertheless, the need for approved MCMs is great. In 2010, President Barack Obama warned that "nuclear terrorism is the gravest threat to global security" [70]. And just this year the President stated that the prospect of Isis or other terrorists getting hold of a nuclear bomb is among the most serious threats faced by the world [71]. We concur with these thoughts and encourage additional development of useful large animal models for acute radiation exposure.

References

1. Williams JP, Brown SL, Georges G E, Hauer-Jensen M, Hill RP, Huser AK, Kirsch DG, MacVittie TJ, Mason KA, Medhora MM, Moulder JE, Okunieff P, Otterson MF, Robbins ME, Smathers JB, McBride WH. Animal models for medical countermeasures to radiation exposure. Radiat Res 173: 557–578, 2010.
2. New Drug and Biological Drug Products; Evidence Needed to Demonstrate Effectiveness of New Drugs When Human Efficacy Studies Are Not Ethical or Feasible, Federal Register, Vol. 67, No. 105, Friday, May 31, 2002, Rules and Regulations, page 37988.
3. National Biodefense Science Board. *Where Are The Countermeasures? Protecting America's Health from CBRN Threats: A Report Of The National Biodefense Science Board*, 2010. http://www.phe.gov/Preparedness/legal/boards/nprsb/meetings /Documents/nbsb-mcmreport.pdf
4. Product Development Under the Animal Rule: Guidance for Industry, October 2015, http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm399217.pdf
5. See 21 CFR 314.610(a) for drugs and 21 CFR 601.91(a) for biological products.
6. As used in the FDA guidance, the term *challenge agent* refers to the substance used to cause the disease or condition in the animal studies, whereas the term *etiologic agent* refers to the substance causing the disease or condition in humans.
7. http://www.fda.gov/emergencypreparedness/counterterrorism/medicalcountermeasures/mcmregulatoryscience/ucm391604.htm
8. Aebersold P. FDA experience with medical countermeasures under the Animal Rule. Adv Prev Med 2012, 507571. http://doi.org/10.1155/2012/507571
9. *Animal Models for Assessing Countermeasures to Bioterrorism Agents*, National Academies Press, ISBN: 0309219094, 9780309219099; 2011.
10. *Nuclear Terrorism and National Preparedness*, edited by S Apikyan, D Diamond. IN: NATO Science for Peace and Security Series B: Physics and Biophysics, Springer, ISBN 9401798915, 9789401798914; 2015.
11. Walker RI, Cerveny RJ, eds. *Medical Consequences of Nuclear Warfare*. Falls Church, VA: Office of the Surgeon General; 1989. Available athttp://www.afrri.usuhs.mil.
12. *Management of Terrorist Events Involving Radioactive Material*. NCRP Report No. 138. Bethesda, MD: National Council on Radiation Protection and Measurements, 125-34; 2001.
13. Mettler FA Jr, Upton AC. *Medical Effects of Ionizing Radiation*, 2nd ed. Philadelphia: WB Saunders; 1995.
14. Farese AM, Cohen MV, Stead RB, Jackson W 3rd, MacVittie TJ. Pegfilgrastim administered in an abbreviated schedule, significantly improved Neutrophil recovery after high-dose radiation-induced myelosuppression in rhesus macaques. Radiat Res 178: 403-413, 2012.
15. Farese AM, Cohen MV, Katz BP, Smith CP, Gibbs A, Cohen DM, MacVittie TJ. Filgrastim improves survival in lethally irradiated nonhuman primates. Radiat Res 179: 89-100, 2013.
16. MacVittie TJ, Bennett A, Booth C, Garofalo M, Tudor G, Ward A, Shea-Donohue T, Gelfond D, McFarland E, Jackson W 3rd, Lu W, Farese AM. The prolonged gastrointestinal syndrome in rhesus macaques: The relationship between gastrointestinal, hematopoietic, and delayed multi-

organ sequelae following acute, potentially lethal, partial-body irradiation. Health Phys 103: 427-453, 2012.
17. MacVittie TJ, Farese AM, Bennett A, Gelfond D, Shea-Donohue T, Tudor G, Booth C, McFarland E, Jackson W 3rd. The acute gastrointestinal subsyndrome of the acute radiation syndrome: A rhesus macaque model. Health Phys 103: 411-426, 2012.
