Exhibit R-2, RDT&E Budget Item Justification: PB 2016 Defense Health Program	Date: February 2015
Appropriation/Budget Activity 0130: *Defense Health Program I* BA 2: *RDT&E*	**R-1 Program Element (Number/Name)** PE 0602787HP *I Medical Technology (AFRRI)*

COST ($ in Millions)	Prior Years	FY 2014	FY 2015	FY 2016 Base	FY 2016 OCO	FY 2016 Total	FY 2017	FY 2018	FY 2019	FY 2020	Cost To Complete	Total Cost
Total Program Element	4.718	1.139	1.241	1.222	-	1.222	1.242	1.331	1.356	1.383	Continuing	Continuing
020: *CSI - Congressional Special Interests*	0.000	-	0.124	-	-	-	-	-	-	-	Continuing	Continuing
241A: *Biodosimetry (USUHS)*	0.963	0.232	0.228	0.249	-	0.249	0.254	0.272	0.277	0.283	Continuing	Continuing
241B: *Internal Contamination (USUHS)*	0.500	0.121	0.119	0.131	-	0.131	0.133	0.143	0.146	0.149	Continuing	Continuing
241C: *Radiation Countermeasures (USUHS)*	3.255	0.786	0.770	0.842	-	0.842	0.855	0.916	0.933	0.951	Continuing	Continuing

A. Mission Description and Budget Item Justification

For the Uniformed Services University of the Health Sciences (USUHS), Armed Forces Radiobiology Research Institute (AFRRI), this program supports developmental research to investigate new approaches that will lead to advancements in biomedical strategies for preventing, treating, assessing and predicting the health effects of human exposure to ionizing radiation. Program objectives focus on preventing or mitigating the health consequences from exposures to ionizing radiation that represent the highest probable threat to U.S. forces in current tactical, humanitarian and counterterrorism mission environments. New protective and therapeutic strategies will broaden the military commander's options for operating within nuclear or radiological environments by minimizing both short-and long-term risks of adverse health consequences. Advances in assessment, prognostication, and therapy in case of actual or suspected radiation exposures will enhance triage, treatment decisions and risk assessment in operational settings.

B. Program Change Summary ($ in Millions)	FY 2014	FY 2015	FY 2016 Base	FY 2016 OCO	FY 2016 Total
Previous President's Budget	1.216	1.117	1.222	-	1.222
Current President's Budget	1.139	1.241	1.222	-	1.222
Total Adjustments	-0.077	0.124	-	-	-
• Congressional General Reductions	-	-			
• Congressional Directed Reductions	-	-			
• Congressional Rescissions	-	-			
• Congressional Adds	-	0.124			
• Congressional Directed Transfers	-	-			
• Reprogrammings	-	-			
• SBIR/STTR Transfer	-0.077	-			

Congressional Add Details ($ in Millions, and Includes General Reductions)	FY 2014	FY 2015
Project: 020: *CSI - Congressional Special Interests*		
Congressional Add: *472A – Program Increase: Restore Core Research Funding Reduction (USUHS)*	-	0.124

Exhibit R-2, RDT&E Budget Item Justification: PB 2016 Defense Health Program	Date: February 2015
Appropriation/Budget Activity 0130: *Defense Health Program I* BA 2: *RDT&E*	**R-1 Program Element (Number/Name)** PE 0602787HP *I Medical Technology (AFRRI)*

Congressional Add Details ($ in Millions, and Includes General Reductions)	FY 2014	FY 2015
Congressional Add Subtotals for Project: 020	-	0.124
Congressional Add Totals for all Projects	-	0.124

Change Summary Explanation

FY 2014: Realignment from Defense Health Program, Research, Development, Test and Evaluation (DHP RDT&E), PE 0602787-Medical Technology (AFRRI) (-$0.077 million) to DHP RDT&E PE 0605502-Small Business Innovation Research (SBIR) Program (+$0.077 million).

FY 2015: Congressional Special Interest (CSI) Additions to DHP RDT&E, PE 0602787-Medical Technology (AFRRI) (+$0.124 million).

FY 2016: No Change.

