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Source: Radiation Research, 176(1)

Published By: Radiation Research Society

URL: <https://doi.org/10.1667/RR0L01.1>

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# Medical Countermeasures for Platelet Regeneration after Radiation Exposure. Report of a Workshop and Guided Discussion Sponsored by the National Institute of Allergy and Infectious Diseases, Bethesda, MD, March 22–23, 2010

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DiCarlo, A. L., Poncz, M., Cassatt, D. R., Shah, J. R., Czarniecki, C. W. and Maidment, B. W. Medical Countermeasures for Platelet Regeneration after Radiation Exposure. Report of a Workshop and Guided Discussion Sponsored by the National Institute of Allergy and Infectious Diseases, Bethesda, MD, March 22–23, 2010. *Radiat. Res.* 176, e0001–e0015 (2011).

The events of September 11, 2001 and their aftermath increased awareness of the need to develop medical countermeasures (MCMs) to treat potential health consequences of a radiation accident or deliberate attack. The medical effects of lethal exposures to ionizing radiation have been well described and affect multiple organ systems. To date, much of the research to develop treatments for mitigation of radiation-induced hematopoietic damage has focused on amelioration of radiation-induced neutropenia, which has long been considered to be the primary factor in determining survival after an unintentional radiation exposure. Consistent with historical data, recent studies have highlighted the role that radiation-induced thrombocytopenia plays in radiation mortality, yet development of MCMs to mitigate radiation damage to the megakaryocyte lineage has lagged behind anti-neutropenia approaches. To address this gap and to foster research in the area of platelet regeneration after radiation exposure, the National Institute of Allergy and Infectious Diseases (NIAID) sponsored a workshop on March 22–23, 2010 to encourage collaborations between NIAID program awardees and companies developing pro-platelet approaches. NIAID also organized an informal, open discussion between academic investigators, product development contractors, and representatives from the U.S. Food and Drug Administration (FDA) and other relevant government agencies about drug development toward FDA licensure of products for an acute radiation syndrome indication. Specific emphasis was placed on the challenges of product licensure for radiation/nuclear MCMs using current FDA regulations (21 CFR Parts 314 and 601) and on the

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importance of animal efficacy model development, design of pivotal protocols, and standardization of irradiation and animal supportive care. © 2011 by Radiation Research Society

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## INTRODUCTION

The Department of Health and Human Services (HHS) is charged with protecting civilian populations by providing leadership in research, development, acquisition, deployment and use of effective medical countermeasures (MCMs) for treatment of injury caused by weapons of mass destruction. This includes the development and procurement of drugs to treat injuries resulting from radiation exposure from a radiological/nuclear accident or incident. HHS has assigned the National Institutes of Health (NIH) to identify, characterize and develop new MCMs against injury caused by a radiological/nuclear attack. On behalf of the NIH, the Division of Allergy and Infectious Diseases (DAIT), National Institute of Allergy and Infectious Diseases (NIAID) is charged with implementing this research and development agenda. A robust research and development program, initiated in 2005, supports the development of many MCMs to treat radiation injury as well as associated diagnostic/radiation dose triage tools.

Acute radiation syndrome (ARS) is caused by exposure of most or all of the body to high-dose radiation in a relatively short period. Depending on the level of radiation exposure, severe neutropenia and thrombocytopenia can result, increasing the risk of death due to opportunistic infections and/or hemorrhage. Both conditions are likely to be major contributors to mortality in untreated individuals; however, little recent work had been done to develop drugs for this indication that target the megakaryocytic lineage. To address this aspect of radiation damage, NIAID has

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**TABLE 1**  
**Invited Workshop Speakers and Areas of Expertise<sup>a</sup>**

Name	Affiliation	Expertise
Amelia Bartholomew, M.D.	University of Illinois, Chicago	Nonhuman primate models of radiation, bone marrow microenvironment
David Cassatt, Ph.D.	DAIT, NIAID	Drug development, immunology, radiation biology
Connie Erickson-Miller, Ph.D.	GlaxoSmithKline	Hematopoietic stem cell biology and megakaryopoiesis
George Georges, M.D.	Fred Hutchinson Cancer Research Center	Hematopoietic cell transplantation, large animal model
Holger Karsunky, Ph.D.	Cellerant Therapeutics	Stem cell biology, preclinical development of cell therapies
Thomas MacVittie, Ph.D.	University of Maryland School of Medicine	Radiation biology, non-human primates
Ronald Manning, Ph.D.	BARDA	Radiochemistry, analytical chemistry, small molecule drug development
James Palis, Ph.D.	University of Rochester	Erythroid and megakaryocyte differentiation
Mortimer Poncz, M.D.	University of Pennsylvania	Megakaryocyte and platelet biology, inhibitors of megakaryopoiesis
Shahin Rafii, M.D.	Weill Cornell Medical College	Stem cell differentiation, angiogenic factors, hematopoietic niche
Kathleen Rodgers, Ph.D.	University of Southern California & US Biotest, Inc.	Angiotensin, thrombocytopenia, radiomitigation, hematopoietic factor
Jui Shah, Ph.D.	DAIT, NIAID	Regulatory affairs, pharmacology, toxicology
Yingfei Wei, Ph.D.	3SBio, Inc.	Drug development, thrombopoietin
C. Ryan Yates, Ph.D.	RxBio, Inc. & University of Tennessee Health Science Center	Combined injury, radioprotector/mitigator drug discovery and development

<sup>a</sup> Invited speakers were given the opportunity to comment on the meeting report before its submission.

funded a portfolio of grants to develop MCMs to mitigate/treat radiation-induced thrombocytopenia and enhance survival. Supported research also includes mechanism of action (MoA) studies for a number of MCM candidates, because such data will be required for licensure of an MCM as a mitigator of radiation-induced platelet depletion by the U.S. Food and Drug Administration (FDA) under current regulations: 21 CFR Part 314 Subpart I – (Approval of New Drugs When Human Efficacy Studies Are Not Ethical or Feasible) and 21CFR Part 601 – Subpart H – (Approval of Biological Products When Human Efficacy Studies Are Not Ethical or Feasible) (7). These regulations are commonly referred to as FDA's Animal Rule.

The NIAID Radiation Countermeasures Program held a workshop on March 22–23, 2010 to bring together representatives from U.S. Government (USG) agencies with researchers who are developing countermeasures and animal models to evaluate approaches to enhance regeneration of platelets after radiation exposure. Scientific presenters included both academic and industry investigators (Table 1). The purpose of the meeting was to (1) allow scientists to update relevant government program staff on their progress in developing MCMs, (2) promote collaborations across the disciplines of radiation biology and megakaryopoiesis, (3) identify any gaps in funded research, and (4) provide a forum for an open, informal discussion between researchers and representatives from USG funding and regulatory agencies, who have been tasked with accelerating the development of MCMs for FDA licensure and potential stockpiling. USG panelists represented during the NIAID-guided discussion that

followed the presentations included representatives from the Radiation/Nuclear Countermeasures Program, NIAID; the Office of Regulatory Affairs, NIAID; the Office of Counterterrorism and Emergency Coordination (OCTEC), Center for Drugs Evaluation and Research (CDER), and the Center for Biologics Evaluation and Research (CBER), FDA; the Biomedical Advanced Research and Development Authority (BARDA), HHS; and the NIAID-funded, advanced product development contract held by the University of Maryland School of Medicine. Several of these panelists also gave talks in the opening session of the meeting (Table 1). The main goals of the discussion session were to (1) clarify issues regarding potential paths forward for FDA licensure of products for ARS indication, (2) encourage discussion about the challenges involved in development of NIAID-funded (and other) MCMs to minimize thrombocytopenia and enhance survival after unintentional radiation exposure, and (3) provide general research guidance to all investigators developing drugs to address radiation-induced thrombocytopenia. This meeting report provides information primarily on approaches currently funded by the NIAID for this indication and is therefore not intended to be an exhaustive listing of all compounds that have promise in the treatment of radiation-induced thrombocytopenia in an accident or threat scenario.

## BACKGROUND

In addition to both published and pre-publication data on the feasibility of a number of approaches to

promote platelet regeneration after exposure to radiation, presentations also focused on the MoA of radiation damage to the hematopoietic compartment and specifically the megakaryocyte lineage as well as the development of animal models that would be representative of expected human responses. Presentation summaries are provided below, along with justifications for addressing amelioration of radiation-induced thrombocytopenia as a means to increase survival after radiation exposure. When pre-publication data are referenced, the name of the presenter is provided in parentheses.

### *Radiation Damage to the Bone Marrow*

Among the most important MCMs are those that will treat or mitigate the hematopoietic component of ARS, since blood-forming cells in the bone marrow are normally the most sensitive to radiation damage. Radiation exposures of only a few Gy can cause substantial damage to the bone marrow, which can have dramatic effects on circulating blood cells, including platelets, neutrophils, lymphocytes and erythrocytes. Platelets play an essential role in hemostasis and thrombosis. As the level of circulating platelets drops, the risk of catastrophic hemorrhage increases. Severe thrombocytopenia contributes to mortality after radiation exposure; however, there are no approved therapeutic drugs currently in the Strategic National Stockpile (SNS) for this radiation-induced complication.

### *Platelet Biology*

Platelets arise from committed, terminally differentiated hematopoietic cells called megakaryocytes (2), which share a common precursor with the red cell lineage (3) and possess highly homologous major cytokines [erythropoietin for red cells and thrombopoietin (TPO) for megakaryocytes] (4, 5). Megakaryocytes initially arise in an osteoblastic niche and then traverse to a vascular niche (6) before releasing large cytoplasmic fragments into the blood (7) that then give rise to platelets. It takes about a week for an immature megakaryocyte progenitor cell to undergo this final differentiation and platelet release (2), with each megakaryocyte capable of shedding up to  $10^2$  platelets (8). Platelets circulate for about 10 days in humans (9).

