NATIONAL INSTITUTES OF HEALTH

STRATEGIC PLAN AND RESEARCH AGENDA FOR MEDICAL COUNTERMEASURES AGAINST RADIOLOGICAL AND NUCLEAR THREATS PROGRESS REPORT: 2005–2011 AND FUTURE RESEARCH DIRECTIONS: 2012–2016

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INTRODUCTION

In 2003, the White House Office of Science and Technology Policy and White House Homeland Security Council jointly convened a Radiological/Nuclear Threat Countermeasures Working Group to develop a list of priority research areas, an assessment of the current status of radiation diagnostics and biodosimetry, and product requirements statements for candidate radiation medical countermeasures (MCMs). The interagency consensus developed through this process was finally published in 2005.

In 2004, building on this interagency consensus, the U.S. Department of Health and Human Services (HHS) directed the National Institute of Allergy and Infectious Diseases (NIAID) to develop a strategic plan and research agenda to guide efforts at the National Institutes of Health (NIH) to develop MCMs against radiological and nuclear threats. Previously, no federal agency had the mission to develop such products for civilian populations. Taking the Working Group Final Report and reports from subsequent focused workshops sponsored by NIAID and the National Cancer Institute (NCI) as a starting point, NIAID developed a draft research agenda for the new NIH Radiation Countermeasures Program (RCP). In October 2004, NIAID convened a Blue Ribbon Panel on Radiological and Nuclear Terrorism and Its Implications for Biomedical Research to review and provide feedback on this draft research agenda. Incorporating the input and recommendations of the Blue Ribbon Panel, the research agenda was revised and published in final form in June 2005. The *NIH Strategic Plan and Research Agenda for Medical Countermeasures Against Radiological and Nuclear Threats* (http://www.niaid.nih.gov/about/whoWeAre/documents/radnucstrategicplan.pdf) described NIH research priorities in this area and was widely disseminated.

NIH has made substantial progress toward addressing the specific research goals and recommendations identified in the four priority areas of the *Strategic Plan and Research Agenda,* thereby increasing the breadth and depth of research on radiation MCMs. These are described as "Continuing Research Goals" in each section of this report. Based on the knowledge and experience gained in the subsequent 5 years, "Expanded/Revised Research Goals" representing a refinement and more in-depth, mature understanding of program requirements have been identified to establish future directions for 2012–2016. As scientific, research and programmatic gaps are identified and assessed, program adjustments are instituted and/or new initiatives are developed.

Sections I through IV of this progress report describe the continuing and expanded/revised goals and programmatic and scientific accomplishments achieved by the RCP in the following four priority research areas:

- Basic and Translational Research
- Radiation Biodosimetry
- Focused Product Development for Radiological Medical Countermeasures
- Infrastructure for Research and Product Development

Section V describes collaborations and partnerships that have been instrumental in implementing the research agenda. These include joint activities and programs within NIH, with other HHS agencies, with other federal agencies, and with private sector organizations.

This report also provides access to

- The RCP business model: The RCP developed and implemented a business model that emphasizes program integration, acknowledges market realities, minimizes administrative requirements, addresses problems proactively, positions the RCP as a strategic partner and investor in radiation MCM development, and delineates approaches to addressing regulatory challenges. The RCP business model was published in *Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science,* Volume 8, Number 4 (2010) and is available at http://www.ncbi.nlm.nih.gov/pubmed/21142762.

- A listing of publications resulting from RCP-funded research (http://www.niaid.nih.gov/topics/radnuc/summariesPubs/Pages/default.aspx)

- A listing of RCP-sponsored meetings (http://www.niaid.nih.gov/TOPICS/RADNUC/Pages/radNucMeetings.aspx)

I. BASIC AND TRANSLATIONAL RESEARCH

STRATEGIC PLAN RATIONALE

Basic and translational research improves our understanding of the mechanisms of radiation injury and accelerates the discovery, development, testing, and licensure of promising MCMs to prevent and treat radiation exposure. The scope of this research includes

- Mechanisms of radiation injury at the system, organ, cell, and molecular levels, with particular focus on the hematopoietic, gastrointestinal, immune, pulmonary, renal, reproductive, and nervous systems, and skin
- Mechanisms of secondary responses that mediate, exacerbate, or ameliorate damage
- Identification and characterization of methods to minimize short- and long-term effects

Summary of Continuing and Expanded/Revised Research Goals

Continuing Research Goals

1. Understand the effects of different levels of radiation exposure, with special emphasis on the moderate dose range, i.e., the dose range anticipated in a radiological or nuclear incident.
2. Define the mechanisms of radiation injury, including secondary responses and multisystem injury.
3. Determine mechanisms of protection, mitigation, and treatment.
4. Identify and characterize animal models relevant to human responses.
5. Understand radiation injury at the molecular and cellular level, including the role of inflammation, chronic oxidative stress, neuroimmune interactions, endothelial injury, and other factors that influence the pathophysiological manifestations of radiation injury.
6. Identify new drug candidates and accelerate the transition from candidate to product.
7. Continue to support research on long-term effects of radiation exposure, including epidemiological and longitudinal medical studies of individuals exposed to radiation.

Expanded/Revised Research Goals

1. Address gaps in understanding of the effects of radiation injury on specific target tissues and the pathogenesis of multi-organ dysfunction syndrome (MODS).
2. Leverage intra-agency agreements to maximize the impact of Radiation Countermeasures Program investments.
3. Leverage the full extent of NIH translational resources to maximize the impact of the Radiation Countermeasures Program investments.

Continuing Research Goals—Programmatic and Scientific Accomplishments

Goal 1: Understand the effects of different levels of radiation exposure, with special emphasis on the moderate dose range, i.e., the dose range anticipated in a radiological or nuclear incident.

Radiation injury is characterized by damage to multiple organ systems that results in extremely complex pathophysiology. Given the rarity and heterogeneity of radiation injury in humans, most

of the knowledge of this physiology must be derived from animal models. The RCP supports multiple research programs and projects focused on animal model development and testing. Specific accomplishments include the following:

- The *Centers for Medical Countermeasures Against Radiation (CMCRs),* established by NIAID in 2005, are contributing to the development and testing of new animal models, which include:
  - A canine model to understand the physiological effects of different levels of radiation exposure and develop medical products that will allow dose-appropriate interventions to mitigate the effects of radiation exposure
  - A non-human primate (NHP) model in cynomolgus macaques
  - Prospective studies in rhesus macaques exposed to radiation doses in the high hematopoietic acute radiation syndrome (HE-ARS) range
  - A canine model of gastrointestinal acute radiation syndrome (GI-ARS) and rat and mouse models of renal, pulmonary, and brain injury
- The *Medical Countermeasures Against Radiological Threats (MCART)* consortium (funded since 2005 under a NIAID contract to provide product development support services) has made substantial progress in developing and characterizing relevant animal models of radiation injury. *MCART* has established radiation dose-response ranges and survival curves for HE-ARS and GI-ARS in mice and NHPs receiving supportive care. *MCART* is also developing a partially shielded NHP model for GI-ARS as well as rodent and canine models of internal contamination with radionuclides.

Goal 2: Define the mechanisms of radiation injury, including secondary responses and multisystem injury.

Specific accomplishments include the following:

- The *CMCRs and other grant programs* have enhanced our understanding of changes in gene and protein expression and the metabolome prompted by radiation exposure, the molecular and cellular determinants of radiation injury physiology, and the response of different tissues to such injury. For example,
  - *CMCR* investigators evaluated superoxide dismutase mimetics in reducing chronic oxidative stress and delayed pulmonary tissue injury in rodent animal models. One antioxidant, a magnesium porphyrin derivative, has shown promise and has transitioned to the HHS Biomedical Advanced Research and Development Authority (BARDA) for advanced development.
  - *CMCR* investigators examined the role of the mitochondria in mediating radiation damage and identified and developed new inhibitors of peroxidase activity of cytochrome c/cardiolipin complexes that have promise as radiation MCMs.

- Other *CMCR* projects have illuminated our understanding of radiation injury to multiple organ systems. One project identified signaling modulators that can control and enhance hematopoietic stem cell (HSC) growth, renewal, and regeneration. Another project focused on the role of three immunologic regulators in radiation-induced acute pneumonitis. A third project has made progress in identifying bacterial components and host cellular pattern recognition receptors that mediate and inhibit radiation-induced enterocolitis and is developing agonists and inhibitors that mitigate or treat GI injury.

- *Other grant programs* are elucidating the mechanisms of radiation-induced normal tissue injury, including the role that stromal cell niches in the bone marrow and intestinal crypt may play in enhancing or minimizing radiation damage of susceptible cell populations, and the role that different signal transduction pathways play in modulating radiation damage. Other studies have enhanced our understanding of the DNA damage response, immune cell involvement in the radiation effect, and the ways in which confounding stressors act synergistically to potentiate radiation-induced damage.

- In 2006, the RCP initiated a collaborative work with the *Armed Forces Radiobiology Research Institute (AFRRI)* to identify promising MCMs and characterize cytokine profiles in a representative model of radiation combined injury (RCI), which is radiation injury and concomitant trauma, hemorrhage, sepsis, and/or burns. In 2007, to further accelerate work on RCI, the RCP developed a targeted solicitation and awarded 10 Phase I exploratory/ developmental research grants in this area. Many of the original grantees are now transitioning to the developmental Phase II grants.

- To better characterize the mechanisms of injury and lethality in their rodent and NHP models, *MCART* investigators have monitored hematopoietic parameters and obtained blood cultures, necropsies, and relevant histopathology specimens from all animals. In the GI-ARS models, investigators are assessing changes in intestinal structure and permeability and monitoring animals for signs of fluid loss or infection. They are also defining the complex interactions between different organ systems that characterize severe radiation injury.

Goal 3: Determine mechanisms of protection, mitigation, and treatment.

Goals 2 and 3 are closely related given that the reason for defining molecular mechanisms of injury is first and foremost to identify strategies for protecting, mitigating, or treating such injury. RCP grantees pursue these objectives in tandem. Specific accomplishments of the *CMCR* program include

- The use of normal and knockout mice to understand the involvement of cyclins and cyclin-dependent kinases in radiation-induced apoptosis in hematopoietic cells. These experiments have shown that ablation of certain cyclins and kinases offers partial protection against radiation-induced GI injury. Several projects have studied the role of cyclin E and together the results suggest that agents that reduced cyclin E levels or activity could mitigate the effects of radiation without compromising animal health. Future work will examine the use of compounds that reduce cyclin E and other cyclin-dependent kinases as therapeutic treatments targeting this pathway.

- The influence of alterations in mitochondrial phospholipids (such as cardiolipin) on the radiation damage response, followed by the demonstration that it is possible to modify radiation injury by modulating the peroxidase activity of the cytochrome c/cardiolipin complex or inhibiting oxidation of mitochondrial cardiolipin. JP4-039—a nitroxide free-radical scavenger conjugated to a hemigramicidin S vehicle—enhances cell repair and inhibits apoptosis in both cycling and quiescent cells. New analogs of JP4-039 with different physicochemical characteristics will be synthesized and evaluated for efficacy.

- The study of somatostatin analogues, such as octreotide and SOM230, has shown that these agents may ameliorate radiation-induced gastrointestinal damage and induce protection against radiation-induced multi-organ dysfunction syndrome (MODS) by reducing the levels of pancreatic enzymes in the intestines. This in turn reduces inflammation of damaged mucosal tissue and bacterial translocation across the injured gut.

- Studies at *MCART* demonstrated the significant therapeutic potential of supportive care alone. Supportive care can reduce mortality related to infection, dehydration, bleeding and blood loss, and anorexia and has demonstrated significant improvements in survival in animal models. MCMs can act synergistically or additively with supportive care to further increase improvements in survival.

Goal 4: Identify and characterize animal models relevant to human responses.

The development of well-characterized animal models is essential to the development of radiation MCMs. Since efficacy in humans cannot ethically be evaluated, the U.S. Food and Drug Administration (FDA) has developed the Animal Efficacy Rule (http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=314&showFR=1&subpartNode=21:5.0.1.1.4.9), which provides a route to licensure by permitting the demonstration of efficacy in well-characterized and predictive animal models. A candidate MCM could be approved if, and only if, all of the following conditions are met:

- There is a reasonably well-understood pathophysiological mechanism of the toxicity of the substance and its prevention or substantial reduction by the product.

- The effect is demonstrated in more than one animal species expected to react with a response predictive for humans, unless the effect is demonstrated in a single animal species that represents a sufficiently well-characterized animal model for predicting the response in humans.

- The animal study endpoint is clearly related to the desired benefit in humans, which is the enhancement of survival or prevention of major morbidity.

- The data or information on the kinetics and pharmacodynamics of the product or other relevant data or information, in animals and humans, allows selection of an effective dose in humans.

Under the provisions of the Animal Efficacy Rule, the FDA will review the evidence of product effectiveness from animal studies as the basis to make a determination of presumed efficacy in

humans. Although the Animal Efficacy Rule provides a pathway for licensure of a candidate MCM, it requires elucidation of the mechanisms of radiation-induced toxicities, injuries, mortality, and pathogenesis in animal species, and the demonstration of the linkage and relevance to human responses. In addition, the mechanisms of action of candidate MCMs need to be understood and linked to human responses. This is an extremely difficult and challenging process because it is impossible to study efficacy in relevant human studies. To date, the FDA has not approved any candidate MCMs for radiation exposure by using the Animal Efficacy Rule.