Exhibit R-2A, RDT&E Project Justification: PB 2016 Defense Health Program			Date: February 2015
Appropriation/Budget Activity 0130 / 2	R-1 Program Element (Number/Name) PE 0602787HP / *Medical Technology (AFRRI)*	Project (Number/Name) 020 / *CSI - Congressional Special Interests*	

COST ($ in Millions)	Prior Years	FY 2014	FY 2015	FY 2016 Base	FY 2016 OCO	FY 2016 Total	FY 2017	FY 2018	FY 2019	FY 2020	Cost To Complete	Total Cost
020: *CSI - Congressional Special Interests*	-	-	0.124	-	-	-	-	-	-	-	Continuing	Continuing

A. Mission Description and Budget Item Justification

The FY15 DHP Congressional Special Interest (CSI) funding is directed toward core research initiatives in Program Element (PE) 0602787 - Medical Technology (AFRRI). Because of the CSI annual structure, out-year funding is not programmed.

B. Accomplishments/Planned Programs ($ in Millions)	FY 2014	FY 2015
Congressional Add: 472A – Program Increase: Restore Core Research Funding Reduction (USUHS)	-	0.124
FY 2014 Accomplishments: No funding programmed. This is an FY 2015 DHP Congressional Special Interest (CSI) spending item.		
FY 2015 Plans: FY 2015 DHP Congressional Special Interest (CSI) spending item directed toward the restoral of core research initiatives in the Medical Technology (AFRRI) Program Element (PE) - 0602787.		
Congressional Adds Subtotals	-	0.124

C. Other Program Funding Summary ($ in Millions)

N/A

Remarks

D. Acquisition Strategy

N/A

E. Performance Metrics

N/A

Exhibit R-2A, RDT&E Project Justification: PB 2016 Defense Health Program			Date: February 2015
Appropriation/Budget Activity 0130 / 2	R-1 Program Element (Number/Name) PE 0602787HP / *Medical Technology (AFRRI)*	Project (Number/Name) 241A / *Biodosimetry (USUHS)*	

COST ($ in Millions)	Prior Years	FY 2014	FY 2015	FY 2016 Base	FY 2016 OCO	FY 2016 Total	FY 2017	FY 2018	FY 2019	FY 2020	Cost To Complete	Total Cost
241A: *Biodosimetry (USUHS)*	0.963	0.232	0.228	0.249	-	0.249	0.254	0.272	0.277	0.283	Continuing	Continuing

A. Mission Description and Budget Item Justification

Biodosimetry (USUHS): For the Uniformed Services University of the Health Sciences (USUHS), the mission and research objectives for biodosimetry are to assess radiation exposure by developing and providing biological and biophysical dosimetry capabilities for acute, protracted, and prior radiation exposures.

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B. Accomplishments/Planned Programs ($ in Millions)	FY 2014	FY 2015	FY 2016
Title: Biodosimetry (USUHS)	0.232	0.228	0.249
FY 2014 Accomplishments: -Established the dosimetry map for protracted (Low-Dose-Rate or LDR) 60Co irradiation for murine model; initiated comparison studies between LDR and prompt radiation on selected biomarkers in murine models. -Completed study evaluating effects of 2 different dose rates on hematology and select proteomic biomarkers. -Began to evaluate protein biomarkers, hematological parameters, and clinical signs ranging 1d – 2d in partial-body irradiated mice. -Continued to evaluate whether epigenetic markers can be used to discriminate low-dose from high-dose radiation. -Determined if there is a chromosomal aberration difference between external radiation and internalized depleted uranium. -Evaluated whether the profile of chromosomal aberrations in human samples are able to discriminate uranium exposure from other toxic exposures. -Determined histological effects of radiation on intestinal organoid cultures. -Sent conditioned media samples from irradiated intestinal organoid cultures for proteomic analysis by liquid chromatography-tandem mass spectrometry to identify biomarkers. -Investigated impact of improving chromosome condensation on the ability to automate detection and counting of interphase chromosome aberrations.			