After irradiation, megakaryocytes remigrate to the osteoblastic niche (10), and it appears that terminally differentiated megakaryocytes are partially resistant to the effects of the radiation (11). Depending on the degree of radiation damage, a drop in platelet count can be observed in less than a week and nadir at 1.5 weeks, with recovery again affected by the type, dose and duration of radiation exposure (12, 13). Platelet counts under  $150,000/\text{mm}^3$  are termed thrombocytopenia. Bleeding complications depend on the degree of

thrombocytopenia, the ability of an individual's platelets to stop bleeding, and the degree of related injury such as vascular damage. Severe thrombocytopenia causes spontaneous small capillary hemorrhages called petechiae and larger bruises called purpura. Mucosal bleeding including nose and gum as well as catastrophic gastrointestinal or intracranial bleeding can occur, the latter being potentially life threatening.

Although local pressure measures can control some bleeds, platelet transfusions are often needed. This measure can be supportive and effective, but platelet product has a short shelf life of a few days and cannot be stockpiled in a frozen state like red blood cells. Moreover, the best-prepared product requires apheresis. This process is lengthy, and it then takes days to weeks for an individual to be fully recovered and available again as a platelet donor. Alternative therapies include recombinant activated Factor VII (14), or a synthetic vasopressin, desmopressin, which enhances platelet function (15), although neither of these treatments is currently approved for an ARS indication. These are often secondary support systems and are less effective than giving platelets. Each also has additional limitations. For example, activated Factor VII is expensive, and it needs to be given intravenously every few hours, whereas desmopressin can cause water intoxication and also is effective for only 1–2 days, after which time the body is unable to respond to this stimulus.

### *Current Status of Mitigators for Radiation-Induced Thrombocytopenia*

Several MCMs are being developed for the management of certain aspects of hematopoietic injury caused by radiation; however, if a radiation incident were to occur tomorrow, the only therapies currently available to patients who experience this form of radiation-induced thrombocytopenia would be platelet concentrates or fresh whole blood transfusions, which represent only a stopgap measure. In small accidents, supportive transfusion is the standard of care; however, logistical requirements to provide transfusions to large numbers of victims after a mass casualty incident are great, and emergency preparedness experts believe that an effective pharmacologic therapy that mitigates or treats radiation-induced thrombocytopenia would offer dramatic advantages. Therefore, NIAID is providing funding to advance the development of drugs that can be easily administered (e.g., preferred routes of delivery are oral, subcutaneous, intramuscular or intranasal). Due to the challenges involved in providing MCMs in the wake of a mass casualty incident, drugs under study are optimized to be given a minimum of 24 h after an otherwise-lethal radiation exposure. In addition, it will be critical to establish an acceptable safety profile of the MCM in healthy subjects, since in the real life scenario, it may not

be possible to separate exposed individuals from unexposed individuals, and the MCM may be administered to healthy individuals who were not exposed to harmful levels of radiation.

#### *Radiation Effects on the Platelet Lineage – Clinical and Preclinical Evidence*

Radiation exposure impacts the hematopoietic system in a number of different ways, including well-documented declines in peripheral blood lymphocyte counts, disruption of the bone marrow niche, and damage to stem and progenitor cells (16). Damage and/or death of these cells results in a decline in the circulating levels of several cell types, including neutrophils and platelets. Neutropenia after radiation exposure has long been considered to be the primary factor leading to mortality; however, historical anecdotal accounts of the aftermath of the 1945 atomic bombings of Hiroshima and Nagasaki highlight the prevalence of platelet/clotting problems (e.g., petechiae and bruising, ecchymoses, gingival, uterine and other organ bleeding) in the immediate survivors (17). In addition, recent animal data suggest that the depletion of platelets plays an important role in radiation-induced death. For example, Stickney *et al.* showed that the severity and duration of platelet loss was a better indicator of eventual survival in rhesus monkeys than neutrophil counts (18).

#### *Animal Models for Hematopoietic ARS*

Licensing pathways provided by the FDA under 21 CFR Parts 314 and 601 as well as the 2009 FDA draft guidance on Animal Rule model development (19) are likely to be used in the licensing of any MCM for mitigation of injury from exposure to lethal radiation. It is therefore critical that appropriate animal models be developed and validated for their relevance to humans to address the injuries caused by radiation exposure to the hematopoietic compartment as well as to other organs. In addition, it must be demonstrated that the mechanism of radiation-induced injury (or specifically, thrombocytopenia) in each selected animal model is the same as that in humans. A number of different models are currently under development to study radiation-induced bone marrow damage and document efficacy of new MCMs (20). For hematopoietic ARS, neutropenia and thrombocytopenia lead to infection and hemorrhage, respectively; therefore, an important component of the treatment of hematopoietic ARS is animal supportive care, in which infection and hemorrhage are treated, and which also includes replacement of lost fluids and nutrient support (21). The goal of antibiotic supportive care is to control bacteremia caused by the intestinal translocation of endogenous gram negative bacteria while preserving gut anaerobic bacteria and is generally

accomplished using antibiotic regimens that include fluoroquinolones (21, 22).

Because of the inherent limitations in providing medical management for mice due to their size, supported murine models of radiation damage to the bone marrow are generally straightforward and are reasonably well established; however, advances in supportive care for larger animals such as dogs and NHPs has led to the need to re-establish mortality curves across a range of radiation exposures. For example, researchers at the Fred Hutchinson Cancer Research Center are developing updated canine models of total-body irradiation with full supportive care for hematopoietic ARS, since dose-response curves for radiation lethality generated previously were carried out with lower levels of medical support (G. Georges). Investigators at the University of Maryland School of Medicine are conducting similar radiation dose-finding studies in the presence of increased medical support in NHPs (T. MacVittie). In addition to updating the baseline mortality with supportive care, which includes intravenous fluids, targeted antibiotics (21, 22) and blood products, these new large animal models are being developed to determine if more advanced medical support, such as might be expected for humans, can enhance survival above the increases seen with the use of medical management alone, and thereby improve on the dose modification factor (DMF) seen for basic supportive care [ $\sim 1.3$  in dogs (23) and  $\sim 1.2$  in NHPs<sup>2</sup>]. These updated models will be important for the efficacy testing of therapies for FDA licensure. For example, historical studies in dogs (24, 25) suggested that 4 Gy was an LD<sub>99/30</sub> radiation dose; however, this survival is much improved with intensive support, with dogs now surviving exposures of up to 8 Gy with no other drug treatments (G. Georges). In earlier studies, researchers used a similar but not as fully supported canine model to look at the survival benefit gained from administration of granulocyte (G) colony stimulation factor (CSF), granulocyte-macrophage (GM) CSF, and stem cell factor (SCF) after radiation exposure (24, 26). Treatment with these cytokines within 24 h postexposure yielded a DMF of 2 or 1.5 over the LD<sub>50/30</sub> of unsupported and supported animals, respectively (16). It has also been shown that the DMF can be as high as 1.7 with bone marrow transplantation (27). In summary, the level of supportive care provided in an animal irradiation protocol can dramatically impact survival and must be carefully considered in designing studies to test the efficacy of potential MCMs.

<sup>2</sup> A. M. Farese *et al.*, Medical management alone increases survival of lethally irradiate nonhuman primates within the hematopoietic syndrome. Presented at the Fifty-fourth Annual Meeting of the Radiation Research Society, 2008.

## APPROACHES TO TREATING RADIATION-INDUCED THROMBOCYTOPENIA

### *Growth Factors and Growth Factor Mimetics*

There is considerable historical evidence that the administration of TPO or megakaryocyte growth and development factor (MGDF – a truncated form of TPO, which may also be pegylated) yields a survival benefit in irradiated animals. For example, recombinant human TPO, when given after radiation exposure and concomitant with recombinant human interleukin (IL)-11 (Numega<sup>®</sup>), increases survival in a mouse model (28), and TPO, delivered in a combined regimen with GM-CSF, works synergistically to improve survival after a lethal radiation exposure in NHPs (29). As mentioned above, further development of supportive care for a canine model of radiation exposure showed that addition of growth factors (alone and in combination) to the intensive supportive care provided to the dogs led to even greater increases in survival, with concomitant improvements in platelet counts, reduced transfusion requirements, and decreased need for antibiotics (G. Georges). Other growth factors being studied in this dog model include flt3 ligand, G-CSF and a TPO mimetic. Another advantage to these growth factor approaches is that since some are already licensed or in clinical trials for other indications [such as idiopathic thrombocytopenic purpura (ITP) or prolonged thrombocytopenia after high-dose chemotherapy], information that might be required for drug label extension for an indication for radiation-induced thrombocytopenia [e.g. safety, toxicity, pharmacodynamics (PD) and pharmacokinetics (PK)] may already be available.

