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Recent advances in medical countermeasure development against acute radiation exposure based on the US FDA animal rule

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Abstract

Recent advances in medical countermeasures (MCMs) has been dependent on the Food and Drug Administration (FDA) animal rule (AR) and the final guidance document provided for industry on product development. The criteria outlined therein establish the path for approval under the AR. The guidance document, along with the funding and requirements from the federal agencies provided the basic considerations for animal model development in assessing radiation effects and efficacy against the potential lethal effects of acute radiation injury and the delayed effects of acute exposure. Animal models, essential for determining MCM efficacy, were developed and validated to assess organ-specific, potentially lethal, radiation effects against the gastrointestinal (GI) and hematopoietic acute radiation syndrome (H-ARS), and radiation-induced delayed effects to lung and associated comorbidities of prolonged immune suppression, GI, kidney and heart injury. Partial-body irradiation models where marginal bone marrow was spared resulted in the ability to evaluate the concomitant evolution of multiple organ injury in the acute and delayed effects in survivors of acute radiation exposure. There are no MCMs for prophylaxis against the major sequelae of the ARS or the delayed effects of acute exposure. Also lacking are MCMs that will mitigate the GI ARS consequent to potentially lethal exposure from a terrorist event or major radiation accident. Additionally, the gap in countermeasures for prophylaxis may extend to mixed neutron/gamma radiation if current modelling predicts prompt exposure from an improvised nuclear device. However, progress in the field of MCM development has been made due to federal and corporate funding, clarification of

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the critical criteria for efficacy within the FDA AR and the concomitant development and validation of additional animal models. These models provided for a strategic and tactical approach to determine radiation effects and MCM efficacy.

Keywords: radiation, medical countermeasures, FDA animal rule, animal model

1. Medical countermeasure (MCM) development against acute and delayed effects of acute radiation exposure (DEARE)

The development of MCM against the effects of radiation, chemotherapeutic drugs and organ-specific disease processes on normal tissues has been ongoing for decades. The potential for nuclear terrorism, military conflict or large sale scale radiation accidents focused renewed efforts to develop MCM against the effects of potentially lethal, acute radiation exposure, as well as prolonged effects due to fallout and cutaneous radiation-induced injury. This objective, to develop MCM that would increase survival from potentially lethal doses of radiation, introduced the federal funding agencies, the Food and Drug Administration (FDA) and corporate sponsors of MCM development to the study of acute, high-dose radiation effects and the concomitant multi-organ injury (MOI) of the acute radiation syndrome (ARS) and the delayed effects of acute radiation exposure (DEARE). The consequent development of the FDA 'animal rule' (AR), funding agency requirements relative to the context of the post nuclear radiation environment and relevant animal models to assess the efficacy of new or repurposed MCM outlined the many variables, hurdles and gaps in knowledge along the critical path of MCM development toward FDA approval.

1.1. The FDA AR for MCM approval

The requirements for FDA approval of MCM to treat personnel against the ARS and/or DEARE are framed by the guidance document that provides critical information and recommendations on MCM development when human efficacy studies are not ethical or feasible. 'The Animal Rule states that the FDA will rely on evidence from animal studies to provide substantial evidence of effectiveness only when all of the following four criteria are met:

(a) The animal model must be well characterized and the mechanism of action of radiation on the specific organ system, as well as how the respective MCM affects that mechanism must be reasonably well understood.
(b) The effect is demonstrated in more than one animal species expected to react to radiation with a response predictive for the human response to radiation and its treatment.
(c) The experimental endpoint is clearly related to the desired benefit in humans, generally the enhancement of survival or prevention of major morbidity.
(d) The pharmacokinetics and pharmacodynamics of the MCM or other relevant information in the animal models and humans allows selection of an effective MCM dose in humans.

If these criteria are met, it is reasonable to expect the effectiveness of the drug in animals to be a reliable indicator of its effectiveness in humans' (US Food and Drug Administration 2015).

The FDA guidance document also provides information on essential elements of animal models that are required to establish a critical path toward approval of the MCM. These elements are underscored with additional consideration of essential elements of the radiobiology and tissue-specific effects of the dose- and time-dependent MOI within the ARS and DEARE within the significant species- and strain-dependent radiation effects.