Exhibit R-2A, RDT&E Project Justification: PB 2016 Defense Health Program		Date: February 2015
Appropriation/Budget Activity 0130 / 2	**R-1 Program Element (Number/Name)** PE 0602787HP / *Medical Technology (AFRRI)*	**Project (Number/Name)** 241A / *Biodosimetry (USUHS)*

B. Accomplishments/Planned Programs ($ in Millions)	FY 2014	FY 2015	FY 2016
-Developed and integrated a spooler for automatic gene expression data inclusion from experiments and literature for indexing into the automated analysis system. -Evaluated applicability of new hardware, imaging tools, and suitability for use of mobile platforms and tablets in the automated chromosome aberration scoring system. -Sustained efforts to provide necessary proof-of-concept dose-response data to transition combined proteomic and hematological concept for further development of diagnostic devices (i.e., hand-held, field deployable). -Reported on evaluation of both radiation induced hematological and plasma protein biomarkers in the early-phase after irradiation partial-body exposure model using x-ray source with lead shielding and mice restrained by holders.			
FY 2015 Plans: -Sustain studies evaluating new radiation-responsive biomarkers in animal models for early-phase and organ-specific bioindicators. -Begin a pilot study using samples from the mouse and NHP total-body irradiation models to permit testing of the measurement of novel tissue- and organ-specific biomarkers in peripheral blood using commercially available antibodies and assays developed at AFRRI. -Begin to analyze blood chemistry data collected in the NHP dose-response study with limited supportive care and in the high-dose study with full supportive care (G-CSF, antibiotics, blood transfusions, etc.) to evaluate radiation damage to specific organs. -Begin to analyze results of necropsies performed on NHPs (limited and full supportive care) to determine the radiation dose-dependent damage to different organs/tissues and correlate those results with levels of tissue/organ-specific protein biomarkers. -Initiate studies to evaluate effects of even lower dose rates on hematology and select radiation biomarkers. -Determine whether epigenetic markers can discriminate between chronic low dose and repeated low dose exposures. -Determine whether epigenetic markers can discriminate between external radiation and internalized depleted uranium.			
FY 2016 Plans: -Establish a partial-body radiation model using mice involving exposure of the abdomen with AFRRI's small animal irradiator to support studies identifying and validating organ (i.e., small intestine, kidney) injury biomarkers. -Establish murine model system to measure low dose epigenetic markers. -Examine radiation-induced mitochondrial DNA (mtDNA) deletion in animal samples from low and high doses of radiation exposure using a nested real-time PCR method, and evaluate the sensitivity and specificity of mtDNA deletion in response to gamma-radiation. -Develop a circulating micro-RNA profile in γ-irradiated animal model.Select the radiation-sensitive micro-RNAs that are stable and easy to calibrate in serum as radiation biomarkers to monitor radiation injury and efficacy of radiation countermeasures. -Evaluate proinflammatory cytokines as biomarkers to monitor ionizing radiation-induced acute and chronic injury and evaluate the efficacy of radiation countermeasures.			

Exhibit R-2A, RDT&E Project Justification: PB 2016 Defense Health Program		Date: February 2015
Appropriation/Budget Activity 0130 / 2	**R-1 Program Element (Number/Name)** PE 0602787HP / *Medical Technology (AFRRI)*	**Project (Number/Name)** 241A / *Biodosimetry (USUHS)*

B. Accomplishments/Planned Programs ($ in Millions)	FY 2014	FY 2015	FY 2016
-Determine the mechanisms of circulating micro-RNA and proinflammatory cytokine release after radiation exposure.			
Accomplishments/Planned Programs Subtotals	0.232	0.228	0.249