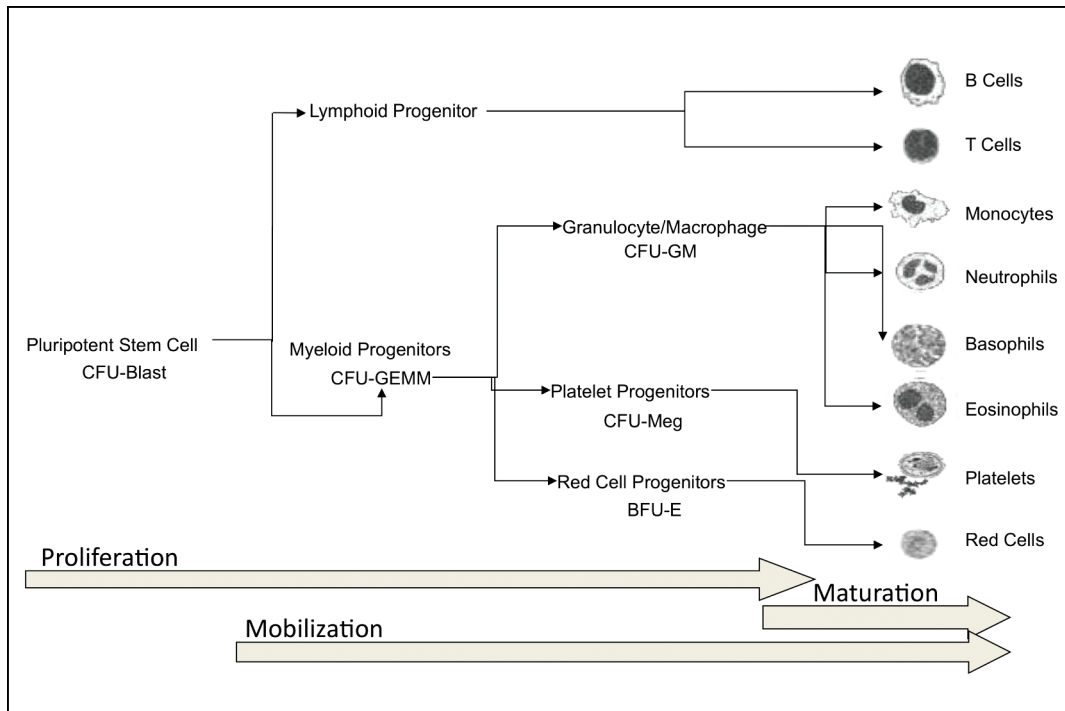
A pegylated form of MGDF (PEG-rHuMGDF) was initially in clinical development at Amgen for several indications, including chemotherapy-induced thrombocytopenia (CIT). Further development of this drug was halted due to the induction of auto-antibodies, which led to severe thrombocytopenia in some healthy controls (30, 31). Although Genentech's full-length, recombinant human TPO was not found to elicit the same autoantibody response, further development of that molecule within the United States was also discontinued. However, clinical development of TPO continued outside of the United States, and one TPO molecule (full-length, glycosylated, recombinant human protein) referred to as TPIAO, produced by the company 3sBio, is currently licensed for clinical use in China. Approved in 2006 for CIT, this molecule decreases the need for platelet transfusions in leukemia patients, and from 2006–2008, the drug was in Phase 2/3 in China for ITP. Information taken from its package insert indicates that when administered to irradiated rhesus macaques at 150 U/kg daily for 20 days, TPIAO increased platelet counts and shortened the duration of thrombocytopenia. A number of animal studies confirm this finding by demonstrated

that recombinant, full-length forms of human or animal TPO, given alone (32–38) or in combination with other drugs (28, 29, 37, 39–41) or bone marrow transplant (29, 43), increase platelet counts and/or improve survival after an acute, total-body radiation exposure. Recent data suggest that TPIAO will have similar efficacy in an animal model (Y. Wei).

Several companies have developed small molecule approaches, targeting binding sites on the TPO receptor. Two TPO mimetics [Nplate<sup>®</sup> (Amgen) and Promacta<sup>®</sup> (Glaxo SmithKline)] are now licensed in the United States to treat ITP, a bleeding condition in which the immune system destroys platelets (43), and Promacta<sup>®</sup> (also known as eltrombopag) is also being evaluated in preclinical radiation exposure models for its ability increase platelet levels in irradiated animals and yield a survival benefit. Eltrombopag is an orally available, small molecule, non-peptide drug that enhances platelet counts in ITP patients and has a good safety profile (44). The drug is also currently in clinical trials as a treatment for CIT and for possible use in bone marrow transplantation. The small molecule binds via thrombopoietin receptors on megakaryocytes in much the same way as native TPO; however, it does not activate the same signal transcription pathways as endogenous TPO (45, 46), and there are differences in binding of eltrombopag compared to native TPO. Eltrombopag binds to the TPO receptor transmembrane domain and activates STAT5 and STAT3 without Akt phosphorylation, whereas native TPO induces Akt phosphorylation (47, 48). In addition, eltrombopag does not appear to activate platelets, while TPO does.

Like other TPO mimetics currently under study, eltrombopag is species-specific, in that it demonstrates activity only in humans and chimpanzees. The species specificity appears to be related to the finding that a histidine residue within the human transmembrane domain that is needed for binding is a lysine in all other animals studied (C. Erickson-Miller). This strong species specificity poses a number of issues, mainly difficulties in conducting animal studies for FDA licensure of the compounds for a radiation mitigation indication. Chimpanzee, the only other species besides human with reactivity, is unavailable for study due to its endangered status (49) and is therefore not a viable model. In other studies using 3D human bone marrow cultures, eltrombopag induces megakaryocyte production (C. Erickson-Miller). The effect of eltrombopag is also currently under study in NOD SCID mice with human bone marrow.

Another approach to the treatment of radiation-induced thrombocytopenia includes the use of growth factors traditionally thought to increase proliferation of other hematopoietic lineages, such as erythroid targets. Researchers at the University of Rochester have shown that erythropoietin, stem cell factor and IL-3, given in



**FIG. 1.** Hematopoietic cell lineages. Used with permission (K. Rodgers). Blast colony-forming unit (CFU-Blast), granulocyte erythrocyte macrophage megakaryocyte colony-forming units (CFU-GEMM), granulocyte-macrophage colony-forming units (CFU-GM), megakaryocyte colony-forming unit (CFU-Meg), erythroid burst-forming units (BFU-E).

combination postirradiation, can rescue megakaryocyte progenitors and precursors (J. Palis). These studies include determining radiosensitivity of erythroid and megakaryocyte lineages (Fig. 1) and assessing the response of the erythroid lineage to sublethal radiation. Although the megakaryocyte and erythroid lineages are derived from a common bipotential progenitor, they demonstrate different kinetics of injury and recovery after irradiation. For example, megakaryocyte precursors are radioresistant but recover slowly, whereas erythroid progenitors are radiosensitive and are quickly depleted (J. Palis). A rational approach to mitigating hematopoietic ARS depends on better understanding the radiosensitivity of the lineage compartments and the kinetics of their cell loss and recovery.

#### *Cell Therapy Approaches and Consideration of the Bone Marrow Niche*

In addition to “traditional” drug treatment approaches for radiation-induced thrombocytopenia, cell therapies are also under study to address radiation effects on platelet lineages. For example, an *ex vivo* expanded megakaryocyte progenitors (MKPs) approach is being pursued by Cellerant Therapeutics (H. Karsunky). The goal of these studies is to optimize the culturing, expansion and cryopreservation of an MKP product for human use. This goal includes demonstrating the ability of cryopreserved MKPs to produce platelets *in*

*vitro* and in xenograft *in vivo* models. MKPs have been described for both mouse (50) and human (51) species as isolatable populations, which can increase numbers of circulating platelets, and megakaryocyte/erythroid progenitors (MEPs) and increase survival when injected into lethally irradiated mice (H. Karsunky). Currently, researchers at Cellerant are optimizing cytokine cocktails and screening resulting cultures for MKP activity and have recently demonstrated that *in vitro*-grown MKPs cultured in an optimized medium are highly enriched in their production of platelets compared to cultures not optimized for MKP proliferation (H. Karsunky).

Other studies focus on the impact of the vascular endothelial cells within the marrow on survival after radiation exposure and the potential use of pro-angiogenic factors as a means of improving survival (S. Rafii). Megakaryocytes in the circulation are known to be more radioresistant than other bone marrow-derived cells; however, their progenitor populations appear to be more radiosensitive (11, 52, 53). Mature megakaryocytes produce both pro-angiogenic factors such as VEGF-A (54) and anti-angiogenic factors including platelet factor 4 (PF4), a chemokine that is expressed mainly in megakaryocytes and platelets (55). Since stem cells reside close to endothelial cells, it is proposed that regeneration of the damaged vascular niche is essential for the reconstitution of stem cells and thrombopoiesis after irradiation (56). The bone marrow

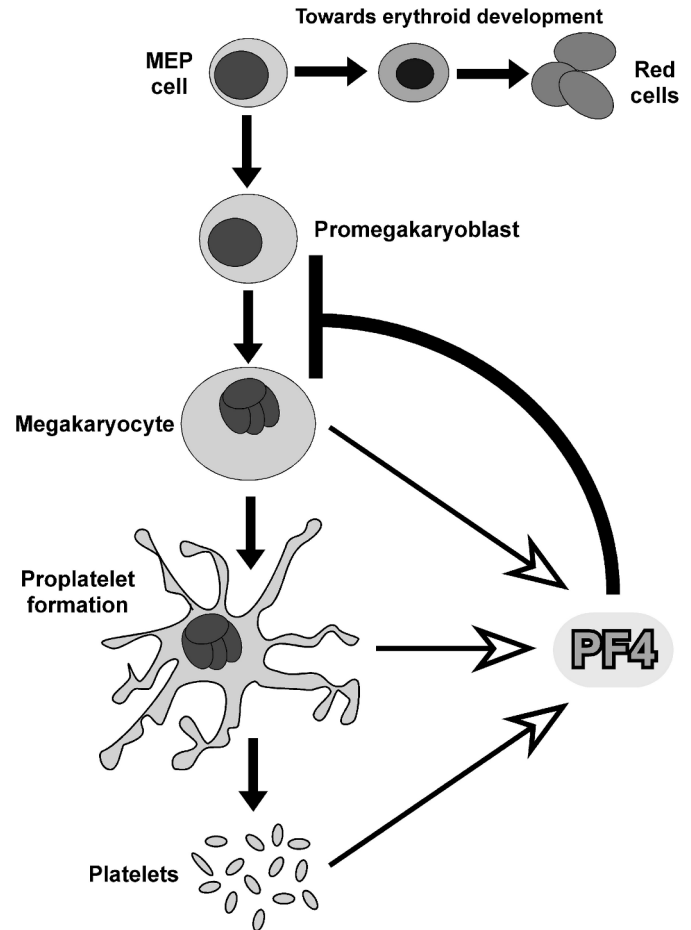
sinusoidal vascular network provides for a vascular niche that supports expansion of the hematopoietic stem cells and differentiation into megakaryocytes and platelets. This has been shown experimentally, in that endothelial cells support long-term expansion of hematopoietic stem and megakaryocyte progenitors in serum-free conditions (57).