RCP-supported investigators have made important contributions to the development of well-characterized animal models, including the following:

- *MCART* has played a critical role in the development and characterization of animal models and has established the capability to perform studies in compliance with Good Laboratory Practice (GLP) regulations in five laboratories. Well-characterized animal models for use in pivotal animal efficacy studies that will support licensure of new radiation MCMs have been developed across multiple species and at doses and dose rates relevant to the radiation exposure scenarios of concern. For example,

  - Animal models of HE-ARS have been established for rodents and NHPs receiving supportive care. Radiation dose response curves for the primary endpoint (survival) have been established and secondary endpoints—such as neutrophil and platelet levels and signs of infection that may help determine the mechanisms of action—have also been studied.

  - A mouse model of GI-ARS has been established, and an NHP model is in development. Because whole-body radiation exposures that precipitate GI-ARS are also lethal due to HE-ARS, approaches that allow for the study of clinically relevant endpoints are being explored, including partial shielding of bone marrow. Secondary endpoints—such as histology, fluid loss, and signs of infection—that may help determine the mechanism of action of potential MCMs have also been studied. The histology of radiation-induced intestinal damage has been examined both in rodents and in NHPs. In NHPs, injury to epithelia from various regions of the GI tract has also been examined endoscopically over the range of GI-ARS radiation exposures. Animals that survive these radiation exposures are being monitored for signs of lung injury in order to provide preliminary data for the establishment of a GLP NHP lung model.

- The *CMCRs* have also contributed to the development of animal models, both directly and in consultation with *MCART* investigators. Using rodents and canines, investigators have characterized models of radiation toxicity (pathophysiology) to define the natural history of GI-ARS after radiation exposure.

- *CMCR and MCART* investigators have collaborated to develop strategies for studying the delayed effects of acute radiation exposure (DEARE), particularly lung injury. Endpoints such as changes in lung function using biweekly breathing rate assessments and lung perfusion studies as an index of overall lung function have been complemented by a variety of other physiological and imaging measurements. In addition, work has been initiated to

better understand other potential late effects from radiation exposure, including skin complications and cardiovascular effects.

- *AFRRI* investigators—funded through an Interagency Agreement (IAA)—have evaluated and characterized the mini-pig as another potential model of the ARS. If successful, the mini-pig could offer another animal species for demonstration of efficacy.

Goal 5: Understand radiation injury at the molecular and cellular level, including the role of inflammation, chronic oxidative stress, neuroimmune interactions, endothelial injury, and other factors that influence the pathophysiological manifestations of radiation injury.

ARS encompasses diverse processes precipitated by exposure to high doses of radiation. Much of the immediate damage at the cellular level caused by radiation is direct DNA damage such as double strand breaks and non-specific damage mediated through the generation of free radicals and peroxides, and ultimately results in changes in the tissue microenvironment promoting inflammation and precipitating cell death. Inflammation and cell death, in turn, lead to tissue and organ damage that, in patients receiving sufficiently high doses of radiation, can trigger a cascade of events leading to multi-organ failure and death. To design the most effective radiation MCMs, a much deeper understanding of the systems biology of radiation injury is required. Specific accomplishments include the following:

- *CMCR* investigators have increased our understanding of the complexity of the DNA damage response. Cellular responses to radiation-induced DNA damage are mediated by a number of protein kinases, including ATM (ataxia telangiectasia mutated) and ATR (ATM and Rad3-related). The outlines of the signal transduction portion of this pathway are known, but little is known about the physiological scope of the DNA damage response. By performing a large-scale proteomic analysis of proteins phosphorylated in response to DNA damage on consensus sites recognized by the ATM and ATR protein kinases, investigators identified more than 900 regulated phosphorylation sites encompassing more than 700 distinct proteins. This network of phosphorylated proteins in response to DNA damage represents many pathways not previously implicated in response to DNA damage.

- Other investigators have examined the role of pro- and anti-inflammatory molecules and pathways of innate immunity in mediating radiation damage. One promising therapeutic approach suggested by this research involves activation of Toll-like receptor (TLR) signaling pathways, particularly TLR-5. The RCP supported multiple grants that led to the early development of CBLB-502, which is a polypeptide drug derived from bacterial flagellin and a potent TLR-5 agonist. Studies evaluating the effect of the drug on different compartments of the immune system (adaptive, innate immunity, and stromal components) in irradiated rodent and NHP models have shown promise in ameliorating tissue damage and potentially improving overall survival. Cleveland BioLabs, the sponsoring company, has gone on to advanced product development with funding from both the Department of Defense (DoD) and BARDA.

- *CMCR* investigators are elucidating the role of inflammatory molecules (IMs) in mediating radiation injury and exploring their potential as biomarkers. The development of IMs as biomarkers could allow treating clinicians to predict radiation damage to organs based on

their levels of IM expression, use IMs as an index to evaluate treatment effects, and rank the mitigation provided by new agents quantitatively in various settings.

Three strains of mice have been evaluated for changes in IMs following irradiation at different doses in models of total and partial body exposure. The results indicate that

- Radiation alters the cytokine expression profiles in a dose-related manner.
- IM response to irradiation is highly time dependent and varies across strains.
- IM profiles are very different if even a small fraction of the bone marrow is protected.
- IMs appear to respond to and reflect intervention.

Cytokine profiles also may prove useful in identifying individuals with heightened natural sensitivity to radiation-related chronic inflammatory toxicity. As a tool to facilitate assessment and early therapeutic intervention, investigators are now developing a panel of cytokine/growth factor proteins that play a critical role in the progression of pulmonary injury following radiation exposure.

- Experimental studies have demonstrated that supportive cells within the bone marrow niche (stromal cells, endothelial cells) are also severely damaged by exposure to high doses of ionizing radiation. Initial studies by *CMCR* investigators suggest that systemic administration of vascular endothelial cells provides support to bone marrow progenitor cells, induces the regeneration of primitive stem/progenitor cell populations, and accelerates the recovery of mature peripheral blood counts. Thus, targeted replenishment of the endothelial cell activity may be a novel and viable strategy to accelerate hematological recovery following myelosuppressive chemotherapy or radiotherapy. Other RCP-supported grantees are studying pro-angiogenic factors, such as SDF-1 and FGF-4, that can reconstitute and/or stabilize the vascular niche, leading to rapid restoration of function and improved engraftment of transplanted stem cells after irradiation.

Goal 6: Identify new drug candidates and accelerate the transition from candidate to product.

RCP-supported research has contributed substantially to the ultimate goal of promoting the development of radiation MCMs. Currently funded efforts range from high-throughput screens of molecule libraries to definitive efficacy evaluations of lead molecules in rigorously controlled trials. Specific accomplishments include the following:

- *CMCR* investigators developed a high-throughput, small-molecule *in vitro* screening program to identify molecules demonstrating radioprotectant effects. They have tested more than 100,000 compounds and identified 26 for further evaluation. Using a mouse model, seven of these compounds have shown promising efficacy in initial *in vivo* screens when administered up to 24 hours after a lethal dose of radiation. In addition, across the *CMCR* program, more than 120 other MCMs in various stages of development have been evaluated in models of hematopoietic, gastrointestinal, lung, skin, kidney, cardiovascular, or central nervous system injury.

- More than 50 other MCMs are being tested and characterized under other grants, contracts, and IAAs. Among these are MCMs to treat or mitigate RCI, GI-ARS, and radiation-induced thrombocytopenia, lung, and skin injury. Other MCMs include novel decorporation agents and oral formulations of diethylenetriaminepentaacetic acid (DTPA), an FDA-approved chelating agent that currently is available in intravenous or inhalation formulations, which are not practical for mass casualty administration.

- *MCART* has completed a non-pivotal GLP study of granulocyte colony stimulating factor (G-CSF; Neupogen) in rhesus macaques that demonstrated a significant survival benefit in animals treated with G-CSF and supportive care as compared with animals receiving supportive care alone. *MCART* is poised to begin two further studies: one with G-CSF to determine whether it retains efficacy when initiation of therapy is delayed until 48 hours (compared to 24 hours) after irradiation and another with pegylated G-CSF (Neulasta) to assess efficacy when administered 24 or 48 hours post-irradiation. *MCART* has also completed several developmental studies of thrombopoietic agents in canines and is mapping out pathways for further work on these agents.

- The RCP has met with approximately 130 companies since 2004 and screened almost 40 compounds in rodent models of HE-ARS, 8 compounds for GI-ARS, and 4 compounds for efficacy in decorporating various radionuclides including americium-241 and plutonium-239. Twenty investigators, groups, or companies that received critical seed funding or other support from the RCP have gone on to receive funding from BARDA for further development of specific products.

Goal 7: Continue to support research on long-term effects of radiation exposure, including epidemiological and longitudinal medical studies of individuals exposed to radiation.

While the main focus of the RCP has been to develop MCMs for acute radiation injury and internal contamination with radionuclides, the program has supported a range of projects investigating the long-term effects and epidemiology of radiation exposure. These projects have encompassed observational and interventional studies in animals and long-term studies in selected human populations, including atomic bomb (A-bomb) survivors in Hiroshima.

An area of particular interest is the long-term effect of radiation on immune function. It is well-established that radiation exposure, whether intentional (e.g., diagnostic tests and radiotherapy cancer treatments) or unintentional (e.g., industrial accidents and terrorist attacks), can accelerate the process of immune aging. This process, also known as immune senescence, causes a decline in the body's ability to fight infections, respond to vaccinations, and suppress cancer development. As such, immune senescence is a major underlying cause of disease and death among the elderly. Specific accomplishments in this area include the following:

- In fiscal year 2009, the RCP awarded a 5-year contract to the *Radiation Effects Research Foundation (RERF),* Japan, to lead a joint United States–Japan effort to study the effects of radiation and aging on the human immune system. Using state-of-the-art technology, this research program employs two complementary approaches to determine how radiation exposure alters the normal age-related decline of the immune system and to identify the cellular and molecular changes that occur. The first approach is to analyze blood samples

from a unique cohort of A-bomb survivors who were exposed to varying levels of radiation in 1945, and the second approach is to validate these observations in a variety of small animal models, including mice. The significance of the program rests on the exploitation of the large and unique database and set of biosamples from a 62-year follow-up of A-bomb survivors by *RERF,* the integration of complementary animal and cellular mechanistic studies to help guide the studies with human samples, and the synergistic efforts of the co-investigators from Japan and the United States.

The program consists of three distinct projects focused on

- The long-term effects of radiation exposure on HSCs and their microenvironment
- Dendritic cell number, response, and function
- The response to immunogenic challenge with influenza vaccine

A fourth project will attempt to develop a comprehensive scoring system aimed at providing predictive tools for radiation effects and aging. A fifth optional project, if funded, will focus on the effects of radiation and aging on the structure and function of the human thymic microenvironment.

- The RCP funded other studies of the long-term effects and risks of radiation exposure through a 5-year IAA with the National Cancer Institute (NCI) that ended in FY 10. This agreement provided funds to the Radiation Epidemiology Branch (REB) within the Division of Cancer Epidemiology and Genetics as well as to NCI intramural investigators. Funded projects leveraged the unique expertise of REB in radiation dosimetry and epidemiology and the expertise of NCI intramural investigators.

  REB dosimetry studies focused on developing, refining, and testing tools and methods to improve dose assessments from radioactive fallout and radioactive materials released into the environment from accidents or damaged nuclear facilities. REB investigators working in the Marshall Islands used a model developed by the National Oceanic and Atmospheric Administration to retrospectively predict fallout trajectories and deposition by year, radionuclide, and atoll. They analyzed historical dosimetry and deposition data, including historical bioassay data, to estimate external dose, internal dose, and cancer risk for exposed Marshallese. REB investigators also continued to improve the NCI I-131 Individual Dose and Risk Calculator for Nevada Test Site Fallout by incorporating external dose and internal dose from radionuclides.

  REB epidemiology studies conducted in the Ukraine and Kazakhstan focused on refining risk estimates for thyroid and other cancers in persons living in proximity to Chernobyl (and exposed to fallout after the 1986 accident) or Semipalatinsk (and exposed to fallout from nuclear weapons testing) and on analyzing the efficacy of potassium iodide in reducing the incidence of thyroid cancer in populations exposed to fallout from Chernobyl. An NCI intramural investigator funded through this IAA has been studying the ability of nitroxides such as Tempol to inhibit radiation-induced carcinogenesis in irradiated mice.

- Through the *CMCRs,* the RCP is also supporting studies of the mid- to long-term effects of varying doses of whole-body gamma irradiation in NHPs and dogs. Using surviving control animals from prior studies, these studies will establish a comprehensive longitudinal database with detailed assessment on the long-term effects of radiation for cohorts of dogs and NHPs that have survived high doses of irradiation.

  CMCR investigators also are evaluating the benefits of metalloporphyrin therapy in mitigating radiation-induced lung injury and the long-term effects of irradiation on immune function in irradiated rhesus and cynomolgus macaques. Other *CMCR* investigators are following dogs that achieved endogenous hematopoietic recovery after total body irradiation with 6 to 9 Gray (Gy). Long-term studies with these animals will monitor survivors for

  - Hematopoietic and immune function
  - Development of leukemia and lymphoma as well as solid tumor cancers
  - Development of radiation fibrosis of the lung, gastrointestinal tract, and kidneys
  - Genomic expression changes
  - Specific clinical abnormalities that affect the health and physiologic function of surviving dogs

  In addition to the long-term observations, the animals from the long-term survivor studies will undergo extensive necropsy and histopathology analysis. Although the number of animals included is modest and studies are observational, the work is significant because there have been few if any long-term studies in large animals receiving high-dose exposures.

Expanded/Revised Research Goals

RCP efforts over the last 5 years have enhanced understanding of radiation-induced changes in gene and protein expression and the metabolome, the molecular and cellular determinants of radiation injury physiology, and the response of different tissues to such injury following whole-body radiation exposure. The scenarios of concern, however, could also result in neutron exposure in addition to gamma exposure, internal contamination with radionuclides, and partial body exposures at different doses and dose rates.