C. Other Program Funding Summary ($ in Millions)

N/A

Remarks

D. Acquisition Strategy

N/A

E. Performance Metrics

By FY14

-Identify radiation biomarkers that are dependent on exposure dose-rate and specific for various ARS subsyndromes.
-Demonstrate accurate radiological detection of radiation biomarkers from biological samples into quartiles of doses 0-1 Gy, 1-3 Gy, 3-6 Gy, 6-10 Gy, and greater than 10Gy.
-Characterize partial-body radiation murine models over a protracted time period and compare results with prompt irradiation on selected biomarkers.
-Provide preliminary analysis of the enhanced utility of combined hematological and protein biomarkers for biodosimetry applications following photon and mixed fieldneutron total-body irradiations in a total-body irradiation murine model.
-Identify subset of biomarkers useful for radiation dose assessment when confounded with thermal burns.
Complete report of select radiation biomarkers that are dependent upon dose-rate.
-Report on gender and age effects as well as partial-body irradiation effects on the evaluated panel of protein biomarkers in mouse model.
-Submit samples from radiation-exposed intestinal epithelial cell organoid cultures for Liquid Chromatography-Tandem Mass Spectrometry analysis for novel radiationbiomarker discovery.
-Score histological injury to intestinal organoid cultures after irradiation.
-Measure specific methylation and histone changes using RT-PCR in low dose and high dose bronchial cells.
-Measure chromosomal aberrations in lymphocytes from gamma ray and depleted uranium exposed mice (spleen tissues).
-Measure intra-chromosomal aberrations using mBAND technology in human samples from individuals potentially exposed to toxic materials during deployment.
-Improve condensation of interphase chromatin into discrete chromosomes capable to be read through high-throughput image capture tools.
-Establish and incorporate Absorption Color Pigment (ACP) method for automated image extractors within CLASP.
-Provide report to validate specificity and sensitivity statistical models for the automated image system and analyses thereby testing CLASP efficiency.
-Evaluate the applicability and efficiency of developed SOPs after inclusion of multi-parametric approaches within CLASP.

By FY15

Exhibit R-2A, RDT&E Project Justification: PB 2016 Defense Health Program		Date: February 2015
Appropriation/Budget Activity 0130 / 2	**R-1 Program Element (Number/Name)** PE 0602787HP / *Medical Technology (AFRRI)*	**Project (Number/Name)** 241A / *Biodosimetry (USUHS)*

-Characterize partial-body radiation murine models over aprotracted time period and compare results with prompt irradiation on selected biomarkers.
-Perform a pilot study using samples from the mouse and NHP total-body irradiation models to permit testing of the measurement of novel tissue- and organ-specific biomarkers in peripheral blood using commercially available antibodies and assays developed at AFRRI.
-Complete analysis of blood chemistry data collected in the NHP dose-response study with limited supportive care and in the high-dose study with full supportive care (G-CSF, antibiotics, blood transfusions, etc.) to evaluate radiation damage to specific organs.
-Complete analysis of results of necropsies performed on NHPs (limited and full supportive care) to determine radiation dose-dependent damage to different organs/ tissues and correlate those results with levels of tissue/organ-specific protein biomarkers.
-Begin to evaluate the identified tissue- and organ-specific biomarkers in partial-body irradiation models.
-Provide necessary proof-of-concept dose-response data to transition combined proteomic and hematological concept for further development of diagnostic devices (i.e.,hand-held, field deployable) and obtain the necessary FDA approval. Prepare preliminary report for FDA on combined utility of hematological and protein biomarkers forbiodosimetry applications in two FDA-required animal models.
-Identify other radiation biomarkers that are dependent on exposure dose-rate.
-Validate dosimetric response of 3 biomarkers from IEC organoids exposed to 0-16 Gy gamma-ray radiation.
-Measure specific methylation and histone changes usingRT-PCR in low dose and high dose murine spleen samples.
-Identify proteomic markers from irradiated organoid cultures for validation by enzyme linked immunosorbent assay.
-Characterize dose profile for partial-body exposures using AFRRI's small animal irradiator (SAARP).
-Establish assays for candidate radiation biomarkers for assessment of injury to specific radiation-sensitive organs.
-Initiate studies to evaluate radiation-induced chromosomal damage in murine radiation model.

By FY16
-Initiate partial-body exposure study to characterize organ specific injury biomarkers using abdomen exposures of mice.
-Report on measurements of miRNA levels to identify organ-specific injury biomarkers.
-Measure the incidence of leukemia development in vivo after chronic or repeated exposure to low dose radiation in a murine model.
-Continue to refine the combination of radiation biomarkers in blood with the best balance of discrimination of sensitivity and specificity.