In addition to the role that vascular growth factors play in enhancing bone marrow recovery, other stromal elements also appear to play a role in hematopoietic and specifically megakaryocytic recovery after irradiation. For example, molecules that have traditionally been targeted for their bone growth and strengthening properties also show promise in increasing platelet numbers after radiation exposure. One example is parathyroid hormone (PTH), which was purified in 1925, was studied in clinical trials in the 1970s, and is currently licensed and in clinical use in the U.S. as a daily injection for osteoporosis (Eli Lilly, Forteo®). When used in conjunction with cell therapies, PTH allows for the use of fewer transplanted bone marrow cells after depletion, to achieve the same effect as a full stem cell transplant (A. Bartholomew). Studies in mice have shown that very small quantities of sub-therapeutic bone marrow followed by PTH treatment can rescue lethally irradiated mice (58, 59). In addition, a single injection of PTH increased survival of irradiated rats when given 3 h after irradiation (60). The PTH molecule is believed to function in this model by preventing damaged hematopoietic progenitors from irreversibly initiating apoptosis during the first few hours after irradiation (61). In studies in both mice and NHPs, PTH also increased platelet counts and overall survival when given at later times postexposure (A. Bartholomew).

#### *Other Novel Approaches to Enhance Platelet Regeneration after Radiation Exposure*

Angiotensin (1–7) [A(1–7)] is a 7 amino acid peptide derivative of angiotensin that has been shown to increase recovery of progenitor cells and stimulate hematopoietic recovery (62). In clinical development by U.S. Biotest, Inc., A(1–7) reduces mucosal lesions after intravenous chemotherapy (63) and reduces the severity of thrombocytopenia when given after total-body irradiation in a mouse model.<sup>3</sup> In addition, studies combining A(1–7) with G-CSF suggest a synergistic treatment effect (K. Rodgers). Another novel compound being tested for platelet regeneration and increased survival after radiation exposure is Homspera, a formulation of the synthetic peptide analog of Substance P made by ImmuneRegen, which appears to mitigate radiation injury to the megakaryocyte lineage (J. Palis).

<sup>3</sup> C. J. Meeks *et al.*, Recovery from radiation induced thrombocytopenia by angiotensin 1–7. Presented at the Fifty-sixth Annual Meeting of the Radiation Research Society, 2010.



**FIG. 2.** PF4 is a negative paracrine of megakaryopoiesis. PF4 inhibits megakaryopoiesis distal to the step at which the megakaryocyte-erythroid progenitor (MEP) cell gives rise to the megakaryocyte-specific lineage and affects cells at the promegakaryoblast/megakaryocyte stage (65). PF4 is normally released from maturing megakaryocytes and may also occur as platelets are formed. Radiation increases the local release of this chemokine in the marrow, worsening the observed thrombocytopenia.

Substance P itself also protects mouse and human bone marrow stem cells during radiation exposure (64).

As mentioned previously, the PF4 chemokine is released in large amounts at sites of platelet activation and is known to play a role in hemostasis/thrombosis and megakaryopoiesis (Fig. 2) (65). One strategy to enhance the regeneration of platelets after radiation exposure is to block the receptor for PF4 on megakaryocytes, thereby blocking the effect of PF4. Endogenous PF4 levels affect baseline platelet count, and in a model of CIT, blocking endogenous PF4 was shown to improve platelet count recovery (65). Endogenous PF4 also appears to affect recovery from radiation-induced thrombocytopenia and is released from injured megakaryocytes. Use of a blocking polyclonal antibody or single-chain fragment to interfere with PF4 binding has shown promise in a mouse model of radiation exposure (M. Poncz).



The final novel approaches presented at this meeting included KZ41, a quinic acid (QA) analog that is orally available, and otadecenyl thiophosphate (OTP), a lysophosphatidic acid analog that has shown promise as a radiation mitigator (66). Both drugs are in development by RxBio, Inc. QA is isolated from the Cat's Claw plant and enhances leukocyte populations in a doxorubicin-induced leukopenia rat model (67). The analog is currently being tested in a combined radiation and vascular injury (nicked vein) model, in which radiation exposure reduces thrombus formation and subsequent flow restoration compared to unirradiated animals (C. Yates). Early data suggest that treatment improves the reduction of clot formation observed in irradiated animals, allowing for more rapid restoration of blood flow. OTP has been shown to enhance platelet counts and increase survival in a mouse model of radiation exposure (66), and both its PK and PD properties have been studied in NHPs.<sup>4</sup> Its MoA is believed to be anti-apoptotic, involving the LPA2 receptor, and the pro-survival nuclear factor-kappaB (NFkB) and ERK1/2 pathways.<sup>5</sup> Another approach involving the NFkB pathway currently under study within the NIAID grants portfolio (although not presented at the meeting) is the flagellin derivative CBLB502. Acting as an agonist to toll-like receptor (TLR) 5 on the cell surface, CBLB502 activates NFkB signaling, resulting in increased platelet (and other hematopoietic cell) levels, and improved survival in irradiated NHPs (68).

### OPEN GUIDED DISCUSSION

In an informal, NIAID-guided discussion session that followed the scientific presentations, representatives from several USG regulatory and funding agencies (listed in the Acknowledgments section) participated in a question and answer session on issues critical to the advanced development of MCMs for radiation-induced damage to the hematopoietic system and in specific, challenges faced in developing pro-platelet therapies. An overview of the discussion, as well as some general guidance for researchers pursuing development of a candidate MCM for this indication, is provided here.

#### *Considerations for Licensure of Pro-platelet Therapies for an ARS Indication*

There are a number of regulatory issues associated with the development of therapeutics that target platelet

regeneration after radiation exposure. Some of these are discussed below, along with guidance regarding possible approaches to addressing these issues.

#### *1. Species specificity and relevance of clinical data for other indications*

The most obvious challenge for some approaches is the species specificity of molecules, as exemplified in this meeting by the TPO-mimetic drugs. As discussed above, several drugs currently licensed for non-radiation mitigation indications work well in humans but have minimal, if any, effects in other animal species tested to date, including canines and NHPs. For one TPO-mimetic drug, for which data were presented, species specificity seems to be due to a single amino acid change in the TPO receptor, which is important for binding of the TPO mimetic. Several approaches are currently being considered to advance the development of these kinds of drugs for FDA licensure. The use of knock-in animals, which have aspects of the human hematopoietic system on which the drug can exert an effect, could represent a path forward to be explored. The possible use of clinical data, derived from use of a drug in other clinical indications, has also been brought forward for discussion. For example, there is a wealth of data for some of these TPO mimetics used to enhance platelet counts in ITP patients or to treat CIT. These types of studies may provide information that would be supportive for licensure; however, data from pivotal efficacy studies in relevant animal models will likely still be required to provide the evidence of efficacy needed for FDA licensure for an ARS indication.

#### *2. Cell therapy approaches*

Cell therapy approaches also face unique challenges, in that the human cells are the "drug product" that must be licensed by the FDA. Under the FDA Animal Rule, the product tested in animals for efficacy must be identical to the product to be used in humans; however, testing human cells in animal models is problematic, because they may not function in the same way they would in humans. Therefore, administration of a homologous animal cell preparation in an animal model may need to be considered. Animal cell preparations would need to be characterized biologically and biochemically in a manner sufficient to establish comparability to the analogous human cell product. Other avenues to explore could include the use of non-obese diabetic/severe combined immune-deficient (NOD-SCID) animals, humanized mice, or syngeneic cells in an animal model. All of these alternative approaches to model development have disadvantages, including differing radiosensitivities. The most critical question is whether studies of a human cellular product in such models would be able to yield data that could

<sup>4</sup> K. E. Thompson *et al.*, Pharmacokinetic and pharmacodynamic studies of the radiomitigant OTP in non-human primates. Presented at the Fifty-sixth Annual Meeting of the Radiation Research Society, 2010.

<sup>5</sup> G. Tigyi *et al.*, Radiomitigative signaling by lysophospholipid receptors. Presented at the Fifty-sixth Annual Meeting of the Radiation Research Society, 2010.

serve as the evidence of effectiveness needed by FDA to license such a product for this indication. Only consultation with the FDA, specific to the approach under consideration will provide an answer.