Given our incomplete understanding of the effects of radiation injury following different exposure scenarios leading to different patterns of organ injury, continued RCP support for basic and translational research is necessary to enhance knowledge of the systemic effects of radiation injury under the following different exposure scenarios:

- Radiation combined with trauma, burn, and sepsis injury
- Radiation injury in special populations including pediatric, geriatric, and immune compromised individuals
- Mechanism of action of promising therapeutics and mitigating agents

Therefore, three expanded/revised research goals have been added.

Expanded/Revised Goal 1: Address gaps in understanding of the effects of radiation injury on specific target tissues and the pathogenesis of multi-organ dysfunction syndrome (MODS).

Although substantial progress has been made in defining mechanisms and pathways for radiation-induced cellular and tissue damage, most studies have focused primarily on identifying treatments for HE- and GI-ARS. Work on other late effects of radiation exposure—such as lung, skin, cardiovascular and kidney injury—is in its early stages and needs to be continued to better define damage pathways (e.g., inflammation and fibrosis) in these other organ systems. Furthermore, additional research efforts are needed to elucidate the pathogenesis of MODS, a hallmark of radiation-induced mortality, in order to develop novel therapeutic approaches.

Expanded/Revised Goal 2: Leverage intra-agency agreements to maximize the impact of Radiation Countermeasures Program investments.

The RCP will maintain existing relationships with NCI, the National Institute on Aging (NIA), and the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), and will seek new opportunities to support intramural and extramural research initiatives that align with the program's mission. These collaborations will maximize the discovery potential of the collective research funding while benefiting common programmatic goals. Specifically, NCI's programs on normal tissue protection and treatment of injuries caused by radiation therapy may uncover MCMs that are useful for radiation injuries resulting from a terrorist attack or accidental exposure, and vice versa. NIA's studies on immune senescence and the long-term effects of radiation exposure in aging populations (e.g., A-bomb survivors), as well as NIDDK's joint investment in defining proliferation mechanisms and surface markers for stem cells within the gastrointestinal crypt, may help explain how radiation causes damage to specific pathways and cell lineages, and identify pathways and opportunities for interventional therapies.

Expanded/Revised Goal 3: Leverage the full extent of NIH translational resources to maximize the impact of Radiation Countermeasures Program investments.

The RCP will seek new partners to pursue joint projects of mutual interest and high value to the NIH mission, while also seeking to take full advantage of the enhanced translational resources and services that NIH now supports. NIH has invested heavily in resources and programs to develop and speed translational research, building up the capabilities of, among others, the National Center for Advancing Translational Sciences (NCATS), the NCI Experimental Therapeutics program, the Bridging Interventional Development Gaps (BrIDGs) program, and Small Business Innovation Research (SBIR) grants. A particularly strong opportunity for collaboration exists at academic institutions that have received both *CMCR* and Clinical and Translational Science Awards. The RCP will seek to leverage these resources fully during 2012–2016, actively link investigators and companies to relevant opportunities, and provide close stewardship to grantees, contractors, and other partners to ensure understanding of the relevance and value of such resources and services to their product development efforts.

II. RADIATION BIODOSIMETRY

STRATEGIC PLAN RATIONALE

Biodosimetry assays and techniques are used to determine and quantify radiation exposure in individuals. Biodosimetry provides biological information, such as dose and or type of radiation-related injury, necessary for medical decision making. There are a few biodosimetry assays and technologies currently available to assess radiation exposure in small numbers of individuals. However, in the event of a mass casualty radiation or nuclear incident, the majority of biodosimetry measurements will take place in acute or emergency care settings. Therefore, biodosimetry assays and technologies need to be developed for rapid dose assessment of large numbers of individuals within a short period of time.

Summary of Continuing and Expanded/Revised Research Goals

Continuing Research Goals

1. Support rigorous quality assurance/quality control studies of current leading biodosimetry technologies to validate their use.
2. Increase the speed and efficiency of current assays to determine radiation doses received due to internal or external contamination with radioactive material.
3. Develop new bioassays that can provide rapid and accurate radiation dose assessments, enabling optimal triage and medical management.
4. Develop biodosimetry tools and assays to evaluate radiation-related injury and the recovery process in different physiological systems.
5. Develop and validate bioassay methods to estimate radiation dose and future risk following exposure to radioactive material by various routes, including inhalation, ingestion, skin contact, or contamination of wounds.

Expanded/Revised Research Goals

1. Address issues relating to biodosimetry and countermeasure use in the setting of heterogeneous exposure, variable health status, differences in gender and age, and other confounders.
2. Support research and development of predictive biomarkers and biodosimetry.

Continuing Research Goals—Programmatic and Scientific Accomplishments
Goal 1: Support rigorous quality assurance/quality control studies of current leading biodosimetry technologies to validate their use.

Radiation experts regard the lymphocyte metaphase-spread dicentric chromosome assay (dicentric assay) as the gold standard for radiation biodosimetry, and the assay has gained renewed importance in this era of heightened concern about mass casualty events involving radiation. In 2004, the International Standards Organization (ISO) issued an international standard, *ISO 19238: Performance Criteria for Service Laboratories Performing Biological Dosimetry by Cytogenetics,* which established regulatory compliance standards for the dicentric assay and addressed quality assurance and quality control to permit laboratory accreditation.

Performance of the dicentric assay, however, requires specialized training and is labor- and time-intensive, which limits the throughput of the current assay in any one laboratory.

The NIH Radiation Countermeasures Program (RCP) played a critical role in validating the dicentric assay for use across laboratories by funding the first major inter-laboratory comparison study of the dicentric assay since 1987. This study involved five laboratories in four countries, was conducted in compliance with the new ISO standard, and measured the performance characteristics, variability in calibration curves, and dose prediction accuracy of the dicentric assay among the different participating laboratories. Dose-blinded samples were irradiated in a single laboratory and provided to the participating laboratories. The percentage error in the estimated doses for individual samples ranged from ±15 percent of the actual radiation dose, with 13 of 20 dose predictions within 10 percent of the true dose. By expert consensus, dose estimations with an error of ±20 percent are considered sufficient for guiding the clinical management of patients. The results of the study validated the dicentric assay and demonstrated the feasibility of an international biodosimetry network as a mechanism for expanding surge capacity for an event producing mass radiation exposure.

In December 2007, the World Health Organization (WHO) convened an expert panel on the development of a global biodosimetry laboratory network (BioDoseNet) that could provide dose estimates for a large number of victims in the event of a radiation emergency. In recommending that WHO adopt the dicentric assay to serve as the basis for dose assessment within the network, the expert panel specifically cited findings of the RCP-funded inter-laboratory comparison study. In 2008, ISO issued a new standard, *ISO 21243: Performance Criteria for Laboratories Performing Cytogenetic Triage for Assessment of Mass Casualties in Radiological or Nuclear Emergencies,* that defines the process and identifies quality control standards for the use of cytogenetic methods to rapidly assess radiation dose and which WHO has adopted as the basis for cytogenetic triage protocols.

Because the dicentric assay requires 3 to 4 days to complete and obtain results and there are only two U.S. labs that could process potentially 50 to 100 samples per day, future activities and efforts will be focused on overcoming these limitations by supporting research and development that will increase throughput and decrease processing time.

Goal 2: Increase the speed and efficiency of current assays to determine radiation doses received due to internal or external contamination with radioactive material.

Because of its inherently limited throughput, the classic dicentric assay is poorly adapted for use in mass casualty events. While validating the assay and creating BioDoseNet clearly enhances our preparedness, these are interim steps. An event resulting in mass radiation exposure could still quickly overwhelm current global laboratory capacity. To meet the requirements for dose assessment that might arise after such an event, overall testing capacity must be greatly increased. The RCP has thus invested in improving and optimizing current techniques. Specific accomplishments include the following:

- Through a long-standing Interagency Agreement (IAA) with the *Armed Forces Radiobiology Research Institute (AFRRI),* the RCP has supported efforts to automate the dicentric assay. *AFRRI* has automated sample preparation steps, which has increased the number of

specimens that can be prepared from 50 specimens per week using the old manual methods to approximately 1,000 samples per week using the new automated techniques. *AFRRI* investigators have developed an automated system for scoring dicentric chromosomes based on current state-of-the art robotic microscopy and other "walk away" hardware and software. Automating and validating techniques for image acquisition and analysis could greatly increase the throughput of existing cytogenetics laboratories, enhancing the value of BioDoseNet.

- The NIAID *Centers for Medical Countermeasures Against Radiation (CMCRs)* are engaged in research to improve current biodosimetry approaches. For example,

  – Electron paramagnetic resonance (EPR) spectroscopy is a well-established physical dosimetry technique that has been used to assess exposures in calcified tissues. Conventional EPR dosimetry involves the extraction and isolation of tooth enamel. Although well-suited for certain applications, this technique is too cumbersome and invasive for large-scale biodosimetry. Simplified approaches allowing *in situ* measurements would greatly expand the potential for using EPR for mass screening after a radiation disaster.

  – Investigators affiliated with the *CMCR* program have developed instruments and procedures for performing EPR dosimetry *in vivo* and are now working to miniaturize and improve the rapidity, accuracy, and other performance characteristics of these instruments. *In vivo* measurements have been performed using molar, premolar, and canine teeth, and current protocols allow individual measurements in 5 minutes with a detection threshold as low as 0.5–1 Gy. Future plans include development of a fully portable version of the biodosimeter for use in the field and techniques to measure clinically significant doses in nail clippings and hair. The EPR spectroscopy system has transitioned to BARDA for advanced development funding.

  – Using advanced robotics and image acquisition technology, *CMCR* investigators have developed a fully automated, modular, ultra-high throughput biodosimetry workstation, called the Rapid Automated Biodosimetry Tool or RABIT. The RABIT automates the well-established micronucleus and γ-H2AX assays that have been shown to be highly accurate and specific over a broad range of radiation doses. The RABIT has achieved a throughput of ~6,000 samples per day and is designed to potentially accommodate as many as 30,000 samples per day, using small volumes of blood obtained from a lancet fingerstick. The RABIT design has already transitioned to BARDA for advanced development funding.

Goal 3: Develop new bioassays that can provide rapid and accurate radiation dose assessments, enabling optimal triage and medical management.

While refining, enhancing, and automating existing biodosimetry assays is important, the RCP has also invested in the development of wholly new approaches to radiation dose and injury assessment. These approaches leverage new tools for genomic, proteomic, and metabolomic analysis as well as (where feasible) advances in miniaturization and lab-on-a-chip technologies to create portable, point-of-care diagnostics. Specific accomplishments include the following:

- Through work conducted at several *CMCRs,* investigators have

  – Established that exposure to radiation causes highly specific and durable changes in gene expression and that clinically useful information can be derived from analyzing peripheral blood samples.

  For example, using gene expression microarrays that evaluated 41,000 different transcripts, investigators have identified gene expression signatures that can predict radiation exposure dose without the need for a pre-exposure sample. The investigators are now developing a series of radiation arrays containing small numbers of genes that will differentiate radiation-related injury types and doses in whole blood and adapting these arrays to a microfluidic platform. The goal is a self-contained, low-cost radiation biodosimeter suitable for deployment in a radiation emergency. The scientific results and technology platform have progressed in parallel, and this project has now transitioned to BARDA for advanced development funding.

  – Characterized gene expression signatures in mouse peripheral blood cells that accurately distinguish irradiated from non-irradiated animals and different levels of radiation exposure in mice and characterize any heterogeneity with respect to gender, genotype, and time from exposure. Extending the analysis to humans, investigators collected peripheral blood samples from healthy individuals, patients who were undergoing total body irradiation as conditioning for hematopoietic stem cell transplantation, and patients receiving alkylator-based chemotherapy conditioning alone. Investigators identified a signature profile of 25 genes that predicted the healthy individuals and pre-irradiation patients with 100 percent accuracy and the irradiated patients with 91 percent accuracy. The profile also discriminated between patients receiving radiation and chemotherapy treatment with a high degree of accuracy. Considering the entire population, the overall accuracy of the signature profile as a predictor of radiation exposure was 90 percent.

  Because the peripheral blood predictors of radiation exposure change over time, the next step is to develop reference lists of genes that are specific for different time points.

  – Performed microarray analyses of human T-cells and whole blood to identify candidate genes with dose-dependent changes in expression at different time points (3, 8, and 24 hours) following *in vitro* radiation exposure. Using these data, quantitative real-time reverse-transcription polymerase chain reaction (qRT-PCR) assays have been developed and optimized for 14 candidate genes identified in the preliminary microarray studies. These genes have now been validated in an *in vitro* human radiation model. The next step is to validate these qRT-PCR assays in an *in vivo* canine radiation model and develop a small cellular phone-sized chamber that can automatically perform qRT-PCR within 2 to 3 hours for the genes induced by specific radiation doses.

  – Explored proteomic approaches to biodosimetry. Using commercially available antibodies, the first phase of this project performed Western blots against protein lysates from both lymphocytes and lymphoblastoid cell lines to identify proteomic changes in response to radiation. From this screen, investigators identified 55 radiation-responsive proteins, including 14 proteins that had not been previously reported. The project is now

developing enzyme-linked immunosorbent assays (ELISAs) to five of these proteins that will be used to evaluate radiation exposure in a lateral flow diagnostic device. cDNA clones of the five proteins have been put into expression vectors that will generate recombinant proteins for use as standards in the ELISAs.

A public-access Web site for this biodosimetry project (http://labs.fhcrc.org/paulovich/biodose_index.html) has been established to display the large amount of generated data.