Exhibit R-2A, RDT&E Project Justification: PB 2016 Defense Health Program			Date: February 2015
Appropriation/Budget Activity 0130 / 2	R-1 Program Element (Number/Name) PE 0602787HP / *Medical Technology (AFRRI)*	Project (Number/Name) 241B / *Internal Contamination (USUHS)*	

COST ($ in Millions)	Prior Years	FY 2014	FY 2015	FY 2016 Base	FY 2016 OCO	FY 2016 Total	FY 2017	FY 2018	FY 2019	FY 2020	Cost To Complete	Total Cost
241B: *Internal Contamination (USUHS)*	0.500	0.121	0.119	0.131	-	0.131	0.133	0.143	0.146	0.149	Continuing	Continuing

A. Mission Description and Budget Item Justification

Internal Contamination (USUHS): For the Uniformed Services University of the Health Sciences (USUHS), the mission and research objective for Internal Contamination is to determine whether the short-term and long-term radiological and toxicological risks of embedded metals warrant changes in the current combat and post-combat fragment removal policies for military personnel. Additionally, the biological effects of internalization of radioactive elements from Radiological Dispersal Devices (RDDs) and depleted uranium weapons, as well as therapeutic approaches to enhance the elimination of radionuclides from the body are being investigated.

B. Accomplishments/Planned Programs ($ in Millions)	FY 2014	FY 2015	FY 2016
Title: Internal Contamination (USUHS)	0.121	0.119	0.131
FY 2014 Accomplishments: -Determined the efficacy of molecularly imprinted polymers on reducing the body burden of internalized radionuclides using a rodent model system. -Validated combinatorial approach of depleted uranium-induced damage to cellular epigenetic machinery using an in vivo model.			
FY 2015 Plans: -Test novel leukemia countermeasures to determine if chemoprevention mechanism involves modification of chromatin regulation in depleted uranium-induced leukemia in vivo. -Design feasibility study to determine if non-radioactive metals can substitute as template molecules for high-specific activity radionuclides in the synthesis of molecularly imprinted polymers.			
FY 2016 Plans: -Initiate study to assess the applicability of molecularly imprinted polymers in the decontamination of skin exposed to radionuclides. -Begin development and validation of a polytrauma model to assess the combined effects of mild traumatic brain injury and low-level radiation exposure, from external or internalized sources, in a rodent model system. -Test novel countermeasure to low dose radiation and determine if chromatin remodeling is involved.			
Accomplishments/Planned Programs Subtotals	0.121	0.119	0.131

C. Other Program Funding Summary ($ in Millions)

N/A

Remarks

Exhibit R-2A, RDT&E Project Justification: PB 2016 Defense Health Program		Date: February 2015
Appropriation/Budget Activity 0130 / 2	**R-1 Program Element (Number/Name)** PE 0602787HP / *Medical Technology (AFRRI)*	**Project (Number/Name)** 241B / *Internal Contamination (USUHS)*

D. Acquisition Strategy

N/A

E. Performance Metrics

By FY14

-Complete assessment of combinatorial approach for assessing depleted uranium-induced damage.

-Conclude evaluation of molecularly imprinted polymers as decorporation agents.

By FY15

-Initiate study to assess feasibility of using non-radioactive templates in the synthesis of molecularly imprinted polymers to radioactive metals.

-Complete in vivo study on the mechanism of depleted uranium-induced leukemia.

By FY16

-Conclude feasibility assessment studies on the possibility of using non-radioactive templates for the synthesis of molecularly imprinted polymers designed to bind radioactive metals.

-Initiate in vitro/in vivo model system study to assess novel countermeasure to low dose radiation leukemia that targets specific chromatin remodeling.

Exhibit R-2A, RDT&E Project Justification: PB 2016 Defense Health Program			Date: February 2015
Appropriation/Budget Activity 0130 / 2	R-1 Program Element (Number/Name) PE 0602787HP / *Medical Technology (AFRRI)*	Project (Number/Name) 241C / *Radiation Countermeasures (USUHS)*	

COST ($ in Millions)	Prior Years	FY 2014	FY 2015	FY 2016 Base	FY 2016 OCO	FY 2016 Total	FY 2017	FY 2018	FY 2019	FY 2020	Cost To Complete	Total Cost
241C: *Radiation Countermeasures (USUHS)*	3.255	0.786	0.770	0.842	-	0.842	0.855	0.916	0.933	0.951	Continuing	Continuing

A. Mission Description and Budget Item Justification

Radiation Countermeasures (USUHS): For the Uniformed Services University of the Health Sciences (USUHS), this program supports developmental, mission directed research to investigate new concepts and approaches that will lead to advancements in biomedical strategies for preventing and treating the health effects of human exposure to ionizing radiation as well as radiation combined with injuries (burns, wounds, hemorrhage). Research ranges from exploration of biological processes likely to form the basis of technological solutions, to initial feasibility studies of promising solutions. Program objectives focus on mitigating the health consequences from exposures to ionizing radiation, in the context of probable threats to U.S. forces in current tactical, humanitarian and counterterrorism mission environments. New protective and therapeutic strategies will broaden the military commander's options for operating within nuclear or radiological environments by minimizing both short- and long-term risks of adverse health consequences.