### *Regulatory and Product Development Strategies.*

The ultimate goal of NIAID's program in this area is licensure of an MCM to increase survival and enhance platelet regeneration after radiation exposure. Because there is an urgency to develop safe and effective drugs for this indication, it is critical for individuals and organizations developing such potential treatments to meet with the FDA early and often. Developing the most optimal regulatory strategy will involve continued discussions with FDA to obtain feedback, refine plans as data are generated, and work toward the goal of licensing therapies for the ARS indication.

#### *1. Animal Rule licensure*

It is assumed that the majority of MCMs developed for radiation-induced thrombocytopenia (in response to a radiation accident or incident) will use licensing pathways provided by the FDA under 21 CFR Parts 314.600-350 – Subpart I and 21 CFR 601.90-96 – Subpart H, as defined above. The Animal Rule can only be used “when adequate and well-controlled clinical studies in humans cannot be ethically conducted and field efficacy studies are not feasible.” These efficacy studies in animals replace pivotal clinical trials and therefore need to be conducted according to Good Laboratory Practice (GLP – 21 CFR 58) and be rigorous as well as reproducible. The following four requirements, described in the Animal Rule CFR, should be met to give FDA confidence in the data obtained from animal efficacy studies:

- (1) Demonstration of a reasonably well-understood pathophysiological mechanism for the toxicity caused by the radiation exposure and its amelioration/mitigation by the MCM in animals as well as in humans. Since demonstration of mechanism of action (MoA) is a key requirement of the FDA Animal Rule, the methodology to be used for evaluating MoA should be agreed upon with FDA.
- (2) Demonstration of the desired effect in at least one (usually more than one) well-characterized animal species, predictive for humans. Usually two relevant species, at least one of which is a non-rodent, will be required to demonstrate the efficacy of the MCM. The animal species selected for efficacy studies may vary with the MCM being developed for the same disease.
- (3) Use of an animal efficacy study end point that is clearly related to the desired benefit in humans, usually prevention of mortality or major morbidity.
- (4) Availability of sufficient PK and PD data in both animals and humans to allow for selection of an appropriate human dose. For most MCMs, safety data obtained from a Phase 1 clinical trial will be needed. The number of human subjects required and population characteristics will have to be negotiated with the FDA.

When planning development of a countermeasure via the Animal Rule pathway, the proposed label claim is critical, since each claim must be supported by data; efficacy data from animal studies and safety data from both animal studies and human clinical trials. The studies must be done in well-characterized models and must include detailed information. Points to consider regarding the radiation include the type of radiation, route of exposure to radiation, radiation dose and dose rate, whether total- or partial-body (e.g. shielded) irradiations will be done, mechanism of radiation injury, etc. Points to consider regarding the MCM include the route of administration of the MCM, time of administration after radiation exposure, dose of MCM, demonstration of the mechanism of action of the MCM, and PK/PD of the MCM in the animal species in which the efficacy studies were done as well as in humans. Other critical elements to consider include the use of medical management (which should be based on objective parameters), the treatment of co-syndromes (which should be clearly delineated), and other disease/syndrome and MCM-specific considerations.

The requirements of the FDA Animal Rule make this approval pathway extremely rigorous. It is therefore imperative for the Sponsor to interact with the appropriate FDA review division early to reach agreement on a suitable development path and requirements and continue the dialogue as data are generated and evaluated. FDA jurisdiction over MCMs for radiation-induced thrombocytopenia (after a radiation incident) that fall into the protein or small molecule category rests within FDA's Division of Medical Imaging Products in the Office of Oncology Drug Products within the Center for Drug Evaluation and Research (CDER), whereas those approaches involving cell therapies or some biologics would be received by the appropriate division within the FDA's Center for Biologics Evaluation and Research (CBER). Interactions with the FDA are designed to be an iterative process, and the guidance given from the discussions at this workshop was to make initial contact with the appropriate liaison group at FDA before approaching the specific FDA review division. For protein and small molecule drug products (under jurisdiction of CDER) the initial contact should be made with FDA's Office of Counter-Terrorism and Emergency Coordination (OCTEC) within CDER. For cell therapies, some biologics, and blood products (falling under jurisdiction of CBER), the applicants are

advised to contact the Senior Advisor for Counterterrorism/Medical Countermeasures, Office of the Director, CBER. These FDA liaison groups can provide potential sponsors with important guidance regarding the licensure pathways for a potential MCM.

Finally, there was discussion about the Emergency Use Authorization (EUA) process. The FDA released draft guidance on EUA of medical products in 2005 (69). The goal of a study drug manufacturer should be complete licensure for the candidate MCM. Per the EUA process, the USG and not the company requests an EUA review. The EUA process does not represent a short cut but could provide the USG with additional options should an incident occur when no licensed products are yet available for the radiation indication.

## *2. Bedside use of multiple drugs, approved combination therapies and multi-utility drugs*

It is highly likely that any drug that will be used to treat radiation injury will be administered concurrently with other MCMs or concomitant treatments, such as routine medical supportive care or perhaps a standard growth factor treatment such as G-CSF (which is already licensed for another indication). The decision to administer multiple drugs to the patient could be made by the treating physician at the bedside. In contrast, combination therapies, as defined by the FDA, involve the administration of two or more FDA-regulated components (i.e., small molecule, biologic or device) that are indicated for use together. These kinds of approaches can lead to a number of regulatory challenges, since to obtain licensure for a “combination product”, many factors must be taken into consideration besides the demonstration of safety and efficacy of the combination, including evaluation of the contributions of each component in the combination to safety and any efficacy noted; the contribution and MoA of each drug separately and together to efficacy; and the interactions of constituents with each other, as they affect safety, efficacy, PK and PD. Adding to these difficulties are intellectual property issues and logistical complications that may be encountered when the different drugs within the combination are manufactured/distributed by different companies. In December 2010, the FDA issued draft guidance for industry on the development of two or more novel drugs for use in combination (70).

Multi-utility refers to the ability of a drug to be used for more than one indication (e.g., given to cancer patients undergoing treatment to protect normal tissues and also to individuals exposed to radiation from an accident or deliberate attack). This is the preferred approach for any MCM being developed for a radiation indication for several reasons: (1) continued production of the drug for another market ensures a “warm base”

for USG procurements that might be spaced apart by years; (2) this development model allows the USG to leverage other funding for aspects of the drug development that might be shared across indications (e.g., safety and PK/PD information, as long as the route, dosage and dose regimens are the same for the different indications); (3) the drug is already in general use in hospitals and distribution centers and therefore inventories may exist locally that could be readily accessed; (4) physicians would already be familiar with clinical use of the drug; and (5) there is value added to the company’s existing market, in that periodic procurements of compound by the USG can contribute to recovery of the development costs of a drug (71). All of the potential regulatory issues discussed above must be taken into consideration when planning the development and FDA licensure pathway for an MCM that will be used in the wake of a radiation incident.

## *Animal Model Development*

### *1. Animal species selection and supportive care*

Efficacy studies conducted under the FDA Animal Rule replace pivotal clinical trials and therefore need to be conducted under GLP. This requires the development of scientifically and rationally designed animal models that parallel both the disease and the response to the therapy under consideration in humans. Therefore, selection of relevant species and development of appropriate animal models is the first and perhaps the most critical step in developing an MCM for a radiation-induced thrombocytopenia indication. Pivotal efficacy studies in two species, at least one of which is non-rodent, will generally be required. The species should exhibit the same mechanism of pathophysiological response to radiation exposure as humans as well as have the same MoA for how the MCM mitigates or treats radiation injury. In choosing end points, it is essential that the primary end point be relevant to human experience, and therefore the optimal primary end point is mortality or major morbidity. In essence, an animal model is already a surrogate for a clinical study in human subjects; therefore, the use of a surrogate end point in animal studies is discouraged. In addition, studies should demonstrate that the mechanism of injury and MoA of the drug across the animal species and humans are identical. The choice of primary and secondary end points will depend on the syndrome being studied and the MoA of the MCM.

Among the considerations during animal model development is the use of supportive care/medical management. Pivotal animal efficacy protocols should clearly delineate the medical management (including the specific elements, timing of administration, and clinically relevant triggers to initiate administration) to be used in the study. These parameters would need to be justified

to FDA. It is likely that medical management used will be different for different species based on what is relevant (i.e., some kinds of medical management, such as choice of antibiotic, may be species specific) and what is feasible (i.e., some forms of medical management are challenging in smaller species), and this should be clearly explained in the justification. For studies in non-rodent, larger animals, supportive care has ranged from little or none (18, 68) to moderate (T. MacVittie) to high (G. Georges). Supportive care is an effective countermeasure on its own (16, 21, 23), and therefore this factor must be taken into account when designing an animal model.

The desired indication for the countermeasure is the driver for the design of the pivotal animal efficacy study. Another key driver for the design of the pivotal animal efficacy studies should be the “scenario for use” of the countermeasure. To this end, it is also important to consider the logistics of the concept of operations after an event, i.e., what type of care is available for given times and locations, the timing of when supportive care measures need to be delivered, and what kind of monitoring can be performed (including determination of an individual’s radiation exposure). An argument could be made that the drug should be tested in the presence and absence of supportive care; however, studies using large animal models in which supportive care is not provided are often difficult to conduct given Institutional Animal Care and Use Committees (IACUCs) guidelines to minimize pain and distress in the animals.