1.2. Animal models and the FDA AR

MCM are focused on prophylaxis, mitigation and therapeutics against acute, potentially lethal, radiation-induced, organ-specific injuries to the bone marrow, gastrointestinal (GI) system and lung. Radiation-induced injury to the kidney and heart have been defined in response to high-dose, acute radiation exposure but not in the context of lethal dose response relationships (DRRs) in the nonhuman primate (NHP). These survivable, dose- and time-dependent sequelae are predominant within the ARS and the DEARE. While these sequelae form organ-specific sub-syndromes, they evolve within the context of concomitant MOI defined as prolonged GI damage and severe immune suppression, delayed injury to the vascular system, kidney and heart, as well as the potential for combined cutaneous radiation injury and associated co-morbidities (Farese *et al* 2012, 2013, Macvittie *et al* 2012a, 2012b, 2014, de Faria *et al* 2015, Plett *et al* 2015, Unthank *et al* 2015, 2019, Fish *et al* 2016, 2020, Cohen *et al* 2017, 2020, Chua *et al* 2019, Jacobs *et al* 2019, Parker *et al* 2019a, 2019b, Miller *et al* 2020). The clinical definition and natural history of the MOI concomitant with the evolution of the ARS and DEARE required validated animal models that mimic the human response to potentially lethal radiation and treatment.

1.3. Where are the MCMs?

The question is focused on the modest development of approved MCM under criteria of the US FDA AR, published in 2002 (US Food and Drug Administration 2002). The final *Guidance for Industry on Product Development Under the Animal Rule* was published in 2015 (US Food and Drug Administration 2015). Additional, critical considerations were recommended by the federal funding agencies based on knowledge of the radiation exposure environment, timely triage and dosimetry, and patient care consequent to a large- scale nuclear terrorist event. The research community is still reacting to the multitude of variables defined by criteria associated with drug development, relevant small and large animal models, study design relative to organ-specific and all-cause mortality, the potential link between acute and delayed effects, validated biomarker analysis and adequate funding.

1.4. Current status

There are four MCM approved via the FDA AR for use to increase survival in personnel exposed to acute, myelosuppressive doses of radiation. These are Neupogen® (filgrastim), Neulasta® (pegfilgrastim), Leukine® and Nplate® (romiplostim, Amgen Inc. 2015a, 2015b, Partner Therapeutics Inc. 2018, 2021). The leucocyte growth factors (LGF), Neupogen, Neulasta and Leukine were approved based on the common mechanism of enhancing recovery of neutrophils, thereby preventing infection and sepsis. Nplate's approval was based on its ability to hasten platelet recovery, thereby mitigating severe thrombocytopenia and haemorrhage. The four MCMs significantly increased survival relative to respective controls in pivotal trials conducted with validated models in rhesus macaques of total-body irradiation (TBI). Efficacy

was assessed when the MCM were administered at least 24 h post exposure through the MCM-specific route and schedule. It is important to recognise that these MCM are not radioprotective and are limited to mitigation against the hematopoietic (H)-ARS. Also, they do not protect or mitigate against other potentially lethal ARS or DEARE sequelae, e.g. the GI-ARS, prolonged immune suppression, and lung, kidney or heart injury characteristic of the DEARE. Thus, there are marked gaps in MCM development for radioprotectants against lethality from all major sequelae and for mitigation against the lethal sequelae of the GI-ARS and DEARE. Singh and Seed and colleagues have provided a cogent series of articles focused on the status of MCMs, strategies for further MCM development and the respective use of animal models (Seed 2015, Singh and Olabisi 2017, Singh and Seed 2017a, Singh *et al* 2017b, 2019). These articles present detailed reviews of prolonged MCM development that underscores the difficulty in solving the issues presented by potentially lethal exposure in the context of a nuclear event.