- NIAID-funded intramural scientists at the National Cancer Institute (NCI) are working to identify novel metabolomic biomarkers of radiation exposure on an ultra-performance liquid chromatography-time-of-flight mass spectrometry (UPLC-TOFMS) platform. Investigators have analyzed more than 2,000 mouse urine samples and demonstrated proof of concept by successfully detecting biomarkers of gamma radiation over a wide range of radiation doses. All of these candidate radiation biomarkers display dose- and time-dependent excretion, peaking in urine at 8 to 12 hours and returning to baseline by 36 hours after exposure. In urine samples obtained from 13 humans, differences were detected in pre- and post-exposure urines, although potential palliative drug treatment confounders could not be ruled out.

  These results suggest that biomarkers identified through metabolomic approaches offer a noninvasive means of screening large populations in relatively short periods, providing that a robust radiation metabolomic signature with unambiguous dose–response characteristics can be developed and a portable device suitable for use in the field by first responders can be designed, built, tested, and validated. The ultimate goal of this project is to develop methods for rapid, noninvasive radiation biodosimetry using easily accessible body fluids such as urine, saliva, or sweat.

- In addition, through individual research project grants, *CMCR* pilot projects, and other mechanisms, the RCP is supporting research on many other approaches to radiation injury assessment and biodosimetry. These include

  – High-throughput flow cytometry of immature red blood cells to assess the radiation dose received by bone marrow

  – Assessment of DNA damage in cells of the dermis and epidermis by measuring micronuclei in skin fibroblasts and accumulations of proteins associated with radiation damage in the nuclei of other cells

  – Assessment of the risk of fatal sepsis in neutropenic animals based on plasma concentrations of soluble CD16b

  – Assessment of radiation dose received by measuring the expression of proteins such as γH2AX and pChk2 in exfoliated oral epithelial cells

- Assessment of radiation-induced mitochondrial DNA deletions and changes in copy number

- Development of rapid diagnostic assays and portable, noninvasive instruments to measure radiation dose to intact teeth based on optically stimulated luminescence

Goal 4: Develop biodosimetry tools and assays to evaluate radiation-related injury and the recovery process in different physiological systems.

In addition to supporting the development of new biodosimetry techniques and devices for overall dose estimation, there is a critical need to develop biomarkers of radiation-related injury and devices that will predict the acute and/or delayed damage to specific organs and tissues. Having such tools will facilitate the accurate and timely administration of radiation medical countermeasures (MCMs).

Several factors account for the difficulty and complexity of evaluating radiation-related injury, including the following:

- Radiation injury can take days or weeks to present clinical manifestations, and some of the delayed radiation injuries (such as fibrosis and carcinogenesis) may not manifest clinically for months or years.

- Individuals may differ in their sensitivity to radiation for a variety of reasons, including age, body size, immune status, and genetic constitution.

- The severity of injury to individual organs and tissues varies with

  - Dose and dose rate

  - Quality of radiation (low versus high linear energy transfer)

  - Heterogeneity of exposure (partial versus total body)

  - Source of exposure (external radiation exposure versus internal contamination)

- The severity of injury to individual organs and tissues may be modulated by the "bystander effect" (radiation-induced effects in unirradiated cells) and the host's adaptive response to prior radiation exposure.

Unfortunately, existing and currently developed biodosimetry techniques and devices cannot assess such variability. Furthermore, these techniques and devices do not predict the severity of injury sustained by specific organs and tissues and thus do not allow for prompt organ- and tissue-directed medical treatment that might be provided by future radiation MCMs. In addition, few if any radiation-specific biomarkers that predict the early acute and chronic delayed radiation injuries to different organs and tissues have been characterized and validated.

Therefore, in addition to supporting the development of new biodosimetry techniques and devices for overall dose estimation, there is a critical need to develop biomarkers of radiation

exposure and injury and devices that will predict the acute and/or delayed damage to specific organs and tissues and facilitate the accurate and timely administration of radiation MCMs. While recognizing the long-term importance of such work, only limited progress in this area has been made since the inception of the RCP. Specific accomplishments include the following:

- *CMCR* investigators are using analysis of plasma cytokines and computerized image analysis of immunostained tissue to develop a model of the delayed effects of acute radiation exposure (DEARE) on the respiratory tract at doses below 10 Gy. The data analysis has identified the combination of two cytokines (IL-6 and KC) as potential biomarkers of radiation exposure > 2Gy. Studies are currently in progress to identify patterns of cytokine expression that may be specific for lung injury. The study of cytokines as biomarkers for lung damage in the presence of radiation and skin burn is also underway.

- A *CMCR* pilot project evaluated the urine proteome to identify biomarkers of radiation injury. Liquid chromatography-mass spectrometry analysis of urinary proteins in rats 24 hours after receiving 10 Gy total body irradiation (TBI) identified significant changes in the urinary proteome without a detectable increase in total urinary protein. In subsequent research evaluating the differential effects of TBI versus local kidney irradiation (LKI) on the urinary proteome, the investigators identified 42 and 103 urinary proteins found only in the settings of TBI and LKI, respectively. Gene ontology analysis of the specific proteins identified also showed differences between the TBI and LKI groups in terms of the molecular function and biological processes of the proteins unique to each group. This pilot study strongly suggests that the urinary proteome may be useful in advancing our understanding of the differences between homogeneous, inhomogeneous, and organ-specific irradiation.

- In an effort to lay the groundwork for future initiatives in this area, the RCP sponsored a two-day workshop titled "Predicting Individual Radiation Sensitivity: Current and Evolving Technologies" at Columbia University in March 2008. The workshop focused on recent research and challenges in predicting acute and long-term radiation injury based on individual radiation sensitivity. Future initiatives in this area could support research and development of novel biomarkers and devices to predict acute and delayed radiation injuries to different physiological systems. Such biomarkers and devices would likely be useful for triage and for making prompt treatment decisions in persons exposed to significant doses of radiation.

Goal 5: Develop and validate bioassay methods to estimate radiation dose and future risk following exposure to radioactive material by various routes, including inhalation, ingestion, skin contact, or contamination of wounds.

The HHS Public Health Emergency Medical Countermeasures Enterprise (PHEMCE) identified the Centers for Disease Control and Prevention (CDC) as the organization with the responsibility to develop and maintain bioassays capable of determining the levels of exposure and/or internal contamination with radionuclides. CDC has developed capabilities to measure levels of internal contamination with a variety of radionuclides in urine samples (see Laboratory Information for Radiation Emergencies at http://emergency.cdc.gov/radiation/labinfo.asp). Therefore, research and development of radionuclide bioassays is under the purview of CDC and is no longer a priority for NIAID.

Expanded/Revised Research Goals

Expanded/Revised Goal 1: Address issues relating to biodosimetry and countermeasure use in the setting of heterogeneous exposure, variable health status, differences in gender and age, and other confounders.

Studies to date have laid the groundwork for high-throughput biodosimetry techniques and radiation MCM development following acute whole-body gamma irradiation of healthy adult individuals. However, given the highly diverse populations that would be affected and the heterogeneous exposures that would occur, diagnosing radiation-induced injury in real-world scenarios would present far greater complexities than this simple scenario suggests, including the following:

- Depending on the scenario, a radiological or nuclear incident could be associated with exposure to gamma rays, beta emitters, neutron/gamma or other mixed field exposures, internal contamination with radionuclides, or a combination of these, at variable dose and duration rates.

- Among the individual factors determining the outcomes associated with a particular exposure are the presence or absence of other injuries, percentage of the body irradiated, which vital organs are irradiated, age and gender of the person exposed, and the presence or absence of other underlying medical conditions.

- Another critical research need is the development of pediatric biodosimetry tools that could be utilized for rapid triage purposes as well as for an accurate assessment of absorbed radiation and the effects on specific organ systems. Where possible, pediatric samples will be characterized and evaluated using the new methods and technologies that are in development.

Thus, there is a critical need to validate the use of high-throughput biodosimetry approaches now under development in variable exposure settings and populations. Relevant animal models need to be developed to validate biodosimetry techniques in special populations.

Expanded/Revised Goal 2: Support research and development of predictive biomarkers and biodosimetry.

Radiation injury can take days or weeks to present clinical manifestations, and some of the delayed radiation injuries (such as fibrosis) may not manifest clinically for months or years. Moreover, individuals may differ in their sensitivity to radiation for a variety of reasons, including age, body size, immune status, and genetic constitution. Because existing and currently developed biodosimetry techniques and devices cannot assess such variability and do not predict the severity of injury sustained by specific organs and tissues, they do not allow for the prompt organ- and tissue-directed medical treatment that might be provided by future radiation MCMs.

After a large scale radiological event, such as the detonation of an improvised nuclear device in which tens of thousands of individuals would be exposed to high doses, it would be highly advantageous to be able to triage individuals based on their actual risk of developing acute radiation syndrome (ARS) or other manifestations of radiation injury. Therefore, in addition to techniques and tools to quantify the radiation dose received, there is a critical need to develop

radiation-exposure biomarkers and devices that have the ability to predict the acute and/or delayed damage to specific organs and tissues and thereby facilitate precise and timely medical interventions.

Specific biomarkers of radiation injury (genomic, proteomic, and metabolomic) need to be developed to assess and diagnose radiation injury, including in special populations. Additionally, relevant animal models need to be developed to identify and characterize such biomarkers of radiation injury in special populations.

III. FOCUSED PRODUCT DEVELOPMENT FOR RADIOLOGICAL MEDICAL COUNTERMEASURES

STRATEGIC PLAN RATIONALE

In 2005, the Strategic National Stockpile—a national repository of vaccines, antibiotics, chemical antidotes, and other essential materials needed for the medical response to a terrorist attack—included only a limited number of medications specific for radiation exposure. Three general categories of promising candidate compounds were defined to be moved expeditiously through development, licensure, and stockpiling:

- Radioprotectants, such as scavengers that help prevent injury from radiation-induced free radicals and other reactive species
- Radiation mitigators that reduce the potential severity of the injury
- Radiation therapeutics that are given after overt symptoms develop to reduce pathophysiological radiation effects, facilitate tissue recovery or repair, or reverse fibrosis and other late effects

Prior to 2005 and the establishment of the NIH Radiation Countermeasures Program (RCP), a limited number of products (the majority of which are chelating or blocking agents) had been approved to prevent or mitigate radiation injury. Prussian blue, calcium diethylenetriaminepentaacetic acid (DTPA), zinc DTPA and a liquid formulation of potassium iodide (liquid KI) had been approved as the result of specific efforts by the Food and Drug Administration (FDA) to expand the armamentarium against radiological and nuclear threats, and all of these products had been added to the Strategic National Stockpile.

The licensure of these products has significantly enhanced our preparedness for incidents involving mass exposures to cesium-137, isotopes of plutonium and americium, and radioiodines. Another approved product, amifostine, is a powerful radioprotectant that is indicated for the prevention of xerostomia in patients receiving radiotherapy for head-and-neck tumors. Unfortunately, amifostine provides little if any benefit when administered following radiation exposure and has been deemed unsuitable for use by first responders because of its substantial toxicity. No products have been approved for the mitigation or treatment of ARS.

Summary of Continuing and Expanded/Revised Research Goals

Continuing Research Goals

1. Initiate studies under the FDA Animal Efficacy Rule to expand the label indications for licensed products that are effective in preventing or treating radiation injury.
2. Facilitate the development of products that are already in the clinical testing stage for other indications and that can prevent or treat radiation injury.
3. Support research on promising compounds that are currently in preclinical development.

4. Identify and develop new drugs to prevent or reduce the severity of radiation-induced damage and to treat irradiation casualties.
5. Develop drugs that will prevent delayed effects, such as pneumonitis and fibrosis, and long-term consequences, such as mutagenesis or fibrosis.

Expanded/Revised Research Goals

1. Provide data to guide the use of granulocyte colony stimulating factor (G-CSF) and/or pegylated G-CSF in a mass casualty situation.
2. Advance oral DTPA and novel decorporation agents to the point of Investigational New Drug (IND) submission.
3. Continue development and validation of pivotal animal models for demonstration of efficacy in radionuclide decorporation.
4. Use animal models developed in multiple species to evaluate the efficacy of candidate radiation medical countermeasures for hematopoietic and gastrointestinal acute radiation syndrome (HE- and GI-ARS), radiation-induced lung and skin injury, radiation combined injury, and radionuclide decorporation and to inform IND submissions.
5. Engage and find innovative ways to collaborate with companies, academia, or government entities in the development of new candidate medical countermeasures.
6. Extend interagency collaborations with the Department of Health and Human Services (HHS) Biomedical Advanced Research Development Authority (BARDA) and the Department of Defense (DoD) to establish seamless pathways for product development and licensure of radiation medical countermeasures.

Continuing Research Goals–Programmatic and Scientific Accomplishments
Goal 1: Initiate studies under the FDA Animal Efficacy Rule to expand the label indications for licensed products that are effective in preventing or treating radiation injury.

Under the provisions of the Animal Efficacy Rule, the FDA will review the evidence of product effectiveness from animal studies to make a determination of presumed efficacy in humans. Understanding the mechanisms of radiation injury and the mechanisms of actions of candidate MCMs is essential to establish linkages across animal species and humans. (See Section 1, Goal 4 for description of the Animal Efficacy Rule).

In the field of radiobiology, a variety of animal models such as rodent, canine, and non-human primate (NHP) have been used to study the effects of radiation exposure and to test the efficacy of candidate MCMs. Among small animal models, mice are commonly used to evaluate the effects of total or partial body external exposure, while rats are used to study the effects of internalized radionuclides. Among large animal models, canine models of stem cell transplantation are well established. For the study of HE-ARS, current practice favors the use of NHPs (specifically, rhesus macaques) as the best large animal model surrogate of human responses to high-dose exposures.

A substantial and consistent body of published and unpublished data demonstrates that radiation damage to the hematopoietic system in the rhesus has a strong clinical resemblance to corresponding injuries in humans. Because rhesus can tolerate clinically relevant treatment and supportive care procedures and withstand sequential, invasive monitoring techniques for assessing clinically relevant endpoints, this species is used in a variety of studies and study

designs. Moreover, numerous investigators have studied the treatment and mitigation of the HE-ARS by recombinant human growth factors in this species.