## 2. Irradiation and monitoring protocols

Considerable thought should be put into the development of real-life irradiation experiments. For example, models that might have clinical relevance for radiotherapy patients (e.g., fractionated exposures involving large doses of radiation localized to one part of the body) are not necessarily appropriate for a radiation counterterrorism indication. By the same reasoning, animal models in which total-body irradiation is mostly homogeneous (e.g., animals are rotated during exposure to ensure complete irradiation) are unlikely to represent exposures anticipated during a radiological or nuclear incident. Although total-body irradiation models are important, for some studies it might be beneficial to consider partial-body irradiations, in which part of the body is shielded, when studying higher radiation exposures. Although not directly relevant for approaches targeting platelets, this kind of exposure allows for the generation of a survivable hematopoietic ARS at dose ranges where gastrointestinal-ARS damage might also be observed. With regard to monitoring protocols, defined loosely as manipulations done to the animal postexposure to obtain additional data (e.g., obtaining body weights and blood samples), researchers must also be mindful of unanticipated stresses that these proce-

dures might cause to the animal. One primary example is the use of the same animals to monitor blood counts and overall survival. Since obtaining blood samples (single or repeated) after radiation exposure can be considered a form of combined injury, especially in small animals, these combined studies can lead to skewed survival data. The use of separate, parallel cohorts to study survival and hematology in small animal species should be considered. There was a discussion about the potential need to standardize not only exposure models (including species, radiation source, dose rate, level of support, acid water) but also data presentation (e.g., presentation of DMF data), with the suggestion that funding agencies might consider requesting these standard models in their funding opportunities.

## 3. Mechanism of action (MoA)

One of the requirements for developing a therapy via the FDA Animal Rule is the establishment of a well-understood, pathophysiological mechanism of toxicity and its amelioration/mitigation by the MCM. For example, since animal data will be relied on to make a determination of potential efficacy in humans, MoA is required to link data from animal studies to human responses. Aside from the requirement that MoA be reasonably well understood for licensure via the Animal Rule pathway, there are a number of reasons why MoA studies are critical for development of a drug for a radiation/nuclear indication. The MoA defined for a drug in nonirradiated animals may be different in irradiated animals. Radiation could also alter the clearance or toxicity of a drug. For this reason, in addition to studying MoA of a drug in normal animals, a cohort of irradiated animals should also be included. In addition, in many cases, understanding the MoA helps to drive the selection of secondary end points for licensure. For example, knowing that a drug exerts its effects on the megakaryocyte lineage might lead to the selection of a clinically relevant, secondary end point such as reduced need for blood and/or platelet transfusions. Especially for some of the delayed effects of acute radiation exposure (DEARE), it is important to understand the MoA of a drug to link events that occur in the period immediately after exposure with biological effects noted at later times.

In summary, FDA guidance on the development of the most relevant animal models should be sought early by investigators, to make optimal use of time and funds for studies that will be acceptable to FDA, for the end goal of product licensure

## *Remaining Unaddressed Research Gaps – Special Populations*

To date, very little research has been done to develop animal models in special populations (pediatrics,

**TABLE 2**  
**Open Discussion Key Points**

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**Licensure challenges** associated with the development of pro-platelet therapies for use after radiation exposure (e.g., species specificity of some approaches, use of animal and/or human-derived cells for cell therapy approaches) exist. Potential means to address these challenges include using novel animal models and/or homologous animal cell preparations as the surrogate human product in an animal model.

The **FDA Animal Rule** is interpreted to mean that a candidate MCM using this licensure pathway should (generally) have efficacy data in at least two animal models (at least one non-rodent) that are predictive for humans. These pivotal efficacy studies must be performed in compliance with GLP and have clinically relevant end points (usually mortality or major morbidity) and MoA of the therapy across species will need to be assessed. Ample PK/PD data should be available to allow for the selection of an appropriate human dose.

**Animal model development** is critical, given the Animal Rule guidance. Species that are selected for study should respond both to radiation and to the candidate MCM in a way that is similar to or predictive of the human response.

**Supportive care** of the animals should also parallel the level of medical management that would be expected for humans. Testing of the MCM in the presence of another treatment that might be considered standard of care for a radiation accident victim could also provide important data.

**Radiation exposures**, to the extent that it is possible to do so, should mimic the anticipated real-world exposure scenarios, including the type of radiation, dose and dose rate. The need to conduct studies in partially shielded animal models should be discussed and agreed upon with FDA. In addition, the dose modification factor (DMF) for the approach should be determined, so as to allow efficacy comparisons between candidate therapeutics.

**Regulatory strategies** should be established early in the development of a drug for a radiation counterterrorism indication. Discussion with FDA review divisions in CDER or CBER should be initiated once proof-of-concept data are obtained by the Sponsor.

**Research gaps** still exist, especially in the area of MCM development for special populations, such as children, the elderly and immunocompromised individuals. Working together, the different components of the U.S. Government must continue to provide guidance and funding to researchers developing pipeline and advanced MCMs for radiation indications.

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geriatrics and immunocompromised patients). This fact represents a critical gap for the HHS Public Health Emergency Medical Countermeasures Enterprise, since according to the U.S. Census Bureau, nearly 37% of the civilian population in 2009 was either under the age of 18 or over the age of 65. Few drugs are being tested in animal models that represent these groups and there is relatively little information about the effects of radiation alone in these groups and how their responses might differ from healthy adults (72). Because adult animal models for the different radiation syndromes are only now being validated, animal model development for pediatric and other special populations is lagging behind. With the pediatric population, it is very difficult to even generate safety data due to the ethics involved in testing drugs in children. Although the Pandemic and All-Hazards Preparedness Act (Public Law 109-417, passed December 19, 2006) stipulates the need to develop MCMs for children, and the 2010 Report to the President and Congress from the National Commission on Children and Disasters recommends “funding and grant guidance for the development, acquisition, and stockpiling of MCMs specifically for children for inclusion in the SNS...” (73), much work still needs to be done.

*Proposed Directions for MCM Development, Acquisition and Use*

Necessary paths forward for future funding of drug development for ARS include not only the development of novel strategies for the USG to fund advanced development of drugs for a radiation indication but also a determination of specific information about how MCMs will be used once stockpiled (e.g., as a fully

licensed drug for the radiation indication or as an EUA). Working together, different government agencies are involved in the basic research and development (NIAID, Department of Defense), procurement (BARDA) and stockpiling of drugs to be used in case of accidental radiation exposure. Overseen by the Centers for Disease Control (CDC), the SNS “...has large quantities of medicine and medical supplies to protect the American public if there is a public health emergency...severe enough to cause local supplies to run out” (74). In the current scarce financial resources environment, it is critical that USG agencies, with roles ranging from strategic partner/investor to eventual customer for a fully licensed MCM, work together to ensure optimal use of funds. As such, the USG must provide a clear understanding of the needs and requirements for MCMs for radiation/nuclear incidents so that academia and industry can focus research and development efforts appropriately. The USG should also (1) facilitate the development of standards for radiation dosimetry and exposure, (2) fund the development of animal models and strategies for efficacy study protocols, and (3) actively support collaborations with industry. The latter includes providing funding for product development activities, including Small Business Innovation Research (SBIR) opportunities, as well as working to minimize market barriers that deter groups or companies with potential countermeasures from working with the USG. Assembled government, academic and industrial participants of the meeting also discussed the need to in some way standardize the science that is proposed by crafting future funding solicitations in such a way as to clearly delineate the kinds of approaches and animal models that are being sought.

## CONCLUSIONS

Key points from the open discussion are provided in Table 2. With recent funding provided by the NIAID, a number of pro-platelet MCMs are being studied for efficacy in a radiation postexposure administration scenario. These approaches include several drugs already licensed for other platelet regeneration indications (e.g. second-generation TPO mimetics) as well as progenitor cell therapies, agents targeting the bone marrow niche, growth factors and other novel compounds. From discussions at this meeting, it is clear that continued collaborations between researchers developing pro-platelet approaches and USG funding, licensure and procurement agencies is critical and should accelerate the development and licensure of drugs to treat thrombocytopenia and increase survival in radiation-exposed victims.

## ACKNOWLEDGMENTS

The authors thank conference participants for contributions to the discussion, especially those who made presentations (Table 1) and assisted with preparing this report. Special thanks to David Cassatt, DAIT, NIAID; Brad Leissa, OCTEC, CDER, FDA; Thomas MacVittie, Ronald Manning, BARDA, HHS; Mercedes Serabian, CBER, FDA; and Jui Shah, Office of Regulatory Affairs, NIAID for serving on the panel and for their valuable contributions to the informal discussion.