2. Continued effort to define the critical path to efficacy and approval

The growth factors previously mentioned were considered the most obvious pharmaceuticals to be submitted for approval as MCMs under the FDA AR, due to the substantial, multi-species preclinical database and extensive clinical database demonstrating efficacy and safety. Consequently, the result is that there are four MCM available for mitigating the lethal H-ARS for both severe neutropenia and thrombocytopenia. Unfortunately, there are no MCM against lethal GI-ARS or delayed effects, characterised by lung, kidney or heart injury. A substantial number of potential MCM have been assessed under the criteria utilised by the FDA and the respective funding agencies with no additional approval by the FDA.

The route to successful approval for ARS for new MCM or repurposed drugs is to understand the rules established by the FDA and the funding agencies considered within the context of potentially lethal exposure in a nuclear terrorist event. There are several hurdles along the critical path to approval under the AR and funding agency considerations. In addition to the four criteria enumerated by the AR, the following variables must be addressed: (a) the differential radiation effect on species, mouse strain and sex, (b) the variable route and schedule of administration to include the stress of handling and anaesthesia relative to small and large animals and (c) efficacy determined by prophylaxis and/or mitigation of clinically relevant parameters that significantly enhance survival and/or mitigation of key signs of major organ-specific morbidity when administered at least 24 h post exposure.

2.1. The timeline for MCM approval under the FDA AR

It is important to recognise the time required for FDA approval of the leucocyte and platelet GF's noted above. Years of preclinical development by the corporate sponsors and collaborative research sites were required. Many experimental efficacy studies were conducted over more than a decade in small and large animal models of sublethal, radiation-induced myelosuppression in multiple species, mice, rats, canines and NHPs (Lord *et al* 1989, Schuening *et al* 1989, Tanikawa *et al* 1989, Macvittie *et al* 1990, 2005, Patchen *et al* 1990, Farese *et al* 1996, Neelis *et al* 1997, Herodin *et al* 2007, Fish *et al* 2016). The LGFs were subsequently FDA-approved for clinical use in 1991 (Neupogen, Leukine) and 2002 (Neulasta) to mitigate cytotoxic therapy-induced myelosuppression. World-wide use in a multitude of patients had proven them to be efficacious and safe in the clinic.

It is of interest that thrombopoietin, the physiologic regulator of platelet production, remains an investigational drug as of 2001. The efficacy of thrombopoietin was shown to significantly enhance platelet production in small and large animal models of radiation-induced myelosuppression and thrombocytopenia. It was shown efficacious as a single agent and when used in combination with granulocyte colony stimulating factor (G-CSF) (Farese *et al* 1996, Neelis *et al* 1997). More recent development of thrombopoietin receptor agonists, Eltrombopag and Romiplostim was initiated in 2008.

Following the 2001 terrorist event in the United States, the enhanced threat of nuclear terrorism changed the course of MCM development. The sequential evolution of these events to include publication of the FDA AR in 2002, set the stage for the predominant LGFs to clear the final hurdles. There were many other potential MCM, such as growth factors, cytokines and biologics, with a similar history of successful preclinical studies yet to falter for various reasons. The FDA AR requirement for efficacy defined as an increase in survival and consideration of initial treatment conditions and schedule—must be able to show efficacy when administered at least 24 h post lethal exposure—in the context of the nuclear terrorist event created a series of critical variables relative to the radiation effect and MCM mechanism of action in a requisite small and large animal species.

The LGFs, due to the large database on mechanism of action, treatment schedule and efficacy in animal models and the clinic were considered ideal candidates for MCM approval under the FDA AR. Their clinical efficacy and safety had been validated for decades. However, they now faced the most difficult criteria for efficacy; the third component of the AR: *the experimental endpoint is clearly related to the desired benefit in humans, generally the enhancement of survival or prevention of major morbidity*. The LGFs were approved for clinical use based on significant mitigation of myelosuppression. They were not approved for clinical use based on animal data that showed a significant increase in survival after whole-body radiation doses that were lethal for at least 50% of the control cohort.