18. Garofalo M, Bennett A, Farese AM, Ward A, Taylor-Howell C, Cui W, Gibbs A, Lasio G, Jackson W 3rd, MacVittie TJ. The delayed pulmonary syndrome following acute high-dose irradiation: A rhesus macaque model. Health Phys 106: 56-72, 2014.
19. Garofalo MC, Ward AA, Farese AM, Bennett A, Taylor-Howell C, Cui W, Gibbs A, Prado KL, MacVittie TJ. A pilot study in rhesus macaques to assess the treatment efficacy of a small molecular weight catalytic metalloporphyrin antioxidant (AEOL 10150) in mitigating radiation-induced lung damage. Health Phys 106: 73-83, 2014.
20. FDA Advisory Committee Briefing Document: A Joint Meeting of the Medical Imaging Drugs Advisory Committee and the Oncologic Drugs Advisory Committee *May 3, 2013, Safety and Efficacy of Currently Approved Leukocyte Growth Factors (LGFs) as Potential Treatments for Radiation-induced Myelosuppression Associated with a Radiological/Nuclear Incident*, http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/MedicalImagingDrugsAdvisoryCommittee/UCM350151.pdf
21. *Animal Models in Toxicology,* Third Edition, edited by SC Gad, CRC Press, ISBN 9781466554290; 2016.
22. Forster R, Bode G, Ellegaard L, van der Laan JW. The RETHINK project: minipigs as models for the toxicity testing of new medicines and chemicals: an impact assessment. J. Pharmacol. Toxicol. Meth. 2010; 62:158-159.
23. Swindle MM, Makin A, Herron AJ, Clubb FJ Jr, Fraizier KS. (2012). Swine as models of research and toxicology testing. Veterinary Pathology 49(2):344-356.
24. Ganderup NC, Harvey W, Mortensen JT, Harrouk W. The minipig as nonrodent species in toxicology – where are we now? Int J Toxicol 31: 507-528, 2012.
25. Moroni M, Port M, Koch A, Gulani J, Meineke V, Abend M. Significance of bioindicators to predict survival in irradiated minipigs. Health Phys 106: 727–733, 2014.
26. Haruna J, Wierzbicki W, Pouliot M, Bassett L, Ascah A, Authier S. *Characterization of a Minipig Model of Gastrointestinal Acute Radiation Syndrome Using Total Body Irradiation and Partial Body Irradiation: A Focus on Intestinal Pathology*. Poster, Radiation Research Society, 2014.
27. Moroni M, Ngudiankama BF, Christensen C, Olsen CH, Owens R, Lombardini ED, Holt RK, Whitnall MH. The Göttingen minipig is a model of the hematopoietic acute radiation syndrome: G-CSF stimulates hematopoiesis and enhances survival from lethal total-body gamma-irradiation. Int J Radiat Oncol Biol Phys 86: 986–992, 2013.
28. Elliott TB, Deutz NE, Gulani J, Koch A, Olsen CH, Christensen C, Chappell M, Whitnall MH, Moroni M. Gastrointestinal acute radiation syndrome in Gottingen minipigs (Sus scrofa domsticus). Comp Med 64: 456-63, 2014.
29. Moroni M, Coolbaugh TV, Lombardini E, Mitchell JM, Moccia KD, Shelton LJ, Nagy V, Whitnall MH. Hematopoietic radiation syndrome in the Gottingen minipig. Radiat Res 176: 89-101, 2011.
30. Moroni M, Elliott TB, Deutz N, Olsen CH, Owens R, Whitnall, MH. Accelerated hematopoietic syndrome after radiation doses bridging hematopoietic (H-ARS) and gastrointestinal (GI-ARS)

acute radiation syndrome: Early hematological changes and systemic inflammatory response syndrome in minipig. Int J Radiat Biol 90: 363-372, 2014.
31. Thrall KD, Lovaglio J, Murphy MK, Cataneo RN, Chaturvedi A, Mundada M, Patel U, Phillips M. A dose-dependent hematological evaluation of whole-body gamma-irradiation in the Gottingen minipig. Health Phys 105: 245-252, 2013.
32. Singh VK, Thrall KD, Hauer-Jensen M. Minipigs as models in drug discovery. Expert Opin Drug Discov 2016 Aug 22: 1-4 (Epub ahead of print].
33. Esker J. *Development Of Models Of Acute Radiation Syndrome In Göttingen Minipigs.* Presentation at BARDA Industry Day, October 16, 2015; https://www.medicalcountermeasures.gov/media/36856/esker_radnuc-bid-2015-508-compliant-slides-v2-508.pdf.