B. Accomplishments/Planned Programs ($ in Millions)	FY 2014	FY 2015	FY 2016
Title: Radiation Countermeasures (USUHS)	0.786	0.770	0.842
FY 2014 Accomplishments: - Evaluated the radioprotective and mitigative/therapeutic effects of nano-gamma-tocotrienol (GT3) in mouse model - Determined acute and late effects of radiation-induced bone damage and prevention by GT3 after whole body radiation. - Analyzed global protein profiling after radiation in mouse spleen and kidney with varying doses and times after radiation. - Evaluated radiation-induced micro-RNA changes in mouse jejunum after GT3 treatment. - Evaluated the efficacy of a combined pharmaceutical regimen against radiation combined injury (irradiation followed immediately by skin wound trauma). -Determined effectiveness of combined therapy of G-CSF and ALXN4100TPO, a thrombopoietin receptor agonist, to prevent, mitigate, or inhibit the long-term deleterious responses to radiation combined injury. -Evaluated the micro-RNA profile in mouse serum after radiation alone and combination with wound trauma. -Evaluated the efficacy of IL-10 as a countermeasure to radiation and combined injury-associated effects on bone microarchitecture, strength, tissue-level cellular mechanisms, biomarkers of bone metabolism and immune effects. -Explored the role of the immune system in bone's response to radiation and combined injury (i.e. osteoimmunology). -Investigated the molecular mechanisms involved in radiation, wounding, hemorrhage, and/or combined injury. -Investigated the effects of mixed neutron/gamma radiation on secondary immune organs (liver, spleen). -Determined the efficacy of CDX-301 as a radiation mitigator after mixed neutron/gamma radiation. -Investigated the effect(s) of CDX-301 on hematopoietic cells in the lung, spleen, bone marrow when administered after mixed neutron/gamma radiation.			

Exhibit R-2A, RDT&E Project Justification: PB 2016 Defense Health Program		Date: February 2015
Appropriation/Budget Activity 0130 / 2	**R-1 Program Element (Number/Name)** PE 0602787HP / *Medical Technology (AFRRI)*	**Project (Number/Name)** 241C / *Radiation Countermeasures (USUHS)*

B. Accomplishments/Planned Programs ($ in Millions)	FY 2014	FY 2015	FY 2016
-Explored the role that sclerostin, an inhibitor of osteoblastogenesis, has on radiation and/or combined injury-associated reductions in bone mass and its effects on Wnt/β-catenin signaling. -Determined whether protection of bone marrow environment epigenetic changes following radiation can prevent radiation leukemia. -Continued study of the mitigation of radiation injury using apoptotic pathway markers in mice receiving TS-mobilized progenitors. -Performed genome-wide transcriptomic and proteomic profiling to elucidate coordinate pathway activation markers associated with tocopherol-mediated bioactivity. -Performed RNA-sequence profiling of small RNA, as well as mRNA transcriptomes, antibody microarray and 2D gel electrophoresis profiling of low and high abundance proteomes with samples obtained after tocopherol succinate (TS) treatment. -Small molecule inhibitors for candidate signaling pathways associated with TS activity were utilized to determine their requirements for CSF family member production, most notably, G-CSF production. -Screened several human primary organ-specific cell types (epithelial, fibroblast, endothelial, etc.) for CSF transcript up-regulation in response to alpha-tocopherol. -Determined radioprotection (drug administered before irradiation) with 10 new compounds. -Tested radioprotection by BB-001 and ODSH. -Determined the efficacy of filgrastim (administered after irradiation) and ALXN4100TPO (administered prior to radiation) on radiation lethality and how the combination influences hematopoietic end points as measured by circulating blood elements. -Tested efficacy of ALXN4100TPO in different mouse strains. -Evaluated microRNAs and inflammatory factors as radiation biomarkers. -Evaluated the radioprotective and mitigative/therapeutic effects of tilorone hydrochloride in in vivo animal model. -Studied the role of inflammatory pathways in ionizing radiation-induced bone marrow failure. -Established 3 dimensional coculture in vitro model to evaluate the effects of bone marrow endothelial cells (BMEC) on hematopoietic stem and progenitor cells (HSPC) in a 3D environment. -Initiated ex vivo culture of murine BMEC for in vivo studies. -Tested hypothesis that EC improve animal survival after gamma irradiation. -Tested functional roles of EC in hematopoietic support after irradiation. -Tested hypothesis that Ang/Tie2 pathway is involved in animal survival after irradiation. -Tested functional roles of Ang/Tie2 pathway in hematopoietic support after irradiation. -Initiated analysis of gene array data from irradiated human marrow endothelial cells and hematopoietic progenitor cells. -Completed establishing the combined injury model with radiation followed by hemorrhage. -Completed evaluation of peg-G-CSF and Alxn4100TPO co-therapy after irradiation and wound combined injury.			
FY 2015 Plans: -Evaluate RANKL-mediated signaling pathways in skeletal tissues after radiation and their modulation by gamma-tocotrienol.			