## REFERENCES

1. Food and Drug Administration, New drug and biological drug products: evidence needed to demonstrate effectiveness of new drugs when human efficacy studies are not ethical or feasible. *Fed. Regist.* **67**, 37988–37998 (2002).
2. A. E. Geddis, Megakaryopoiesis. *Semin. Hematol.* **47**, 212–219 (2010).
3. O. Klimchenko, M. Mori, A. Distefano, T. Langlois, F. Larbret, Y. Lecluse, O. Feraud, W. Vainchenker, F. Norol and N. Debili, A common bipotent progenitor generates the erythroid and megakaryocyte lineages in embryonic stem cell-derived primitive hematopoiesis. *Blood* **114**, 1506–1517 (2009).
4. S. Lok, K. Kaushansky, R. D. Holly, J. L. Kuijper, C. E. Lofton-Day, P. J. Oort, F. J. Grant, M. D. Heipel, S. K. Burkhead and D. C. Foster, Cloning and expression of murine thrombopoietin cDNA and stimulation of platelet production in vivo. *Nature* **369**, 565–568 (1994).
5. F. J. de Sauvage, P. E. Hass, S. D. Spencer, B. E. Malloy, A. L. Gurney, S. A. Spencer, W. C. Darbonne, W. J. Henzel, S. C. Wong and D. L. Eaton, Stimulation of megakaryocytopoiesis and thrombopoiesis by the c-Mpl ligand. *Nature* **369**, 533–538 (1994).
6. H. G. Kopp and S. Rafii, Thrombopoietic cells and the bone marrow vascular niche. *Ann. NY Acad. Sci.* **1106**, 175–179 (2007).
7. T. Junt, H. Schulze, Z. Chen, S. Massberg, T. Goerge, A. Krueger, D. D. Wagner, T. Graf, J. E. Italiano, Jr. and U. H. von Andrian, Dynamic visualization of thrombopoiesis within bone marrow. *Science* **317**, 1767–1770 (2007).
8. R. Fuentes, Y. Wang, J. Hirsch, C. Wang, L. Rauova, G. S. Worthen, M. A. Kowalska and M. Poncz, Infusion of mature megakaryocytes into mice yields functional platelets. *J. Clin. Invest.* **120**, 3917–3922 (2010).
9. M. R. Dowling, E. C. Josefsson, K. J. Henley, P. D. Hodgkin and B. T. Kile, Platelet senescence is regulated by an internal timer, not damage inflicted by hits. *Blood* **116**, 1776–1778 (2010).
10. M. Dominici, V. Rasini, R. Bussolari, X. Chen, T. J. Hofmann, C. Spano, D. Bernabei, E. Veronesi, F. Bertoni and E. M. Horwitz, Restoration and reversible expansion of the osteoblastic hematopoietic stem cell niche after marrow radioablation. *Blood* **114**, 2333–2343 (2009).
11. S. Monzen, K. Osuda, Y. Miyazaki, N. Hayashi, K. Takahashi and I. Kashiwakura, Radiation sensitivities in the terminal stages of megakaryocytic maturation and platelet production. *Radiat. Res.* **172**, 314–320 (2009).
12. W. M. Brown, Wide field irradiation and the platelet count. *Acta Radiol.* **32**, 407–427 (1949).
13. T. P. McDonald, M. Cottrell and R. Clift, Hematologic changes and thrombopoietin production in mice after X-irradiation and platelet-specific antisera. *Exp. Hematol.* **5**, 291–298 (1977).
14. I. Hers and A. Mumford, Understanding the therapeutic action of recombinant factor VIIa in platelet disorders. *Platelets* **19**, 571–581 (2008).
15. N. L. Kobrinsky and H. Tulloch, Treatment of refractory thrombocytopenic bleeding with 1-desamino-8-D-arginine vasopressin (desmopressin). *J. Pediatr.* **112**, 993–996 (1988).
16. N. Dainiak, J. K. Waselenko, J. O. Armitage, T. J. MacVittie and A. M. Farese, The hematologist and radiation casualties. *Hematology Am. Soc. Hematol. Educ. Program*, 473–496 (2003).
17. M. Hachiya, *Hiroshima Diary*. University of North Carolina Press, Chapel Hill, 1955.
18. D. R. Stickney, C. Dowding, S. Authier, A. Garsd, N. Onizuka-Handa, C. Reading and J. M. Frincke, 5-Androstenediol improves survival in clinically unsupported rhesus monkeys with radiation-induced myelosuppression. *Int. Immunopharmacol.* **7**, 500–505 (2007).
19. Guidance for Industry: Animal Models – Essential Elements to Address Efficacy Under the Animal Rule. Food and Drug Administration, U.S. Department of Health and Human Services, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER), 2009.
20. J. P. Williams, S. L. Brown, G. E. Georges, M. Hauer-Jensen, R. P. Hill, A. K. Huser, D. G. Kirsch, T. J. MacVittie, K. A. Mason and W. H. McBride, Animal models for medical countermeasures to radiation exposure. *Radiat. Res.* **173**, 557–578 (2010).
21. J. K. Waselenko, T. J. MacVittie, W. F. Blakely, N. Pesik, A. L. Wiley, W. E. Dickerson, H. Tsu, D. L. Confer, C. N. Coleman and N. Dainiak, Medical management of the acute radiation syndrome: recommendations of the Strategic National Stockpile Radiation Working Group. *Ann. Intern. Med.* **140**, 1037–1051 (2004).
22. I. Brook, T. B. Elliott, G. D. Ledney, M. O. Shoemaker and G. B. Knudson, Management of postirradiation infection: lessons learned from animal models. *Mil. Med.* **169**, 194–197 (2004).
23. T. J. MacVittie, A. M. Farese and W. Jackson, Defining the full therapeutic potential of recombinant growth factors in the post radiation-accident environment: the effect of supportive care plus administration of G-CSF. *Health Phys.* **89**, 546–555 (2005).
24. F. G. Schuening, F. R. Appelbaum, H. J. Deeg, M. Sullivan-Pepe, T. C. Graham, R. Hackman, K. M. Zsebo and R. Storb, Effects of recombinant canine stem cell factor, a c-kit ligand, and recombinant granulocyte colony-stimulating factor on hematopoietic recovery after otherwise lethal total body irradiation. *Blood* **81**, 20–26 (1993).
25. F. G. Schuening, R. Storb, S. Goehle, T. C. Graham, F. R. Appelbaum, R. Hackman and L. M. Souza, Effect of recombinant human granulocyte colony-stimulating factor on hematopoiesis of normal dogs and on hematopoietic recovery after otherwise lethal total body irradiation. *Blood* **74**, 1308–1313 (1989).
26. R. A. Nash, F. G. Schuening, K. Seidel, F. R. Appelbaum, T. Boone, H. J. Deeg, T. C. Graham, R. Hackman, M. Sullivan-Pepe and R. Storb, Effect of recombinant canine granulocyte-macrophage colony-stimulating factor on hematopoietic recovery after otherwise lethal total body irradiation. *Blood* **83**, 1963–1970 (1994).