The AR was approved in 2002 following consideration that MCM would likely be used to treat potentially lethally irradiated personnel consequent to a terrorist event. Neupogen and Neulasta were FDA-approved under the AR in March and November of 2015, respectively and Leukine-approval followed in March 2018. Each MCM was approved based on pivotal efficacy studies performed using lethal models of the H-ARS in rhesus macaques (Farese *et al* 2013, Hankey *et al* 2015, Amgen 2015a, 2015b, Clayton *et al* 2016, Partner Therapeutics 2018, Zhong *et al* 2020). Although these LGFs were approved for clinical use in the treatment of myelosuppressed patients, gaining FDA-approval for the treatment of potentially lethally irradiated personnel, occurred 13 years after the publication of the FDA AR. Similarly, Nplate, Romiplostim, gained FDA approval in 2008 for mitigating idiopathic thrombocytopenia purpura however, subsequent approval for the treatment of potentially lethally irradiated personnel via the FDA AR did not follow until 2021.

2.2. The FDA AR and other critical criteria, requisite for MCM efficacy. The context of the post nuclear exposure environment

The following requisites set the path for efficacy and approval of MCM to be approved and administered in the context of the post exposure environment: (a) the ability to significantly enhance survival, given dose- and time-dependent mortality in animal models, (b) the radiation exposure environment that strictly limits the initial time to triage and treatment for many personnel, (c) schedule of administration post exposure in an animal model, (d) route of administration, (e) a stable pharmacokinetic and pharmacodynamic for the MCM relative to the route

of and concurrent, organ-specific sequelae in the irradiated animal, (f) considered radiation exposure geometry and likely differential dose distribution relative to survival and organ injury and (g) the use of medical management post exposure.

2.3. The critical hurdles in MCM development

2.3.1. Continued advancement in MCM development requires a clear focus on severalcriteria identified within the FDA AR. These are the animal model(s), dose- and time-dependent organ-specific injury, achieving statistically significant improvement in survival and the continued use of multiple species and strains. Each animal species must establish mortality and DRRs relative to constructing the model that defines the relevant organ-specific injury to assess MCM efficacy. A cautionary note, 'an animal model is only an animal model', underscored the difficulty in establishing the predictive validity of multi-species and strain-dependent animal models irradiated using variable exposure protocols. To date, only a single predominant exposure model that requires an organ-specific DRR, has been used to assess organ-specific radiation effects and efficacy under the criteria of the FDA AR. Specifically, it is uniform, bilateral, TBI to assess H-ARS mortality in the NHP, rhesus macaque.

Several models and exposure protocols have been used to show MCM efficacy in small and large animals. These are: unilateral, nonuniform TBI, whole thorax lung irradiation (WTLI) and bilateral, partial-body exposure with bone marrow sparing. Published DRRs have not been established for unilateral, nonuniform TBI using a moderate dose rate for small animals or the NHP. A pulse dose rate was used to establish a DRR for mixed neutron/gamma radiations from a nuclear reactor several decades ago (Turbyfill *et al* 1968, Wise and Turbyfill 1968). A nuclear test site series was also reported for two cohorts of rhesus macaques exposed to prompt mixed neutron/gamma radiation (Zellmer and Pickering 1960). Pulse rate exposures to mimic a nuclear detonation have not been reported on since Operation Plumbbob. The respective LD50/60 were estimates at 395 and 403 rad. A limited data set was used to estimate the LD50/60 in response to uniform TBI with Co-60 radiation at 800 cGy min^{-1}. The LD50/60 was estimated at approximate 438 cGy midline tissue dose (Allen *et al* 1960). Additional exposure protocols using nonuniform, unilateral TBI were established to assess the efficacy of potential MCM, as well as mimic the nonuniform exposure from a nuclear terrorist event. Unfortunately the exposure protocols did not establish respective DRRs and only used a single radiation dose at a moderate dose rate to assess MCM efficacy against the H-ARS (Chapel *et al* 2003, Drouet *et al* 2004, 2008). The partial-body exposure with variable BM-sparing and variable dose rate has been used to evaluate MCM efficacy in several protocols using Co-60 gamma radiation (Monroy *et al* 1988, Bertho *et al* 2005a, 2005b). Monroy *et al* used a bilateral, partial body exposure model at a high dose rate of 500–735 cGy min^{-1} to expose NHP to 800 cGy with variable shielding sparing the partial iliac crest, femora and tibiae. However, only a single series of DRRs were developed for GI- and H-ARS as well as DEARE-lung and MOI at a moderate dose rate using the PBI/BM-sparing protocol (Macvittie *et al* 2012a, 2015b, 2019, Farese *et al* 2019). The FDA's Division of Imaging and Radiation Medicine is responsible for reviewing MCM efficacy under the FDA AR and have approved models of uniform TBI and PBI/BM-sparing to assess pivotal MCM efficacy.