Building on this base, the RCP has dedicated significant resources to developing reliable animal model protocols and a research and development infrastructure for the performance of pivotal trials under the FDA Animal Efficacy Rule. Progress includes the following:

- As reported in Section I, the *Medical Countermeasures Against Radiological Threats (MCART)* consortium has completed a non-pivotal Good Laboratory Practice (GLP) study of G-CSF (Neupogen) in rhesus macaques that demonstrated a significant survival benefit in animals treated with G-CSF and supportive care as compared with animals receiving supportive care alone. A pivotal GLP study in mice receiving supportive care is also planned. *MCART* is poised to begin further studies with G-CSF (Neupogen) to determine whether it retains efficacy when initiation of therapy is delayed until 48 hours (compared to 24 hours) after irradiation and with pegylated G-CSF (Neulasta) to assess efficacy when administered 24 to 48 hours post-irradiation.

- Licensed products for which at least preliminary data have been or will be obtained in animal models under existing RCP grants, contracts, and Interagency Agreements (IAAs) include the following:

  - Keratinocyte growth factor
  - Human growth hormone
  - Interleukin-11
  - Captopril
  - Enalapril
  - Fosinopril
  - Ramipril
  - Losartan
  - Atorvastatin
  - Doxycycline
  - Tetracycline
  - Ciprofloxacin
  - Levofloxacin
  - Celecoxib

- Valproic acid
- Activated protein C
- Parathyroid hormone
- Lithium carbonate

A few of these products have demonstrated promise and warrant further testing, but none has reached a stage at which it would be appropriate to perform pivotal GLP studies.

There are additional licensed products that appear promising and warrant further evaluation in animal models of radiation injury. The RCP has initiated discussions with the companies that manufacture several of these products and is exploring options for evaluating them in appropriate animal models.

The RCP has encountered some significant obstacles and setbacks in this area of research, including the following:

- Products have shown promise for aspects of radiation injury for which well-established large animal models do not exist. An example is captopril for use in kidney or lung radiation injury.
- For models that do exist, establishing reproducible animal models and negotiating the design of the proposed studies with the FDA have taken longer than anticipated.
- Some relevant commercial partners have declined to support the design and execution of the necessary studies.
- Products are off patent and lack commercial sponsorship for development as MCMs.
- Mechanisms for expanding the label indications of licensed products absent the support of product manufacturers are not well defined.

Goal 2: Facilitate the development of products that are already in the clinical testing stage for other indications and that can prevent or treat radiation injury.

RCP's perspective regarding products that target host factors influencing tissue regeneration and repair has made it comparatively easy to identify compounds in clinical development with therapeutic potential for an indication that also may prevent or treat radiation injury. The major advantage of concentrating effort on such compounds is that the preliminary advanced development work (e.g., pharmacokinetics, toxicity, and formulation) has already been completed, greatly facilitating work on the radiation injury indication. Such compounds also will have demonstrated their ability to attract other sources of funding, reducing the draw on limited U.S. government biodefense resources.

In addition, where markets for the stipulated clinical indication are insufficient in themselves to drive product development, adding a biodefense indication and associated procurements may

influence product development and investment decisions for industry or other potential sponsors. To the extent that the RCP expedites the development of such products while succeeding in its stated mission of delivering effective radiation MCMs, it provides an enhanced return on the funds invested. These advantages notwithstanding, a number of caveats and qualifications—both those listed in the section above as well as others specific to unapproved multi-use products—apply to these products.

Products in the clinical stage of development for which at least preliminary animal model data have been or will be obtained under existing RCP grants, contracts, and IAAs include the following:

- AEOL 10150 (a metalloporphyrin catalytic antioxidant)
- Angiotensin-(1-7)
- ARA-290 (an erythropoietin mimetic)
- Curcumin
- CYT107 (glycosylated recombinant human IL-7)
- Ghrelin
- Manganese superoxide dismutase plasmid/liposome complex
- MAXY-G34 (a "next-generation" pegylated G-CSF)
- Mesenchymal stem cells
- NOV-002 (a proprietary formulation of oxidized glutathione)
- Oral beclomethasone diproprionate
- RWJ-800088 (a thrombopoietin-receptor agonist)
- SOM230 (a multiligand somatostatin analogue)
- TP508 (the receptor binding domain of native human thrombin)
- Velafermin (a fibroblast growth factor)

The diversity of indications for which these products are being developed gives some indication of the broad potential utility of host-directed MCMs. For example,

- CYT107 is being evaluated as an adjunct to PEG-interferon alpha/ribavirin therapy for hepatitis C, as immunotherapy for melanoma, and as an agent to promote immune reconstitution in patients with HIV or allogeneic bone marrow transplant recipients.

- SOM230 is currently undergoing Phase III clinical trials as a treatment for acromegaly, gastroenteropancreatic neuroendocrine tumors, and Cushing's disease.

- TP508 has demonstrated positive effects in the healing of diabetic ulcers and the repair of fractures and now is being evaluated in disorders that involve vascular endothelial dysfunction such as acute myocardial infarction and chronic myocardial ischemia.

Challenges and Risks
Focusing on products being developed for other indications is not without challenge or risk. Many small companies lack the staff, expertise, and resources to pursue more than one indication at a time, and the specific requirements of biodefense research are viewed as exacting a heavy opportunity cost. Even for companies that do choose to pursue a biodefense indication, products may fail in Phase II or Phase III clinical trials for the primary indication while continuing to show promise as radiation MCMs. Faced with a loss of their primary market (or occasionally for business reasons), firms may choose to cut their losses and cancel all clinical development programs for the product in question.

Goal 3: Support research on promising compounds that are currently in preclinical development.

The RCP has used the same rationale described under Goal 2 to select and support work on compounds that are in preclinical development, with the emphasis on products that target host factors influencing tissue regeneration and repair. The major difference between products in preclinical compared to clinical development is the amount of work that remains to be done. Therefore, for products in preclinical development, the risks as well as the costs of development assumed by the U.S. government will be substantially higher and the timelines until delivery of a product correspondingly longer than drugs already advanced to the clinical development stage.

Understanding the costs, risks, and timelines, the RCP is pursuing work on promising compounds at this stage of development and can report substantial progress. In addition to the early development of CBLB-502, a truncated flagellin TLR-5 agonist (see Section 1, Goal 5), the RCP has supported the development of many other promising compounds and approaches in preclinical stages of development, including the following:

- Rx 100, which inhibits apoptosis and enhances survival in mice following a single subcutaneous dose administered up to 72 hours after lethal, whole-body gamma irradiation. Rx100 is believed to protect critical stem cells following radiation exposure and appears to protect multiple tissue types against radiation injury. It preserves bone marrow in animals receiving doses of radiation that induce HE-ARS and crypt architecture in the guts of animals receiving doses of radiation that induce GI-ARS. This compound is now being tested in NHPs to determine if its efficacy in rodents can be repeated in an animal model more closely related to humans.

- R-spondin1 (Rspo1), a potent intestinal mitogen, which is being evaluated as a mitigator of GI-ARS under the auspices of an NIH Grand Opportunities (GO) grant. Previous work had shown that, when administered prior to exposure, Rspo1 protected mice from chemotherapy or radiation-induced oral mucositis and experimental GI-ARS, and when administered

following exposure to dextran sulfate sodium, it ameliorated experimental colitis. Prophylactic Rspo1 also was found to decrease the number of apoptotic nuclei and induce the proliferation of crypt stem cells in mice receiving whole-body irradiation, resulting in improved gastric function.

- ALXN4100, which is a human antibody fragment against the protective antigen of anthrax toxin. When administered as prophylaxis, it enhances survival in rabbits exposed to a lethal aerosol challenge of *Bacillus anthracis* spores. Interestingly, grafting a thrombopoietin mimetic peptide into the ALXN4100 antibody confers activity as a thrombopoietin receptor agonist, and RCP-supported studies have shown that this product enhances survival in lethally irradiated mice. Studies with this product are ongoing.

- Hydroxypyridinonate (HOPO) compounds, such as octadentate 3,4,3-LI-1,2-HOPO and tetradentate 5-LIO-Me-3,2-HOPO, which are novel radionuclide decorporation agents with greater potency or a broader spectrum of activity than currently licensed compounds. These rationally designed biomimetic compounds share structural and chemical properties with naturally occurring iron-chelating molecules produced by plants and bacteria, and they are extremely potent, orally bioavailable chelating agents with no overt toxicity.

  Work supported by the RCP has confirmed the efficacy of these compounds in reducing body burdens of americium-241 in rodent models. Through a jointly funded effort with the NIH Rapid Access to Interventional Development (RAID) Program, the RCP is now funding scale-up synthesis and stability/formulation work on these compounds that will provide sufficient material for full preclinical safety and efficacy evaluations. Through a separate NIH GO grant, the RCP is supporting preclinical efficacy, pharmacology, and toxicology studies of these compounds.

Goal 4: Identify and develop new drugs to prevent or reduce the severity of radiation-induced damage and to treat irradiation casualties.

The escalating costs of pharmaceutical research and development, coupled with the broad mission and finite resources of the RCP, have made it critical to concentrate on projects that can leverage private investment to defray the costs of product development. Consequently, the RCP has focused to a much greater extent on extending the indications for existing products than on developing new drugs *per se.* However, several promising compounds have emerged from the *Centers for Medical Countermeasures Against Radiation (CMCRs)* that warrant mention in regard to this stated program goal. Specifically, *CMCR* investigators have

- Pioneered the development of mitochondria-targeted disruptors and inhibitors of peroxidase activity of cytochrome c/cardiolipin complexes. JP4-039, the lead compound in this class, is a nitroxide compound free-radical scavenger conjugated to a hemigramicidin S vehicle, and it enhances cell repair and inhibits apoptosis in both cycling and quiescent cells. As reported in Section I, these investigators performed subsequent work on this compound under a contract from BARDA.

- Established a high-throughput DNA deletion (DEL) recombination assay in *Saccharomyces cerevisiae* that is comparable in accuracy to, but much faster than, standard assay platforms

for the *in vitro* detection of chromosomal breakage. Using this screening platform, several new compounds with apparent activity as radioprotectors have been identified, for example Yel1 and Yel2. Mice that received high doses of radiation followed by 5 days of treatment with Yel1 and Yel2 had enhanced survival at 30 days and decreased rates of leukemia development.

- Conducted groundbreaking work on pleiotrophin, a developmentally regulated, heparin-binding neurite growth factor. This work has identified pleiotrophin as a regenerative growth factor for hematopoietic stem cells (HSCs)—a previously wholly unknown activity of the peptide. Thus, pleiotrophin has potential clinical utility as a radiation injury therapeutic and as a means of accelerating hematopoietic recovery in patients receiving myelotoxic chemotherapy or radiotherapy. Moreover, by virtue of its ability to expand HSCs *ex vivo,* pleiotrophin may have potential application for another area of unmet clinical need: the production of cord blood grafts capable of accelerated engraftment in adult transplant patients who lack a histocompatible adult donor.

Goal 5: Develop drugs that will prevent delayed effects, such as pneumonitis and fibrosis, and long-term consequences, such as mutagenesis or fibrosis.

The long-term consequences of radiation exposure include both deterministic and stochastic effects. The deterministic effects, frequently referred to as the "delayed effects of acute radiation exposure" (DEARE), include pulmonary and cutaneous fibrosis, declines in immune and renal function, and cataract formation. Stochastic effects are attributable to radiation-induced mutagenesis, have long latencies and no identified threshold dose, and include cancer and genetic effects. As the dose to an individual increases, the probability that cancer or a genetic effect will occur also increases. The stochastic risks of radiation exposure are of broad interest because even effective radiological protection programs cannot wholly eliminate them, whereas protection programs can greatly reduce or eliminate the risk of deterministic effects.

There are several barriers to the approval and clinical applications of compounds demonstrating promise for one or more components of DEARE, including

- Basic gaps in our understanding of mechanisms of injury

- Lack of well-validated animal models for pivotal efficacy studies under the FDA Animal Efficacy Rule

- Time and expense that would be associated with such studies, even assuming the existence of appropriate animal models

- Paucity of reliable, pre-symptomatic biomarkers that can identify patients at greatest risk and facilitate early initiation of therapy

In spite of these barriers, a number of RCP-supported investigators are dissecting the cellular and molecular mechanisms of injury responsible for the late effects of radiation injury. The value of such work is twofold: in addition to suggesting approaches to the development of effective

countermeasures, it may lead to the identification of clinically relevant biomarkers. For example, *CMCR* investigators have

- Evaluated alterations in mouse lung cytokine expression following whole-lung and total-body irradiation between 0–10 Gy and found dose-related changes in IL1B, IL1R2, and CXCR2 gene expression at 1 and 6 hours after irradiation concurrent with increases in plasma protein levels of KC/CXCL1 and IL6.

- Measured circulating cytokines and cellular inflammatory infiltrates in the lungs of rats exposed to 10 and 15 Gy thoracic radiation and found a dramatic (13-fold) and comparatively isolated rise in mast cell infiltration of affected areas at the higher dose. This mast cell infiltration was associated with a marked increase in the severity of observed pneumonitis, suggesting that circulating levels of mast cell products may be useful markers of severe pneumonitis. These promising results only underscore the amount of work that is needed in this area.

- Reported promising results with curcumin and esculentoside A as mitigators of radiation-induced skin fibrosis and with captopril, genistein, and the manganese porphyrin superoxide dismutase mimetic MnTE-2-PyP5+ as mitigators of radiation-induced pneumonitis and/or lung fibrosis.