27. J. J. Broerse, D. W. Van Bekkum, C. F. Hollander and J. A. Davids, Mortality of monkeys after exposure to fission neutrons and the effect of autologous bone marrow transplantation. *Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med.* **34**, 253–264 (1978).
28. A. Van der Meeren, M. A. Mouthon, M. H. Gaugler, M. Vandamme and P. Gourmelon, Administration of recombinant human IL11 after supralethal radiation exposure promotes survival in mice: interactive effect with thrombopoietin. *Radiat. Res.* **157**, 642–649 (2002).
29. K. J. Neelis, S. C. Hartong, T. Egeland, G. R. Thomas, D. L. Eaton and G. Wagemaker, The efficacy of single-dose administration of thrombopoietin with coadministration of either granulocyte/macrophage or granulocyte colony-stimulating factor in myelosuppressed rhesus monkeys. *Blood* **90**, 2565–2573 (1997).
30. J. Li, C. Yang, Y. Xia, A. Bertino, J. Glaspy, M. Roberts and D. J. Kuter, Thrombocytopenia caused by the development of antibodies to thrombopoietin. *Blood* **98**, 3241–3248 (2001).
31. R. L. Basser, E. O'Flaherty, M. Green, M. Edmonds, J. Nichol, D. M. Menchaca, B. Cohen and C. G. Begley, Development of pancytopenia with neutralizing antibodies to thrombopoietin after multicycle chemotherapy supported by megakaryocyte growth and development factor. *Blood* **99**, 2599–2602 (2002).
32. K. J. Neelis, L. Qingliang, G. R. Thomas, B. L. Cohen, D. L. Eaton and G. Wagemaker, Prevention of thrombocytopenia by thrombopoietin in myelosuppressed rhesus monkeys accompanied by prominent erythropoietic stimulation and iron depletion. *Blood* **90**, 58–63 (1997).
33. K. J. Neelis, T. P. Visser, W. Dimjati, G. R. Thomas, P. J. Fielder, D. Bloedow, D. L. Eaton and G. Wagemaker, A single dose of thrombopoietin shortly after myelosuppressive total body irradiation prevents pancytopenia in mice by promoting short-term multilineage spleen-repopulating cells at the transient expense of bone marrow-repopulating cells. *Blood* **92**, 1586–1597 (1998).
34. M. A. Mouthon, A. Van der Meeren, M. H. Gaugler, T. P. Visser, C. Squiban, P. Gourmelon and G. Wagemaker, Thrombopoietin promotes hematopoietic recovery and survival after high-dose whole body irradiation. *Int. J. Radiat. Oncol. Biol. Phys.* **43**, 867–875 (1999).
35. E. G. Stefanich, C. C. Carlson-Zermeno, K. McEvoy, M. Reich and P. J. Fielder, Dose schedule of recombinant murine thrombopoietin prior to myelosuppressive and myeloablative therapy in mice. *Cancer Chemother. Pharmacol.* **47**, 70–77 (2001).
36. M. A. Mouthon, M. H. Gaugler, A. Van der Meeren, M. Vandamme, P. Gourmelon and G. Wagemaker, Single administration of thrombopoietin to lethally irradiated mice prevents infectious and thrombotic events leading to mortality. *Exp. Hematol.* **29**, 30–40 (2001).
37. A. Van der Meeren, M. A. Mouthon, M. Vandamme, C. Squiban and J. Aigueperse, Combinations of cytokines promote survival of mice and limit acute radiation damage in concert with amelioration of vascular damage. *Radiat. Res.* **161**, 549–559 (2004).
38. M. A. Mouthon, A. Van der Meeren, M. Vandamme, C. Squiban and M. H. Gaugler, Thrombopoietin protects mice from mortality and myelosuppression following high-dose irradiation: importance of time scheduling. *Can. J. Physiol. Pharmacol.* **80**, 717–721 (2002).
39. F. Herodin, P. Bourin, J. F. Mayol, J. J. Lataillade and M. Drouet, Short-term injection of antiapoptotic cytokine combinations soon after lethal gamma-irradiation promotes survival. *Blood* **101**, 2609–2616 (2003).
40. A. Grossmann, J. Lenox, T. A. Deisher, H. P. Ren, J. M. Humes, K. Kaushansky and K. H. Sprugel, Synergistic effects of thrombopoietin and granulocyte colony-stimulating factor on neutrophil recovery in myelosuppressed mice. *Blood* **88**, 3363–3370 (1996).
41. S. C. Hartong, K. J. Neelis and G. Wagemaker, Co-administration of Flt-3 ligand counteracts the actions of thrombopoietin in myelosuppressed rhesus monkeys. *Br. J. Haematol.* **121**, 359–367 (2003).
42. G. Wagemaker, K. J. Neelis, S. C. Hartong, A. W. Wognum, G. R. Thomas, P. J. Fielder and D. L. Eaton, The efficacy of recombinant thrombopoietin in murine and nonhuman primate models for radiation-induced myelosuppression and stem cell transplantation. *Stem Cells* **16**, 375–386 (1998).
43. D. J. Kuter, New thrombopoietic growth factors. *Blood* **109**, 4607–4616 (2007).
44. J. M. Jenkins, D. Williams, Y. Deng, J. Uhl, V. Kitchen, D. Collins and C. L. Erickson-Miller, Phase I clinical study of eltrombopag, an oral, nonpeptide thrombopoietin receptor agonist. *Blood* **109**, 4739–4741 (2007).
45. D. Bouscary, C. Lecoq-Lafon, S. Chretien, S. Zompi, S. Fichelson, O. Muller, F. Porteu, I. Dusanter-Fourt, S. Gisselbrecht and C. Lacombe, Role of Gab proteins in phosphatidylinositol 3-kinase activation by thrombopoietin (Tpo). *Oncogene* **20**, 2197–2204 (2001).
46. J. Garcia, J. de Gunzburg, A. Eychene, S. Gisselbrecht and F. Porteu, Thrombopoietin-mediated sustained activation of extracellular signal-regulated kinase in UT7-Mpl cells requires both Ras-Raf-1- and Rap1-B-Raf-dependent pathways. *Mol. Cell. Biol.* **21**, 2659–2670 (2001).
47. J. A. Erhardt, C. L. Erickson-Miller, M. Aivado, M. Abboud, K. Pillarisetti and J. R. Toomey, Comparative analyses of the small molecule thrombopoietin receptor agonist eltrombopag and thrombopoietin on in vitro platelet function. *Exp. Hematol.* **37**, 1030–1037 (2009).
48. C. L. Erickson-Miller, E. Delorme, S. S. Tian, C. B. Hopson, A. J. Landis, E. I. Valoret, T. S. Sellers, J. Rosen, S. G. Miller and J. M. Jenkins, Preclinical activity of eltrombopag (SB-497115), an oral, nonpeptide thrombopoietin receptor agonist. *Stem Cells* **27**, 424–430 (2009).
49. International Union for Conservation of Nature, IUCN Red List of Threatened Species. Version 2010.3.
50. T. Na Nakorn, D. Traver, I. L. Weissman and K. Akashi, Myeloerythroid-restricted progenitors are sufficient to confer radioprotection and provide the majority of day 8 CFU-S. *J. Clin. Invest.* **109**, 1579–1585 (2002).
51. D. Clay, E. Rubinstein, Z. Mishal, A. Anjo, M. Prenant, C. Jasmin, C. Boucheix and M. C. Le Bousse-Kerdiles, CD9 and megakaryocyte differentiation. *Blood* **97**, 1982–1989 (2001).
52. I. Kashiwakura, O. Inanami, Y. Abe, T. A. Takahashi and M. Kuwabara, Different radiosensitive megakaryocytic progenitor cells exist in steady-state human peripheral blood. *Radiat. Res.* **164**, 10–16 (2005).
53. I. Kashiwakura, M. Kuwabara, O. Inanami, M. Murakami, Y. Hayase, T. A. Takahashi and Y. Takagi, Radiation sensitivity of megakaryocyte colony-forming cells in human placental and umbilical cord blood. *Radiat. Res.* **153**, 144–152 (2000).
54. R. Mohle, D. Green, M. A. Moore, R. L. Nachman and S. Rafii, Constitutive production and thrombin-induced release of vascular endothelial growth factor by human megakaryocytes and platelets. *Proc. Natl. Acad. Sci. USA* **94**, 663–668 (1997).
55. J. Kisucka, C. E. Butterfield, D. G. Duda, S. C. Eichenberger, S. Saffaripour, J. Ware, Z. M. Ruggeri, R. K. Jain, J. Folkman and D. D. Wagner, Platelets and platelet adhesion support angiogenesis while preventing excessive hemorrhage. *Proc. Natl. Acad. Sci. USA* **103**, 855–860 (2006).
56. J. M. Butler, H. Kobayashi and S. Rafii, Instructive role of the vascular niche in promoting tumour growth and tissue repair by angiocrine factors. *Nat. Rev. Cancer* **10**, 138–146 (2010).
57. S. Yildirim, A. M. Boehmler, L. Kanz and R. Mohle, Expansion of cord blood CD34+ hematopoietic progenitor cells in coculture with autologous umbilical vein endothelial cells (HUVEC) is superior to cytokine-supplemented liquid culture. *Bone Marrow Transplant.* **36**, 71–79 (2005).
58. D. T. Scadden, The stem-cell niche as an entity of action. *Nature* **441**, 1075–1079 (2006).

59. L. M. Calvi, G. B. Adams, K. W. Weibrecht, J. M. Weber, D. P. Olson, M. C. Knight, R. P. Martin, E. Schipani, P. Divieti and D. T. Scadden, Osteoblastic cells regulate the haematopoietic stem cell niche. *Nature* **425**, 841–846 (2003).
60. R. H. Rixon and J. F. Whitfield, The radioprotective action of parathyroid extract. *Int. J. Radiat. Biol.* **3**, 361–367 (1961).
61. J. F. Whitfield, Parathyroid hormone (PTH) and hematopoiesis: new support for some old observations. *J. Cell Biochem.* **96**, 278–284 (2005).
62. S. Heringer-Walther, K. Eckert, S. M. Schumacher, L. Uharek, A. Wulf-Goldenberg, F. Gembardt, I. Fichtner, H. P. Schultheiss, K. Rodgers and T. Walther, Angiotensin-(1–7) stimulates hematopoietic progenitor cells in vitro and in vivo. *Haematologica* **94**, 857–860 (2009).
63. K. E. Rodgers, J. Oliver and G. S. diZerega, Phase I/II dose escalation study of angiotensin 1–7 [A(1–7)] administered before and after chemotherapy in patients with newly diagnosed breast cancer. *Cancer Chemother. Pharmacol.* **57**, 559–568 (2006).
64. Y. S. An, E. Lee, M. H. Kang, H. S. Hong, M. R. Kim, W. S. Jang, Y. Son and J. Y. Yi, Substance P stimulates the recovery of bone marrow after the irradiation. *J. Cell Physiol.* **226**, 1204–1213 (2011).
65. M. P. Lambert, L. Rauova, M. Bailey, M. C. Sola-Visner, M. A. Kowalska and M. Poncz, Platelet factor 4 is a negative autocrine in vivo regulator of megakaryopoiesis: clinical and therapeutic implications. *Blood* **110**, 1153–1160 (2007).
66. W. Deng, E. Shuyu, R. Tsukahara, W. J. Valentine, G. Durgam, V. Gududuru, L. Balazs, V. Manickam, M. Arsuru and G. Tigyi, The lysophosphatidic acid type 2 receptor is required for protection against radiation-induced intestinal injury. *Gastroenterology* **132**, 1834–1851 (2007).
67. Y. Sheng, C. Akesson, K. Holmgren, C. Bryngelsson, V. Giamapa and R. W. Pero, An active ingredient of Cat's Claw water extracts identification and efficacy of quinic acid. *J. Ethnopharmacol.* **96**, 577–584 (2005).
68. L. G. Burdelya, V. I. Krivokrysenko, T. C. Tallant, E. Strom, A. S. Gleiberman, D. Gupta, O. V. Kurnasov, F. L. Fort, A. L. Osterman and A. V. Gudkov, An agonist of toll-like receptor 5 has radioprotective activity in mouse and primate models. *Science* **320**, 226–230 (2008).
69. Draft Guidance: Emergency Use Authorization of Medical Products. Office of Counterterrorism Policy and Planning (HF-29), Office of the Commissioner, Food and Drug Administration, Washington, DC, 2005.
70. Food and Drug Administration, Draft Guidance: Codevelopment of Two or More Unmarketed Investigational Drugs for Use in Combination. Office of Communications, Division of Drug Information, Food and Drug Administration, Washington, DC, 2010.
71. N. Hafer, B. W. Maidment and R. J. Hatchett, The NIAID Radiation Countermeasures Program Business Model. *Biosecur. Bioterror.* **8**, 357–363 (2010).
72. Radiological and nuclear terrorism. Chapter 6 in *Pediatric Terrorism and Disaster Preparedness: A Resource for Pediatricians* (G. L. Foltin, D. J. Schonfeld and M. W. Shannon, Eds.). American Academy of Pediatricians, Elk Grove Village, IL, and Agency for Healthcare Research and Quality, Rockville, MD, 2006.
73. National Commission on Children and Disasters, 2010 Report to the President and Congress. Rockville, MD, 2010.
74. Centers for Disease Control, Emergency Preparedness and Response: Strategic National Stockpile. 2010.