A critical variable that introduced yet another set of hurdles (concerns) is the markedly different radiation sensitivity of species and strain. The multiplicities of these factors lessen the number of available, validated models since established DRRs that define the mortality/dose relationship are minimal. It is of interest that a workshop on animal models for MCM recommended, '…that every laboratory establish the lethal dose-response relationship for each of

its strains at least twice a year. Additionally, it is also appropriate to design each study testing agent efficacy using radiation doses across the hematopoietic syndrome, …' (Williams *et al* 2010).

2.3.2. TBI, WTLI and PBI/BM-sparing protocols. Uniform TBI does not mimic the likely exposure geometry in a nuclear terrorist event. The threshold dose range (9–11 Gy) required for the GI-ARS and delayed lung injury characteristic of the DEARE, does not allow survival for the valued analysis of prolonged GI injury, immune suppression or lung injury due to the concomitant, 100% lethal H-ARS. Furthermore, the WTLI model is focused on radiation exposure to the lung and heart but negates exposure to the major volume of bone marrow and the GI system, thus eliminates the ARS. Additionally, the WTLI model due to its selected organ-focus may lack predictive validity relative to the nuclear scenario. The FDA has projected concern that the WTLI model does not provide a valid exposure geometry for a nuclear event and is thus marginalised relative to the conduct of pivotal studies to assess MCM efficacy against lethal lung injury (Laniyonu and Marzella 2018). The FDA's Division of Imaging and Radiation Medicine has indicated that 'PBI/BM5 is a superior model to WTLI given its allowance for full evolution of DEARI-lung within the context of multi organ injury'.

2.3.3. PBI/BM-sparing protocols. PBI/BM-sparing protocols were developed to provide relevant animal models, small and large, that allowed focus on all three, potentially lethal, organ-specific sequelae, the GI-, H-ARS and lung-DEARE, during the 180 d in-life study duration (Booth *et al* 2012a, 2015, Macvittie *et al* 2012a, 2019, Fish *et al* 2016, 2020, Farese *et al* 2019, Accardi *et al* 2020). The PBI/BM-sparing models also permitted analysis of the concomitant prolonged GI injury and cellular and functional immune recovery, as well as kidney and heart injury (Cohen *et al* 2017, 2019, de Faria *et al* 2015, Macvittie *et al* 2012a). The animal model research platform provides essential information for interpreting and defining the complex interrelationships in clinically relevant MOI models of the human response to potentially lethal irradiation and treatment.

2.3.4. Added value of respective protocols; PBI/BM-sparing and high-dose, uniform TBI survivors. The PBI/BM-sparing models allowed analysis of concurrent, potentially lethal MOI of the GI- and H-ARS and associated early immune suppression, kidney injury and concomitant co-morbidities. Furthermore, the MOI of the ARS occurred during the latent period characteristic of the dose- and time-dependent MOI of the DEARE. The most predominant injury within the DEARE is the characteristic radiation-induced pneumonitis and fibrosis in lung injury and associated species- and strain-dependent incidence and severity of kidney and heart injury. Note, that the MOI of the DEARE is concomitant with continued prolonged immune suppression and GI injury (Macvittie *et al* 2012a, 2014, Booth *et al* 2012a, 2015, de Faria *et al* 2015, Fish *et al* 2016, Cohen *et al* 2017, 2019, Medhora *et al* 2019, Jacobs *et al* 2019).

2.3.5. Time of administration, combined MCM. The attendant MOI within both the ARS and DEARE allows the efficacy testing of organ specific MCM on potential influential effects on other organ injury. Additionally, the efficacy testing of combined, organ specific MCMs, on early survival due to the ARS sequelae will allow assessment of deleterious or positive effects on the latency, incidence and severity of the DEARE. The time of administration of MCMs against the DEARE is a critical question that continues to be evaluated. Specifically, if MCMs

against the ARS are administered early post exposure, is it possible to delay administration of a MCM against the DEARE, until a more delayed, selected trigger point for intervention? This may increase the MCM utility and logistics with a more efficient combined treatment efficacy. Additional questions can be asked relative to the effect of the early administration of MCMs against the ARS on the latency, incidence, severity of the DEARE.