- Performed a comparative analysis (including breathing rate measurements, micro-computerized tomography, lung tissue weight, pleural fluid weight, and histopathology) of the radiosensitivity of lungs of three different strains of mice to determine which strain was best suited for the development of an appropriate mouse model of lung injury and entered into a subcontract with *MCART* to develop such a model.

This research was an outgrowth of a two-day workshop on "Animal Models for Medical Countermeasures" (San Antonio, January 2008) and subsequent deliberations in organ-specific working groups. The resulting publication became a major consensus document that mapped out pathways for the development of standardized animal model systems, which would allow products to be compared for efficacy in prophylaxis, mitigation, and treatment of radiation injury in the major organ systems of interest in a manner consistent with FDA requirements (Williams JP, *et al.* Animal Models for Medical Countermeasures to Radiation Exposure. Radiation Research 2010;173:557–578 available at http://www.ncbi.nlm.nih.gov/pubmed/20334528).

In a jointly funded initiative with BARDA, the RCP awarded a series of Challenge Grants focused on the development of therapeutics for radiation-induced skin and lung injury. Numerous drugs being evaluated for efficacy against lung injury under this initiative include the IL-1 receptor antagonist anakinra, simvastatin, and MnTE-2-PyP5+. Approaches being evaluated for efficacy against skin injury include the administration of anakinra or angiotensin-(1-7), and the application of topical preparations of JP4-039 and mesenchymal stem cells affixed to fibrin microbeads.

RCP efforts are focused on the delayed effects of radiation exposures such as pneumonitis and organ fibrosis, which represent potentially lethal sequelae in the weeks to months after radiation exposure. Efforts on mutagenesis and carcinogenesis are secondary. RCP's investments in this

secondary area have been to support National Cancer Institute (NCI) intramural investigators to evaluate the ability of nitroxides such as Tempol to reduce the incidence of cancers in mice following radiation exposure. This large, lifetime study is ongoing and will include detailed pathology reports for all mice in each arm of the study. Preliminary data suggest that dietary Tempol is quite effective in reducing or delaying the incidence of certain cancers following radiation exposure.

Expanded/Revised Research Goals

Over the last 5 years, research and early development sponsored by the RCP has identified a number of candidate therapeutic and mitigating agents, primarily for HE- and GI-ARS. A continuing goal of the program will be to translate these promising drugs into advanced stages of product development and to expand the pipeline for other forms and manifestations of radiation injury. The *CMCRs* and other grantees are beginning to transition some of their lead compounds to BARDA and/or *MCART,* a process that will accelerate over the next 5 years. A number of specific product development goals can be articulated for the next 5 years of the program.

Expanded/Revised Goal 1: Provide data to guide the use of granulocyte colony stimulating factor (G-CSF) and/or pegylated G-CSF in a mass casualty situation.

G-CSF is approved for the treatment of neutropenia in patients receiving myelosuppressive chemotherapy and certain other settings and has been shown to increase survival of animals exposed to lethal irradiation. Studies in mice, dogs, and NHPs demonstrate that treatment can be effective even when initiated 24 hours post-exposure, but there are little data to guide therapy if longer delays in initiating treatment occur, such as following the detonation of an improvised nuclear device. The RCP will support the performance of Good Laboratory Practice (GLP) non-pivotal efficacy studies with G-CSF in relevant animal models to better delineate the window of efficacy of post-exposure treatment. GLP non-pivotal efficacy studies with the pegylated form of G-CSF (which offers the operational advantage of intermittent dosing) to determine efficacy and establish the optimal dosing interval will also be supported.

Expanded/Revised Goal 2: Advance oral DTPA and novel decorporation agents to the point of Investigational New Drug (IND) submission.

Significant progress has been made in developing oral DTPA and other improved radionuclide decorporation agents from 2005 to 2010, and new contracts supporting the continuation of this work were awarded in FY 10. Investigators developed and evaluated three techniques to increase the oral bioavailability of DTPA: a pro-drug, a nanoparticle approach, and an enhanced formulation with surfactants. Each technique significantly increased oral bioavailability when evaluated in rodent models, and preliminary rodent efficacy studies showed the increased elimination of a radionuclide, americium. The investigator for the nanoparticle DTPA technology has filed an IND with the FDA and has received a contract with BARDA for further advanced development.

The RCP has built a strong collaborative relationship with contractors and grantees working in this area, and RCP program managers will continue to work closely with contractors and grantees to ensure that studies are completed on time and within stipulated budgets. The RCP

anticipates bringing additional oral DTPA candidates or novel decorporation agents to IND submission within the next 5 years.

Expanded/Revised Goal 3: Continue development and validation of pivotal animal models for demonstration of efficacy in radionuclide decorporation.

Validated small and large animal models suitable for testing oral DTPA and other improved decorporation agents are essential for the approval of these MCMs under the FDA Animal Efficacy Rule. Such animal models were originally developed and used extensively in the 1950s and 1960s, but interest in updating the models deteriorated as trained personnel retired and facilities have fallen out of use. The RCP is committed to bringing these models into the 21st century by training a new generation of scientists in their use and developing procedures and cutting-edge analytical techniques that are compatible with current instrumentation and regulatory requirements.

Expanded/Revised Goal 4: Use animal models developed in multiple species to evaluate the efficacy of candidate radiation medical countermeasures for hematopoietic and gastrointestinal acute radiation syndrome (HE- and GI-ARS), radiation-induced lung and skin injury, radiation combined injury, and radionuclide decorporation and to inform IND submissions.

The FDA Animal Efficacy Rule requires manufacturers to demonstrate efficacy of candidate MCMs in well-characterized animal models of radiation injury. The RCP has thus invested significant resources in developing mouse, canine, and NHP models for HE-ARS, mouse and NHP models for GI-ARS, mouse models for radiation-induced lung injury, and mouse and canine models for internal contamination with radionuclides. Much of this work is ongoing and will be extended over the next 5 years, and development of a large animal model for radiation-induced lung injury and development of models for other radiation injury syndromes will be initiated. Candidate radiation MCMs that demonstrate efficacy in screening studies will undergo further testing in mice to optimize dose, dose route, and timing of administration. The goal is to have a drug or biologic approved for the emergency radiation injury indication and available for stockpiling and use in a mass casualty situation. Further IND submission-enabling studies for lead candidates will include mechanistic studies to examine secondary endpoints, and animal safety studies to evaluate MCM pharmacokinetics and pharmacodynamics.

Expanded/Revised Goal 5: Engage and find innovative ways to collaborate with companies, academia, or government entities in the development of new candidate medical countermeasures.

Over the last 5 years, the RCP has interacted with more than 130 companies interested in developing radiation MCMs with multi-use potential. Most of these interactions were company-initiated. Over the next 5 years, the RCP will expand its outreach efforts to both small and large pharmaceutical and biotechnology companies with extensive preclinical research pipelines. RCP program managers and senior regulatory staff will seek to leverage fully the wide array of translational resources and services now provided by NIH. They will also continue to provide guidance to investigators and proactively engage the FDA on issues regarding the approval of products for an emergency radiation injury indication.

Expanded/Revised Goal 6: Extend interagency collaborations with the Department of Health and Human Services (HHS) Biomedical Advanced Research Development Authority (BARDA) and the Department of Defense (DoD) to establish seamless pathways for product development and licensure of radiation medical countermeasures.

The efforts of the RCP are increasingly well-integrated with other government agencies engaged in radiation MCM development activities through cross-participation in program review, technology watch, contract technical evaluation, and project management panels. These relationships have resulted in joint projects and strategic alignment on resource allocation in many areas and the successful transition of numerous projects from the RCP to BARDA or DoD stewardship. RCP program managers and senior regulatory staff will continue to place a high priority on transparency and coordination of effort with BARDA and DoD and will seek to reduce any remaining gaps with these key partners.

IV. INFRASTRUCTURE FOR RESEARCH AND PRODUCT DEVELOPMENT

STRATEGIC PLAN RATIONALE

In order to carry out the research outlined in the Strategic Plan, the development of required expertise as well as modernization and expansion of the research infrastructure will be necessary. Specific requirements include large animal irradiation and animal husbandry facilities that can safely care for animals during and after radiation exposure.

Summary of Continuing and Expanded/Revised Research Goals

Continuing Research Goals

1. Establish a collaborative research network focused on the identification and development of medical countermeasures to radiation exposure.
2. Promote integration and collaboration in radiobiology research and training among government organizations, academia, and private industry.
3. Support centralized facilities for efficient, carefully standardized testing and validation of new products.
4. Attract a new generation of scientists to radiobiology research and radiation chemistry through training and mentoring programs.
5. Support bioinformatics and specialized technology centers for radiobiology research.

Expanded/Revised Research Goals

1. Work with the Food and Drug Administration (FDA) to achieve consensus on GLP, pivotal animal model protocols, primary endpoints to determine efficacy, and secondary endpoints to support mechanism of action.

Continuing Research Goals—Programmatic and Scientific Accomplishments

Goal 1: Establish a collaborative research network focused on the identification and development of medical countermeasures to radiation exposure.

The NIH Radiation Countermeasures Program (RCP) has emphasized the creation of formal and informal collaborative research networks as a major programmatic priority since its inception in 2005. The *Centers for Medical Countermeasures Against Radiation (CMCRs)* program is the most important formal network established by the RCP. From 2005 to 2010, eight *CMCRs* were funded and then re-competed in 2010 with awards made to seven CMCRs. The list of CMCRs funded in 2005 and 2010 can be found at http://www.niaid.nih.gov/topics/radnuc/funding/Pages/awardees.aspx

***CMCR* Research Focus**

Each *CMCR* has its own scientific areas of expertise and range of projects it supports. For example, the Columbia University *CMCR* focuses exclusively on the development of high-throughput radiation biodosimetry and dose assessment. The University of California—Los Angeles (UCLA) *CMCR* focuses heavily on high-throughput, *in vitro* screening of compound libraries and *in vivo* confirmation of promising leads. The Pittsburgh *CMCR* has concentrated on the role of mitochondria in mediating radiation injury. The CMCRs have also identified areas of common interest and/or complementary expertise, leading to ongoing collaborations across the

network of CMCRs. Such collaborations are facilitated by RCP program officers and have contributed to the sharing and advancement of common goals.

CMCR Collaborative Interactions

The interactions between and across *CMCRs* have been fruitful. *CMCR* investigators convene annually for a two-day scientific conference that has facilitated cooperative activity, and the *CMCR* Steering Committee (comprised of the *CMCR* principal investigators and RCP program managers) holds monthly conference calls. In addition, the *CMCRs* work jointly on education and training activities, including the development of a radiobiology lecture series, and have sponsored symposia at annual meetings of the Radiation Research Society and other relevant scientific organizations. *CMCR* investigators have presented lectures and seminars at the invitation of other centers and served on each other's pilot project review panels.

The RCP also sponsored two well-attended workshops for *CMCR* investigators on the FDA Animal Efficacy Rule and pre-market regulatory process. *CMCR* investigators held a two-day animal models workshop, as well as a follow-up meeting hosted by the *MCART* consortium on animal models of radiation-induced lung injury. *CMCR* investigators continue to work closely with *MCART* on specific projects.

CMCR Scientific Collaborations

In addition to helping establish a sense of shared mission, *CMCR* interactions have resulted in a number of scientific collaborations, which include the following:

- In establishing its studies of the mid- to long-term effects of irradiation in non-human primates (NHPs), the Duke University *CMCR* invited input on study design and requests for necropsy specimens from the other *CMCRs*.

- The Duke University *CMCR* Primate Core maintains a sample bank of more than 2,500 tissue samples and has drawn more than 3,000 blood samples for investigators. Sample sharing, particularly for biodosimetry projects, has occurred between *CMCRs,* including a joint *CMCR* effort at product development and commercialization of a diagnostic device.

- The UCLA *CMCR* has evaluated compounds from the Rochester *CMCR* in its *in vitro* screens and worked with investigators from the Dana-Farber *CMCR* to delineate mechanisms of action for compounds found to have activity.

Other *CMCR* Activities

CMCR investigators have demonstrated their commitment to the mission of the RCP and to the larger goal of protecting the American public from radiological and nuclear threats in a number of ways. For example, *CMCR* investigators have

- Applied for advanced development funding from the Department of Health and Human Services (HHS) Biomedical Advanced Research and Development Authority (BARDA) or the DoD

- Participated in more than 30 meetings, workshops, and symposia sponsored by the RCP since 2004 (http://www.niaid.nih.gov/TOPICS/RADNUC/Pages/radNucMeetings.aspx)

- Joined a delegation that traveled to Hiroshima, Japan in November 2007 to participate in the workshop on "Radiation and Age-Associated Immunosenescence," which led to the United States–Japan joint effort to study the effects of radiation and aging on the human immune system

- Served as members of a delegation that visited Delhi, India in August 2008 to participate in the Indo-U.S. Workshop "Medical Countermeasures for Radiation Injury: Current and Evolving Technologies" and to meet with Indian scientists, academicians, and policy makers

- Served as consultants to the World Health Organization (WHO) on issues relating to radiological and nuclear emergency preparedness and on the steering committee of the Radiation Injury Treatment Network, an alliance of transplant centers, donor centers, and cord blood banks that stand ready to treat victims of a radiological or nuclear event

The raised awareness and deepening involvement of *CMCR* investigators in matters relating to national and global preparedness for radiological and nuclear events is a significant benefit of the program.

Goal 2: Promote integration and collaboration in radiobiology research and training among government organizations, academia, and private industry.

The costs of drug discovery and development have significantly increased in recent decades and vastly exceed the resources government can bring to bear on the development of specialized, niche products. The RCP recognized that promoting integration and collaboration between government organizations, academic institutions and investigators, and the private sector would be essential for the successful development of radiation countermeasures.