34. Farese AM, Cohen MV, Katz BP, Smith CP, Jackson W 3rd, Cohen DM, MacVittie TJ. A nonhuman primate model of the hematopoietic acute radiation syndrome plus medical management. Health Phys 103: 367-382, 2012.
35. Thrall KD, Love R, O'Donnell KC, Farese AM, Manning R, MacVittie TJ. An interlaboratory validation of the radiation dose response relationship (DRR) for H-ARS in the rhesus macaque. Health Phys 109: 502–510, 2015.
36. Whitnall and M. Moroni (2014). Gastrointestinal acute radiation syndrome in Gottingen minipigs (*Sus scrofa domesticus*). Comparative Medicine 64(6): 456-463.
37. Esker J, Moyer B, Raulli R, Grace M, Homer M, Weber W, Doyle-Eisele M, Melo D, Guilmette R, Thrall K, Lovaglio J, Moroni M, Bartholomew A, Lindeblad M, Lyubimov A.: *Development of a Large Animal Model of h-ARS in Göttingen Minipigs Under Minimal Supportive Care: Natural History and Biomarker Results for Radiation Injury from Multiple Institutions using a Harmonized Model. Demonstration of Reproducibility for Regulatory Acceptance;* poster at Annual Meeting of the Radiation Research Society, September 2014.
38. Esker J, Moyer B, Raulli R, Grace M, Weber W, Doyle-Eisele M, Melo D, Guilmette R, Thrall K, Lovaglio J, Barthalomew A, Lindeblad M, Lyubimov A. *Development of a Large Animal Model of h-ARS in Göttingen Minipigs Under Minimal Supportive Care: Results from Three Institutions and Progress Toward Establishing a Harmonized Model for Regulatory Acceptance*; poster at Annual Meeting of the Radiation Research Society, September 2013.
39. Esker J, Moyer B, Raulli R, Homer M. *Animal Model Development of Gastrointestinal-Acute Radiation Syndrome (GI-ARS) in Minipigs: Approaches to Model Development, and Harmonization*; poster at Annual Meeting of the Radiation Research Society, October 2015.
40. Vigneulle RM, Rao S, Fasano A, MacVittie TJ. Structural and functional alternations in the gastrointestinal tract following radiation-induced injury in the rhesus monkey. Dig Dis Sci 47: 1480-1491, 2002.
41. Sun H, Tsai Y, Nowak I, Liesveld J, Chen Y. Eltrombopag, A thrombopoietin receptor agonist, enhances human umbilical cord blood hematopoietic stem/primitive progenitor cell expansion and promotes multi-lineage hematopoiesis. Stem Cell Res 9: 77–86, 2012.
42. Gluzman-Poltorak Z, Vainstein V, Basile LA. Recombinant interleukin-12, but not granulocyte-colony stimulating factor, improves survival in lethally irradiated nonhuman primates in the absence of supportive care: Evidence for the development of a frontline radiation medical countermeasure. Am J Hematol 89: 868–873, 2014.
43. https://www.medicalcountermeasures.gov/phemce/cdc.aspx

44. http://www.cdc.gov/phpr/stockpile/stockpile.htm .
45. Coleman CN, Adams S, Adrianopoli C, Ansari A, Bader JL, Buddemeier B, Caro JJ, Casagrande R, Case C Jr, Caspary K, Chang AS, Chang HF, Chao N, Cliffer KD, Confer D, Deitchman S, DeRenzo EG, Dobbs A, Dodgen D, Donnelly EH, Gorman S, Grace MB, Hatchett R, Hick JL, Hrdina C, Jones R, Kane E, Knebel A, Koerner JF, Laffan AM, Larson L, Livinski A, MacKinney J, Maidment BW, Manning R, Marinissen MJ, Martin C, Michael G, Miller CW, Murrain-Hill P, Nemhauser JB, Norwood AE, Nystrom S, Raheem M, Redlener I, Sheehan K, Simon SL, Taylor TP, Toner E, Wallace KS, Weinstock DM, Whitcomb RC Jr, Wieder J, Wiley AL Jr, Yeskey K. *Medical planning and response for a nuclear detonation: A practical guide*. Biosecur Bioterror 10: 346-371, 2012.