The analysis of MOI after MCM administration are described in the following PBI models. A PBI/BM model used 'leg-out' BM-sparing in the WAG/RijCmer rat strain that enabled definition of the concurrent ARS and DEARE, e.g. the GI-, H-ARS and lung, kidney and cardiac injury (Fish *et al* 2016). The PBI/BM-sparing model allowed single and combined analysis of delayed administration of a potential MCM, lisinopril, to mitigate lung, kidney injury, cardiac remodeling and additional combined treatment of lisinopril and G-CSF, to include early efficacy against the H-ARS. The effect of organ-specific LGFs, Neupogen and Neulasta, were investigated in NHP models of PBI/BM-sparing to assess mitigation of potentially lethal H-ARS and evidence of the LGF's on the latency, incidence and severity of delayed lung and kidney injury (Macvittie *et al* 2015b, 2019, Cohen *et al* 2019, Farese *et al* 2019).

2.3.6. Murine high-dose, H-ARS survivors: MOI at <10 Gy TBI. TBI is the established model for the direct approach to ascertain MCM efficacy against the lethal H- and GI-ARS. Recent advances have been made through a more strategic approach that investigates both the durability of delayed radiation effects and efficacy of MCM in survivors of the H-ARS. The use of medical management in combination with approved MCM will increase survival from the potentially lethal H-ARS. Orschell and colleagues have expanded the value of the TBI model by investigating the prolonged, residual hematopoietic stem cell injury and delayed MOI of cohorts that survived the high-lethal effects of the H-ARS (Chua *et al* 2012, 2014, Unthank *et al* 2015). This strategic approach defined marked impairment of hematopoietic stem cell function to produce multi-lineage reconstitution, prolonged, skewed recovery of the immune system and the development of significant delayed effects in lung, kidney and cardiovascular injury in the heart in long-term survivors of the H-ARS. The approach to determining the role of MCM against the H-ARS evolved to defining the durability of long-term recovery of hematopoietic stem cell function, the latency and incidence of modified dose- and time-dependent thresholds for the MOI of the DEARE and long-term survival effects.

2.3.7. A continued challenge; the ARS and DEARE, animal models, species and strain differences. The systematic approach to understand acute and delayed radiation effects of acute exposure relative to MCM development and utility in the context of the nuclear exposure environment, required as noted above, a different set of questions. The ability to address the challenge of efficacy for varied MCMs is exacerbated data obtained in multiple mouse and rat strains and a single NHP, the rhesus macaque. MCM efficacy evaluation under the criteria of the FDA AR requires validated animal models relative to TBI and PBI/BM-sparing.

The published database relative to animal models required for efficacy testing provided an organised view of species-, strain, organ sequelae-, time-, radiation quality- and dose-dependent MOI within the ARS and DEARE relative to established exposure protocols. The comparative value of multi-species data sets is dependent on the established DRR and natural history from each well-characterised model. The DRR provides the relative dose-dependent values for mortality, the slope for the DRR and characteristic LD50 or any LD value for comparison of the equivalent biological effect, e.g. organ-specific sequelae and MOI.

The persistent challenge is the ability for movement among research sites that lack published, peer-reviewed, validated models for TBI-induced GI- and/or H-ARS and PBI/BM-sparing protocols to assess the MOI of the ARS and DEARE. The available research sites and qualified investigators have developed 'models' or exposure protocols in different animal species, strains, sex, radiation quality and exposure geometry. There are currently only four research sites that have published models for PBI/BM-sparing exposure protocols in the mouse and rat, and NHP (Macvittie *et al* 2012a, Booth *et al* 2012b, 2015, Fish *et al* 2016, 2020, Accardi *et al* 2020).