This goal specifically focuses on efforts to promote integration and collaboration among RCP-supported academic investigators who are not actively affiliated with a *CMCR.* Section V of this report describes partnerships between the RCP and the NIH, other government agencies, and international partners. More information can be found in the RCP Business Model on program integration and facilitation of partnerships at http://www.ncbi.nlm.nih.gov/pubmed/21142762.

Specific accomplishments to promote integration and collaboration among RCP-supported academic investigators (not *CMCR* affiliated) include the following:

- Targeted Solicitations: Since its inception in 2005, the RCP has sponsored or contributed to the following targeted solicitations focused on specific organ systems or, in the case of therapeutics for contamination with radionuclides, on classes of MCMs:

  – Protecting the Immune System against Radiation (2004)

  – Development of Improved Diethylenetriaminepentaacetic Acid (DTPA) for Radionuclide Chelation (2005)

  – Radionuclide Decorporation Agents for Radiation/Nuclear Emergencies (2006)

  – Medical Countermeasures to Restore Gastrointestinal Function after Radiation (2007)

- Medical Countermeasures to Enhance Platelet Regeneration and Increase Survival Following Radiation Exposure (2008)

- Radiation Combined Injury: Radiation Exposure in Combination with Burn, Wound, Trauma, or Infection (2008)

- Medical Countermeasures to Mitigate and/or Treat Ionizing Radiation-Induced Cutaneous Injury (2008) [co-funded with BARDA]

- Medical Countermeasures to Mitigate and/or Treat Ionizing Radiation-Induced Pulmonary Injury (2008) [co-funded with BARDA]

- Intestinal Stem Cell Consortium (2009) [co-funded with the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)]

- Development of an Oral Form of Diethylenetriaminepentaacetate (DTPA) for Use in Radionuclide Decorporation in Radiological Emergencies (2010)

- Predictive Biodosimetry: Discovery and Development of Biomarkers for Acute and Delayed Radiation Injuries (2012)

- Topical Meetings: Coordination and collaboration between grantees and integration with the RCP has been promoted through close interactions with program managers and carefully designed topical meetings (for more information, see http://www.niaid.nih.gov/TOPICS/RADNUC/Pages/radNucMeetings.aspx). The RCP schedules topical meetings 12 to18 months after the relevant grants are awarded in response to the targeted solicitations and publishes meeting reports, often co-authored with meeting participants, in *Radiation Research* or other appropriate journals. The format for these meetings ensures that all investigators receive baseline information about current priorities and pathways to approval for their products. They also have an opportunity to meet with academic colleagues and representatives of government and the private sector to exchange ideas and seek counsel.

Goal 3: Support centralized facilities for efficient, carefully standardized testing and validation of new products.

Designing and validating animal models is a long, arduous, and expensive process, and studies to confirm the efficacy of radiation MCMs are expensive, requiring highly specialized facilities and personnel. In addition, few companies have access to facilities with the requisite capabilities or to radiobiologists with appropriate technical expertise. The establishment of centralized facilities can alleviate these issues by

- Providing standardized approaches to enhance the reliability of the testing platform
- Making it easier to design and predict study cost and duration
- Facilitating review by the FDA
- Reducing economic costs (most important)

The *MCART* consortium was established in 2005 under a NIAID contract to provide support services and facilities for product development and has a critical role within the RCP's business model. The University of Maryland—Baltimore (UMB) serves as the prime contractor for the *MCART* consortium, which currently includes 14 subcontractors. In addition, *MCART* has established relationships with world-renowned investigators and institutions to perform specialized studies of internal contamination with radionuclides (Lovelace Respiratory Research Institute), radiation-induced hematopoietic injury (Indiana University), radiation-induced gastrointestinal injury (Epistem, Ltd. and the UMB Mucosal Biology Research Center), and radiation-induced lung injury (Duke University).

The consortium's goal is to provide definitive efficacy testing of candidate therapeutics for hematological, gastrointestinal, and pulmonary injury, as well as internal contamination with radionuclides, in a variety of Good Laboratory Practice (GLP) animal models (murine, canine, and/or NHP). *MCART* can currently test products for each of these in murine models and for hematological injury in canines and NHPs. NHP models of radiation-induced gastrointestinal injury are in a late stage of development; NHP models of radiation-induced pulmonary injury are in the early stage of development; and canine models of internal contamination with radionuclides are planned.

MCART has assembled expertise and resources that provide support to academic and private sector partners with candidate radiation MCMs. The central UMB facility provides

- Access to
  - Eight fully equipped investigative laboratories
  - Cytokine and flow cytometry core facilities
  - Clinical pharmacology, bacteriology, parasitology, and histology laboratories
- A fully serviced GLP animal care and housing facility (10 experimental animal rooms and 1 quarantine room, with a capacity of >200 NHPs at any one time)
- A state-of-the-art linear accelerator (LINAC) facility, wholly dedicated to radiation research, to increase throughput for large animal studies. Its imaging capability allows researchers to monitor the progression of radiation injury in the gut, lungs, and other organ systems in real time.

MCART subcontractor facilities and product development services include

- Capacity for GLP efficacy studies in murine, canine, and NHP models of ARS and in murine and canine models of internal contamination with radionuclides
- Capability to perform GLP non-clinical studies (general toxicology, general pathology, and directed toxicity studies)
- Two clinical sites to conduct Phase I human clinical safety studies

- Chemistry, manufacturing, and control testing (CMC) support services
- Access to current Good Manufacturing Practice-compliant facilities
- Regulatory affairs guidance
- Statistical support

Goal 4: Attract a new generation of scientists in radiobiology research and radiation chemistry through training and mentoring programs.

A major consequence of the reduced investment in radiobiology research toward the end of the 20th century has been the lack of recruitment of new scientists into the field. This has led to an aging workforce and a diminishing number of scientists studying the mechanisms and treatment of normal tissue after radiation injury. However, concerns about nuclear proliferation and the use of radiological or nuclear weapons by terrorists have significantly increased since 2001. In parallel, there has been a resurgence of interest and investment in nuclear power as an alternative to carbon-based energy production, and, more recently, heightened international concerns about the safety of nuclear power plants.

Drawing young scientists to radiobiology and promoting the field's cross-fertilization by attracting scientists from other disciplines to the problem of normal tissue injury are critical to revitalizing research in this area. The RCP has supported this goal primarily through the *CMCRs,* which have been required to include training/education cores and pilot project programs and new solicitations to attract applications from scientists outside of the traditional radiation research community, as follows:

CMCRs

- Training and education program

  Because many *CMCR*-affiliated investigators come with academic pedigrees obtained outside the field of radiation biology, the *CMCRs* have been required to include training and education cores in their programs, and core supervisors have been elected to coordinate efforts in this area. The goals of the CMCR Training and Education Program are to

  – Increase the numbers and capabilities of researchers and other personnel in radiobiology and related areas

  – Provide multidisciplinary short-term training and education programs to technicians, medical/graduate students, postdoctoral fellows, and independent researchers within and beyond the *CMCRs*

  – Train researchers in assays, methods, reagents, animal models, and technologies to study radiobiology and/or develop new products

  – Train researchers in principles of radiobiology, radiation epidemiology, or radiation safety

- Support seminar series and symposia in radiobiology or radiation epidemiology
- Promote interaction between centers

To achieve these goals, the *CMCRs* have

- Enhanced or expanded existing courses
- Introduced new seminar series
- Sponsored specialized training workshops and wet labs
- Convened symposia
- Created a web-based "virtual campus" consisting of an educational program, videotaped seminar series, and webcasts for trainees

In addition, all of the *CMCRs* maintain dedicated websites where training and education materials are publicly available, enhancing their impact. Some of the *CMCRs* support graduate students and post-doctoral trainees under the terms of their award. More specific information should be available on the CMCRs websites, which can be accessed from http://www.niaid.nih.gov/topics/radnuc/funding/Pages/awardees.aspx.

- Pilot project program

The *CMCRs* have also supported pilot projects that provide seed funding for young investigators or specialists from other fields interested in an expanded research area. Collectively, the eight *CMCRs* awarded 130 pilot projects between 2006 and 2010. Awardees receive up to $100,000 per year for up to two years of research, an amount and period that should be sufficient to generate data to justify follow-on funding for promising projects. Prior to 2008, at least eight of these awardees have competed successfully to become principal investigators for RCP-funded grants, suggesting that the program has offered a solid return on investment.

New Solicitations

Another indicator that the RCP is succeeding in attracting new researchers to the study of normal tissue injury is the number of non-radiobiologist principal investigator grants awarded through targeted solicitations that are non-*CMCR*. Of approximately 70 such grants, at least 20 have been awarded to investigators whose primary expertise is in areas other than radiobiology.

There also is accumulating circumstantial evidence that RCP investments are having a positive influence on the career direction of funded investigators: more than a quarter of the grants awarded under subsequent targeted solicitations have been awarded to investigators already affiliated with a *CMCR*. This trend suggests a commitment on the part of these investigators to addressing the problem of radiation-induced normal tissue injury.

Goal 5: Support bioinformatics and specialized technology centers for radiobiology research.

To date, RCP efforts to support bioinformatics and specialized technology requirements have been directed through the *CMCRs* to specific bioinformatics and technology cores. Two of the *CMCRs* have bioinformatics/computational biology cores and two have biostatistics cores. The *CMCR* program also supports medicinal chemistry, chemical process development, microfluidics, functional genomics, and proteomics cores. Currently, the programs focused on biodosimetry have generated the greatest need for bioinformatics and specialized technology development support.

The RCP does not plan to establish freestanding bioinformatics or specialized technology centers in the near- to mid-term. However, RCP program managers are familiar with, and in the future will make greater use of, other translational and product development resources supported by NIH, which include

- Biomedical Technology Research Centers (BTRCs) Program, formerly managed by the National Center for Research Resources (NCRR), now transferred to various NIH Institutes
- Rapid Access to Interventional Development (RAID) program
- National Chemical Genomic Center (NCGC)
- Molecular Libraries Program

Expanded/Revised Research Goals

As a result of the efforts of the RCP over the last five years, there are now multiple GLP facilities that can perform rodent, canine, and NHP radiation studies, and a radionuclide research program that has been re-established at the Lovelace Respiratory Research Institute after lying dormant for nearly 20 years. Looking forward, it will be crucial to maintain and possibly expand these facilities to accommodate the anticipated increase in the number of radiation countermeasures available for testing.

Expanded/Revised Goal 1: Work with the Food and Drug Administration to achieve consensus on GLP, pivotal animal model protocols, primary endpoints to determine efficacy, and secondary endpoints to support mechanism of action.

The RCP must confirm that the animal models used to test candidate MCMs appropriately reflect human disease and are robust enough to show that such MCMs effectively alter mortality or major morbidity through the same mechanisms that cause the human disease and pathology. As animal model protocols for the pivotal safety and efficacy studies are established, and as primary and secondary endpoints that demonstrate MCM safety and efficacy and elucidate the mechanism of action are identified, the RCP will present these findings to the FDA for regulatory review and concurrence. In the future, as the number of animal models under development increases, the RCP will work proactively with FDA to ensure ample regulatory input and review on animal model protocols and share these protocols with the research community.

V. COLLABORATIONS AND PARTNERSHIPS

Resources allocated by the U.S. government to radiobiology research and the development of radiation medical countermeasures (MCMs) are finite and distributed across a number of departments and agencies. To facilitate the efficient and effective use of these resources, the NIH Radiation Countermeasures Program (RCP) has established a network of collaborations and partnerships that promote interagency coordination of research on radiation-induced normal tissue injury and on the discovery and development of radiation MCMs.

Collaborations and partnerships have helped foster a new self-awareness and common sense of purpose among the community of policy makers and program managers. This sense of common purpose, in turn, has resulted in increased transparency between the programs and better informed allocation of effort and resources. With the costs, risks, and complexity of drug development so high, the value of enhanced efficiency cannot be overstated. The most important of these collaborations and partnerships are summarized in this section.

NATIONAL INSTITUTES OF HEALTH COLLABORATIONS AND PARTNERSHIPS

Research and Development Partnerships

Since its inception in 2005, the RCP has established direct research and development partnerships with three National Institutes of Health (NIH) Institutes: the National Cancer Institute (NCI), National Institute on Aging (NIA), and the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), which are ongoing as follows:

- *National Cancer Institute (NCI)*

  - NCI subject matter experts played an important role in the conception and early development of the RCP, and the National Institute of Allergy and Infectious Diseases (NIAID) and NCI have maintained a close working partnership ever since. The RCP has drawn on the skills, insight, and expertise of NCI extramural staff; funded the research of intramural investigators working to develop improved diagnostics and other MCMs; and begun to leverage NCI's powerful clinical trials network.

  - The RCP and NCI have cooperated on research and development projects in the following general areas: development of safe and effective MCMs to mitigate/treat the immediate and long-term medical effects of ionizing radiation; improvement of the basic understanding of radiation-related health risks associated with types and levels of radiation exposure, mechanisms of radiation injury, and host responses; and development of biology-based diagnostic assays or biomarkers to assess cellular and tissue damage following exposure to ionizing radiation. An Intra-Agency Agreement (IAA) was established in 2005 that has specifically supported the identification of radiation biomarkers, the development of MCMs, and the study of radiation epidemiology.

  The RCP is working closely with NCI to explore the development of products with efficacy as emergency radiation MCMs for routine supportive oncology indications, thus establishing larger markets for these products. A joint workshop was held on January 25, 2010, to

examine how compounds being developed for a radiation injury indication might be developed for a radiation therapy indication. Consequently, RCP program managers are working with the NCI Clinical Trials Groups to identify candidates for clinical trials. NCI released a Funding Opportunity Announcement in August 2011 for a Small Business Innovation Research (SBIR) program that included the development of agents that decrease or ameliorate the toxicity of radiotherapy or radiochemotherapy. The SBIR also will support pre-clinical studies, consultations with the FDA, and early-phase human trials. The applications were due in early November 2011.