46. Kodell RL, Lensing SY, Landes RD, Kumar KS, Hauer-Jensen M. Determination of sample sizes for demonstrating efficacy of radiation countermeasures. Biometrics 66: 239-248, 2010.
47. Moyer BR. *Symposium 10: Translating from the Laboratory to the Patient: Advanced Development Requirements – The Animal Rule and a Clinical Indication*, Annual Meeting of the Radiation Research Society, http://www.brmassocllc-org.com/RadRes_Symposium10_MOYER_FINAL.pdf September 2015.
48. *Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers*, http://www.fda.gov/downloads/drugs/guidances/ucm078932.pdf, July, 2005.
49. Duffus JH, Worth HGJ. *Fundamental Toxicology*, Royal Society of Chemistry, ISBN: 0854046143, 9780854046140; 2006.
50. Festing MF, Altman DG. Guidelines for the design and statistical analysis of experiments using laboratory animals. ILAR J (Institute for Laboratory Animal Research) 43: 244-258, 2002.
51. Oehlert GW. *A first course in design and analysis of experiments* ISBN 0-7167-3510-5; 2010.
52. Myers RH, Montgomery DC, Anderson-Cook CM. *Response Surface Methodology: Process and Product Optimization Using Designed Experiments*, 4th Edition, ISBN: 978-1-118-91603-2, 856 pages, Wiley; 2016.
53. Suntharalingam N. *Chapter 14 BASIC RADIOBIOLOGY - Nuclear Sciences and Applications*, www-naweb.iaea.org/nahu; International Atomic Energy Agency.
54. http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Post-marketingPhaseIVCommitments/
55. ASPR Strategic Plan, February 2014, http://www.phe.gov/about/aspr/strategic-plan2014/Documents/stratplan-2014.pdf
56. Progress Report on the Implementation of Provisions Addressing At-Risk Individuals, August 2008; http://www.phe.gov/Preparedness/legal/pahpa/Documents/pahpa-at-risk-report0901.pdf .
57. Thompson R, *Life Span Effects of Ionizing Radiation in the Beagle Dog*, Pacific Northwest Laboratory report PNL-6822, 323 pages, 1989.
58. Goans RE, Flynn DF, *Medical Consequences of Radiological and Nuclear Weapons*, Chapter 2, Acute Radiation Syndrome In Humans; 2013, http://www.cs.amedd.army.mil/borden/filedownloadpublic.aspx?docid=423f63d0-dc11-4ab1-99f2-31a5b47cc5ca .
59. http://www.phe.gov/Preparedness/legal/pahpa/pages/default.aspx
60. https://www.medicalcountermeasures.gov/barda/core-services.aspx

61. *NIH Strategic Plan and Research Agenda for Medical Countermeasures against Radiological and Nuclear Threats*, June 2005, https://www.niaid.nih.gov/sites/default/files/documents/radnucstrategicplan.pdf
62. https://www.sri.com/newsroom/press-releases/sri-international-awarded-100-million-niaid-contract-develop-treatments .
63. *Planning Guidance for Response to a Nuclear Detonation*, Second Edition, June 2010, https://www.remm.nlm.gov/PlanningGuidanceNuclearDetonation.pdf
64. See the Radiation Emergency Medical Management website: https://www.remm.nlm.gov/ .
65. https://www.medicalcountermeasures.gov/media/14697/22_bid_ron_manning.pdf
66. Berger ME, Christensen DM, Lowry PC, Jones OW, Wiley AL. Medical management of radiation injuries: Current approaches. Occup Med 56: 162-172, 2006.
67. https://www.medicalcountermeasures.gov/barda/cbrn/cbrn-diagnostics-and-biodosimetry.aspx .
68. Wallace R. *Radiation Diagnostic Tools in Development at the Biomedical Advanced Research and Development Authority*", http://www.nationalacademies.org/hmd/~/media/76DA2A6ABD1B423C822E4BEE6FEF15D3.ashx; 2012.
69. Manning R. *Emergency Medical Countermeasures Development and Acquisition: BARDA's Role and Biodosimetry*, https://justnet.org/pdf/5_Manning.ppt; 2007.
70. http://www.telegraph.co.uk/news/worldnews/northamerica/usa/7580210/Nuclear-terrorism-is-gravest-threat-to-global-security-Barack-Obama-warns.html .
71. http://www.independent.co.uk/news/world/politics/isis-nuclear-bomb-is-a-serious-threat-warns-barack-obama-a6964621.html .