2.3.8. Model constraints relative to small and large animal species. An established DRR within species, strain, sex, and age necessitates the knowledge and control of several factors critical for a well-characterised model; e.g. radiation physics, veterinary conditions, Institutional Animal and Care Use Committee criteria for euthanasia and blood volume limitations for assays, animal behaviour, MCM route and schedule of administration, and medical management. Knowledge of these conditions permits relevant assessment of MCM efficacy and extrapolation to the NHP and human database (Plett *et al* 2012, 2015, Fish *et al* 2016). The route and schedule of MCM administration, e.g. oral or intravenous for long durations, will cause considerable design problems for small animal models. Stress to the animal may result in increased morbidity and mortality and shift the DRR. Small animals also limit the use of medical management that will be used in NHP models and considered as standard of treatment for humans relative to the context of use. Medical management shifts the DRR to the right and will be the standard of care relative to context of use and enhanced MCM efficacy (Taketa 1962, Macvittie *et al* 1991, 2005, Plett *et al* 2012, 2015, Booth *et al* 2012b, Yu *et al* 2015).

2.3.9. Model constraints, acute, pulse rate, mixed neuron/gamma radiation exposure, the ARS and DEARE. The renewed effort to model the radiation exposure environment consequent to the prompt exposure from an improvised nuclear device has suggested that survival form blast and thermal effects is possible in an urban environment (Kramer *et al* 2016). The research community is lacking small and large animal models of unilateral, nonuniform, mixed neuron/gamma, pulse dose rate, exposure protocols and validated DRRs for the ARS and DEARE (Macvittie *et al* 2020a). Several variables are critical to relevant model development, these are, (a) the neutron energy, (b) the neutron/gamma ratio, (c) the depth dose consequent to the unilateral, nonuniform exposure, (d) the dose delivered to critical organs, (e) variable exposure geometry to include partial-body exposure with marginal bone marrow sparing, determination of the DEARE, (f) relative biologic effect and (g) biomarkers for organ involvement.

The research database for NHP consists predominantly of male, rhesus macaque, *Macaca mulatta*. This fact has been an advantage relative to validation and accumulation of a large database on required models, respective DRRs for each model, varied radiation quality and organ subsyndromes, use of medical management to mimic human clinical support, differential dose distribution to organ volume, and a comparison of the hematopoietic syndrome to human radiation accident cases (Doerr *et al* 2014, Graessle *et al* 2015, Macvittie *et al* 2015a, 2020a, 2020b). A potential concern is the lack of research studies that have used female rhesus. Another concern is that there is a marginal database relative to the rhesus in the less commonly studied cynomolgus macaque. Therefore, additional model development and validation would be required for them to join the rhesus in enhancing the effort in MCM approval. The focus on the rhesus has created a potential dilemma, if a national research program required use of all available rhesus due to sex and age, the research using rhesus macaque NHP for MCM

development would halt or be significantly reduced and require consideration of expanding model development to include the cynomolgus macaque.

3. Animal model consolidation

A cohesive approach to animal models, small and large, is required to marginalise the use of animals, especially the NHP. This will reduce cost and effort and increase research efficiency. The current effort to identify established and validated NHP models has taken the form of evidence-based reviews. The reviews have focused on models of the ARS and DEARE that include critical variables such as radiation source, nuclear exposure context, FDA consideration, exposure geometry, species of rhesus macaque and medical management. The reviews have provided the context for assessing lethality within an established DRR relative to exposure by, (a) predominant LINAC-derived photons and ^{60}Co gamma radiation and use of medical management as population-based or subject-based care (Farese *et al* 2012, Yu *et al* 2015, Singh *et al* 2017b, Thrall *et al* 2020, Beach *et al* 2021) and (b) mixed gamma/neutron radiations via unilateral reactor-based pulse rate exposure and prompt nuclear weapon exposure (MacVittie *et al* 2015a, 2020b). Other reviews have focused on, (a) the comparative analysis of lung injury, the predominant, lethal sequelae characteristic of the delayed effects of acute exposure relative to exposure geometry (MacVittie *et al* 2020b), (b) the evidenced-based comparative analysis of the H-ARS dose response and myelosuppression between the macaque species, *M. mulatta* and *Macaca fasicularis* (Farese *et al* 2021b) and (c) the natural history of the MOI within the lethal H-ARS (Farese *et al* 2021a). The extent of these published, evidence-based reviews has provided considerable, validated information for the conduct of focused research toward MCM development and approval under the criteria of the FDA AR and federal funding agencies.