- *National Institute on Aging (NIA)*

  An IAA was established between NIAID and NIA in 2009 to promote the study of immune senescence occurring as a result of natural aging and exposure to ionizing radiation and to identify countermeasures and strategies to retard this process. Three projects focusing on how ionizing radiation and natural aging affect the ability of the immune system to fight off infections and respond to vaccinations are supported under the IAA.

- *National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)*

  In 2009, the RCP partnered with NIDDK to develop the Intestinal Stem Cell Consortium (ISCC), consisting of a network of research teams and a data coordinating center. The goals of the ISCC are to isolate, culture, characterize, validate, and eventually transplant intestinal stem cell populations as a means of restoring mucosal integrity in damaged or injured intestine. The RCP provided funding to support one of eight awarded cooperative agreement grants and partial funding for the ISCC data coordinating center.

Programmatic or Policy Collaborations
The RCP also has consulted or collaborated on specific programmatic or policy issues with several others institutes, including

- National Library of Medicine
- National Heart, Lung, and Blood Institute
- National Institute of Arthritis and Musculoskeletal and Skin Diseases
- National Institute of Child Health and Human Development
- National Institute of Environmental Health Sciences
- National Institute of Biomedical Imaging and Bioengineering

Other Relationships
The RCP maintains a close relationship with the NIAID biodefense program and the NIH CounterACT (Countermeasures Against Chemical Threats) program. With the former, the RCP shares a common interest in antimicrobials, and with the latter it shares a common interest in therapeutics for treating lung, skin, and bone marrow injury.

Interagency Collaborations and Partnerships
The RCP maintains direct "bilateral" relationships and partnerships with several components of the U.S. government that fund research and development of radiation MCMs. In addition, RCP program managers also participate actively in a number of interagency committees that facilitate communication and policy coordination.

Research and Development Partnerships

- *Department of Health and Human Services (HHS) Biomedical Advanced Research and Development Authority (BARDA)*

  The single most important interagency relationship maintained by the RCP is with BARDA, which was established by the Pandemic and All-Hazards Preparedness Act of 2006 (http://www.niaid.nih.gov/TOPICS/RADNUC/Pages/radNucMeetings.aspx) to support the advanced research and development of critically needed MCMs against chemical, biological, radiological, and nuclear (CBRN) and pandemic threats. BARDA provides critical funding to help move candidate MCMs through the late stages of product development (the so-called "valley of death"). It is the major transition partner for products emerging from the RCP.

  The RCP and BARDA have undertaken a number of joint research and development projects, including the following:

  – BARDA has provided critical support for infrastructure development projects, enabling the RCP (through the *Medical Countermeasures against Radiological Threats (MCART)* consortium) to re-establish the radionuclide research program at the Lovelace Respiratory Research Institute for the testing and evaluation of candidate decorporation agents.

  – BARDA has provided essential continuation funding for the development of the oral formulations of diethylenetriaminepentaacetic acid (DTPA) that may be tested in the Lovelace Respiratory Research Institute.

  – The RCP and BARDA co-funded grants in the areas of radiation-induced lung and skin damage, and subsequently BARDA provided additional funding to these awards. Although RCP program managers have primary responsibility for scientific oversight of these grants, all progress reports and other documentation received from the awardees are shared with BARDA project officers.

  – The RCP provides testing and evaluation services to BARDA for confirmation of efficacy of some of the candidate MCMs in their contract portfolio.

  In addition, RCP program managers have contributed to the development of BARDA initiatives and serve as subject matter experts for BARDA technical evaluation panels. BARDA project officers participate in all RCP special topic and general program conferences and workshops including the RCP twice-monthly Radiation/Nuclear Group meetings and advanced product development meetings.

- *Department of Defense (DoD)*

  Prior to the establishment of the RCP, DoD was responsible for most of the U.S. government's investment in this area. Historically, military requirements for MCMs against nuclear threats differed from civilian requirements, placing a greater emphasis on radioprotectants. However, DoD's requirements have evolved to include mitigating and therapeutic agents and now largely overlap with those of civilian responders. This evolution has both facilitated and heightened the value of coordination and collaborations across departmental and organizational lines, and the RCP has established close working relationships with each of the major DoD components that support radiation MCM development.

- *Armed Forces Radiobiology Research Institute (AFRRI)*

  The relationship between NIAID and AFRRI predates the establishment of the RCP under the auspices of NIAID. In 2003, NIAID and AFRRI established an IAA to recharge AFRRI's terminally depleted cobalt irradiator. This agreement has evolved over the years to support major research and development projects in the following areas: automation of dicentric assay sample processing and analysis; screening of candidate radiation MCMs to mitigate and treat the effects of ionizing radiation; and identification, characterization, and testing of MCMs for radiation combined injury. NIAID also has supported studies of the effects of neutron/gamma mixed field irradiation and the development of a radiation injury model in Gottingen mini-pigs.

- *Defense Threat Reduction Agency (DTRA) and Defense Advanced Research Projects Agency (DARPA)*

  DTRA maintains a small portfolio of basic research grants focused on the development of MCMs against radiological or nuclear threats, and DARPA has supported programs on both radiation biodosimetry and countermeasures development. Although the RCP and DTRA/DARPA have not developed or funded any joint initiatives, RCP program managers routinely serve on DTRA review panels. Informal program reviews between RCP, DTRA, and DARPA are conducted annually to minimize overlap or duplication of effort.

- *Joint Program Executive Office for Chemical and Biological Defense (JPEO-CBD)*

  In late 2006, having consulted RCP and others, the JPEO-CBD Chemical Biological Medical Systems Joint Project Management Office for Medical Identification and Treatment Systems made a strategic decision to concentrate advanced product development efforts on mitigators and therapeutics for gastrointestinal acute radiation syndrome (GI-ARS). RCP program managers served as advisers to the JPEO-CBD Technical Evaluation Panel during the contract review process and serve on the Integrated Project Teams supervising the awarded contracts.

  To complement this program, the RCP developed a GI-ARS initiative focused on products at an earlier stage of development, thus creating candidate MCMs that could be advanced if the initial products supported by JPEO-CBD failed to achieve approval. To facilitate such

approvals, the RCP, working through *MCART,* accelerated its efforts to develop Good Laboratory Practice (GLP) murine and non-human primate models of GI-ARS.

In addition, JPEO-CBD program managers and staff participate in all RCP special topic and general program conferences and workshops, including RCP twice-monthly Radiation/Nuclear Group meetings and advanced product development meetings.

- *National Aeronautics and Space Administration (NASA)*

  NASA established the *National Space Biomedical Research Institute (NSBRI)* as a non-profit scientific partnership to engage academic, industrial and government researchers and educators and the resources of the nation's leading biomedical research institutions to provide solutions to reduce the significant health risks associated with human space travel and long-duration spaceflight. NSBRI has identified the development of effective MCMs against radiation as a programmatic priority.

  In recognition of their overlapping missions, the RCP and NSBRI signed a Memorandum of Understanding (MOU) in 2007, outlining research areas of mutual interest. This MOU stated the intention of both institutions to communicate openly and coordinate research in these common interest areas. Although budget limitations have prevented the development of joint programs, RCP and NSBRI program managers have assisted each other in the review of grants and contracts and participate in each other's program meetings.

Interagency Committees
RCP program managers also actively participate in a number of interagency committees that include

- The Public Health Emergency Medical Countermeasures Enterprise (PHEMCE) Enterprise Executive Committee, which meets regularly to establish overall PHEMCE policy objectives and review progress. PHEMCE was established to coordinate interagency goals, objectives, and efforts to develop MCMs against chemical, biological, radiological, and nuclear (CBRN) and pandemic threats.

- The NIH Biodefense Research Coordinating Committee, which serves as a forum for information exchange among the different NIH Institutes and Centers

- The Radiobiology Bioterrorism Research and Training Group, which is convened quarterly by NCI and brings together representatives from all Departments and Agencies that perform or support radiobiology research

The RCP promotes interagency awareness of recent scientific advances through its periodic Radiation Nuclear Group meetings, at which NIAID-funded investigators and other scientists present cutting-edge work to RCP program managers and interagency partners.

International Collaborations and Partnerships
As with its domestic collaborations and partnerships, the RCP seeks through its international outreach to foster a sense of common purpose, enhance transparency and coordination of effort,

and improve the allocation of limited resources. Increasing international interest in and markets for radiation MCMs is an important component of the RCP business model.

RCP program managers have visited international facilities and engaged in exploratory discussions about the possibility of establishing joint research programs with colleagues at several national radiation research centers. Although there are considerable logistical and organizational challenges to establish and maintain such collaborations, these have not hindered the development of a successful collaboration with the *Radiation Effects Research Foundation (RERF).* RCP has also leveraged multilateral forums to promote awareness and interest in radiation MCMs, serving as a Liaison Institution of the *World Health Organization (WHO)/Radiation Emergency Medical Preparedness and Assistance Network (REMPAN)* and participating in the *Global Health Security Initiative (GHSI) Radiological and Nuclear Threats Working Group.* These efforts are described below.

- *Radiation Effects Research Foundation (RERF)*

  RERF is a binational Japan–U.S. scientific organization dedicated to studying the health effects of atomic bomb radiation for peaceful purposes. RERF's long-term follow-up of atomic bomb survivors provides unparalleled opportunities to study the effects of atomic bomb radiation on human populations. See Section 1, Goal 7 for a description of the collaborative research scope.

- *World Health Organization (WHO)/Radiation Emergency Medical Preparedness and Assistance Network (REMPAN)*

  REMPAN was established in 1987 to fulfill WHO's responsibility under the two international conventions on Early Notification and Assistance. By joining REMPAN and participating in the WHO/REMPAN Biennial Meetings since 2006, the RCP has increased its profile internationally, enabled networking between RCP program managers and colleagues and peers abroad, and provided an international forum to communicate program priorities. The RCP provided financial support for the 2008 Biennial Meeting and expert consultations in Geneva on radiation MCM stockpile development and the harmonization of clinical treatment protocols for patients with acute radiation syndrome (ARS).

- *Global Health Security Initiative (GHSI) Radiological and Nuclear Threats Working Group (WG)*

  The GHSI provides a forum for countries to discuss and coordinate efforts to prepare for and respond to the threats of CBRN terrorism and pandemic influenza. The GHSI was formed in October 2001 and includes Canada, the European Union, France, Germany, Italy, Japan, Mexico, the United Kingdom, and the United States, with WHO serving as an expert advisor. The WG meets annually and has been instrumental in elevating the priority of establishing a global infrastructure for MCMs. RCP program managers participate in all WG meetings and have given presentations about RCP activities.

LIST OF ACRONYMS

A-bomb	Atomic Bomb
AFRRI	Armed Forces Radiobiology Research Institute
ARS	Acute Radiation Syndrome
BARDA	Biomedical Advanced Research and Development Authority
BioDoseNet	Biodosimetry Laboratory Network
BrIDGS	Bridging Interventional Development Gaps Program
BTRCs	Biomedical Technology Research Centers
CBRN	Chemical, Biological, Radiological and Nuclear
CDC	Centers for Disease Control and Prevention
CMCRs	Centers for Medical Countermeasures Against Radiation
CounterACT	Countermeasures Against Chemical Threats
DARPA	Defense Advanced Research Projects Agency
DEARE	Delayed Effects of Acute Radiation Exposure
DoD	Department of Defense
DTPA	Diethylenetriaminepentaacetic Acid
DTRA	Defense Threat Reduction Agency
ELISA	Enzyme-Linked Immunosorbent Assay
EPR	Electron Paramagnetic Resonance
FDA	Food and Drug Administration
G-CSF	Granulocyte Colony Stimulating Factor
GHSI	Global Health Security Initiative
GI-ARS	Gastrointestinal Acute Radiation Syndrome
GLP	Good Laboratory Practice
Gy	Gray
HE-ARS	Hematopoietic Acute Radiation Syndrome
HHS	Department of Health and Human Services
HOPO	Hydroxypyridinonate
HSC	Hematopoietic Stem Cell
IAA	Intra-Agency Agreement or Interagency Agreement
IM	Inflammatory Molecules
IND	Investigational New Drug
ISCC	Intestinal Stem Cell Consortium
ISO	International Standards Organization
JPEO-CBD	Joint Program Executive Office for Chemical and Biological Defense
LKI	Local Kidney Irradiation
MCART	Medical Countermeasures Against Radiological Threats
MCMs	Medical Countermeasures
MODS	Multi-Organ Dysfunction Syndrome
MOU	Memorandum of Understanding
NASA	National Aeronautics and Space Administration
NCATS	National Center for Advancing Translational Sciences
NCGC	National Chemical Genomic Center
NCI	National Cancer Institute
NDA	New Drug Application

NHP	Non-Human Primate
NIA	National Institute on Aging
NIAID	National Institute of Allergy and Infectious Diseases
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases
NIH	National Institutes of Health
NSBRI	National Space Biomedical Research Institute
PHEMCE	Public Health Emergency Medical Countermeasures Enterprise
qRT-PCR	Quantitative Real-Time Reverse-Transcription Polymerase Chain Reaction
RABIT	Rapid Automated Biodosimetry Tool
RAID	Rapid Access to Interventional Development
RCI	Radiation Combined Injury
REB	Radiation Epidemiology Branch
REMPAN	Radiation Emergency Medical Preparedness and Assistance Network
RERF	Radiation Effects Research Foundation
Rspo1	R-spondin1
SBIR	Small Business Innovation Research
TBI	Total Body Irradiation
TLR	Toll-Like Receptor
UCLA	University of California—Los Angeles
UMB	University of Maryland—Baltimore
WHO	World Health Organization