4. Knowledge gaps

4.1. The continued advancement of MCM development and FDA approval under the criteria of the AR will require a strategic approach to close the critical gaps in knowledge

There remain clear gaps in knowledge relative to the *in vivo* effects of acute radiation exposure on the MOI of the ARS and DEARE. These are focused on:

(a) The durability of organ-specific effects on the ARS and DEARE. Unless we know the progression and duration of organ-specific effects, we do not know the 'true effect' and therefore do not know the 'true' DRR, as well as the 'true efficacy' of a selected MCM. Defining the 'true duration' of an organ injury may result in several time-dependent descriptive LD values, e.g. LD50/60 LD50/180, LD50/250, etc. To this end, we need to know the natural history of the organ-specific injury, i.e. the latency, incidence, severity, progression AND resolution post exposure, (b) can key signs of morbidity predict clinical outcome and satisfy the key criteria of the FDA AR? To date, the use of key signs of morbidity has not been shown to be predictive of MCM efficacy. A critical definition of the natural history of organ-specific sequelae my support the use of keys signs of morbidity, (c) what is the optimal model(s) for early efficacy and pivotal efficacy trials for FDA approval? PBI/BM, Unilateral, non-uniform exposure, other? Should models of unilateral exposure be considered? These non-uniform models will support consideration of prompt exposure of mixed neutron/gamma radiations from an IND, (d) do we need to know the 'true effect' of organ injury for appropriate study design to assess 'true efficacy' of MCMs. In this context, will the longer study duration, required to assess the

true effect of selected organ injury, reveal other lethal, radiation-induced organ injury to the kidney, heart? (e) There are no validated FDA-approved biomarkers for DEARE. Does the MOI of the ARS, manifest during the latent or early active phase of DEARE effect definition of valid biomarkers? Does the administration of MCM against the ARS affect biomarker definition for DEARE? (f) Radiation effect scenario. If prompt exposure is relevant to the nuclear radiation effect scenario, then, additional research (acute and delayed effects, MOI, biodosimetry, biomarkers, organ dose, MCM efficacy) is required relative to non-uniform, unilateral or partial-body exposure consequent to pulsed, mixed neutron:gamma dose and ratio of mixed neutron:gamma and neutron energy (Kramer *et al* 2016). (g) The database for acute radiation-induced ARS and the use of MCM are marginal in the cynomolgus macaque. Model development is required for the ARS and DEARE sequelae.

5. Conclusions

Recent advances in MCM development require validated animal models, small and large. The lack of new MCM are due to the strict requirements of the FDA AR and those of the federal funding agencies relative to the treatment schedule within the nuclear terrorist environment. Recent advances point to the continued development of animal models that permit strategic and tactical approaches to assessing MCM efficacy. Well characterised mouse, rat or NHP models that have established DRRs, relative to the context of use, i.e. (a) characterise the animal response to acute radiation exposure; (b) ensure consistent radiation physics and prescribed dose delivery; (c) have clearly defined primary, secondary and tertiary endpoints; (d) established natural history over the study duration to include latency, incidence, severity and progression of organ-specific sequelae and trigger points for pathophysiology and intervention and/or treatment, will provide a comparative small and large animal database to support MCM development predictive of human radiation effects and treatment.

The FDA AR and guidance document is critical for approval of MCM that prevent and/or mitigate the lethal consequences of acute radiation exposure ‘when human efficacy studies are not ethical’ (US Food and Drug Administration 2002, 2015). Success in the efficient use of the FDA Guidance document, linked to federal funding agency requirements adherent to the context of the nuclear exposure environment is dependent on all criteria noted above. The efficient use of effort, funds and animals is dependent on knowledge of the field of radiation effects and MCMs relative to the focused effort to utilise the most relevant animal models in concert with recommendations of the FDA’s Division of Imaging and Radiation Medicine.

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