Exhibit R-2A, RDT&E Project Justification: PB 2016 Defense Health Program		Date: February 2015
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B. Accomplishments/Planned Programs ($ in Millions)	FY 2014	FY 2015	FY 2016
-Examine radiation-induced neuronal damage and mitigation by GT3 using cell culture and mouse brain. -Evaluate the role of nrf2 pathway after radiation in microglial cells and its modulation by GT3. -Evaluate intracellular signaling pathways in mechanisms of efficacy of GT3 in different mouse tissues after radiation. -Determine the role of hedgehog signaling in hematopoietic recovery following sub-lethal dose of radiation (in vitro and in vivo study). -Determine the role of HIF-1a and HIF-2a in the regulation of erythropoiesis after radiation, and effect of GT3. -Continue to evaluate micro-RNA profiles in mouse serum after both radiation alone and combination with wound trauma with treatment with countermeasures. -Determine the potential efficacy of a sclerostin antibody, which inhibits radiation-induced reductions in bone formation. -Continue to explore the role of the immune system in bone's response to radiation and combined injury. -Determine whether phenylbutyrate-induced suppression of neoplastic transformation of bronchial tissue is radiation dose dependent (low versus high) and whether epigenetic or genetic processes are predominant. -Study transcriptomics in various subsets of TS-mobilized progenitors. -Continue to evaluate changes in hematopoietic cell populations in multiple organs (spleen, lung, liver, bone marrow) in irradiated mice treated with bone marrow endothelial cells. -Evaluate alterations in signaling pathways and cytokine profiles in response to bone marrow endothelial cell induced responses to gamma radiation. -Complete analysis of gene array data from irradiated human marrow endothelial cells and hematopoietic progenitor cells. -Characterize mTOR-AKT and MAPK signal mediation of radiation-hemorrhage combined injury. -Identify dynamic changes in circulatory blood cell counts, bone marrow cellularity and ileum structure morphology after radiation-wound combined injury. -Evaluate systemic bacterial infection after radiation-wound combined injury. -Screen 10-15 drugs in a mouse model for their radiation countermeasure potential. ***FY 2016 Plans:*** -Continue to correlate mTOR-AKT and MAPK signaling network and ATP production after radiation-hemorrhage combined injury. -Continue to elucidate mechanisms underlying ghrelin efficacy on survival improvement after radiation-wound combined injury by profiling cytokine/chemokine, signal transduction pathway activation, and miRNA regulation. -Improve low dose risk assessment knowledge base by determining whether chronic or repeated low dose exposure in a murine model induces leukemia in comparison to a high dose radiation exposure. -Study efficacy biomarkers for Ex-RAD using in vitro and in vivo systems. -Study whether elevated levels of pAkt are associated with survival. -Investigate various signaling pathways for Ex-RAD biomarkers.			
Accomplishments/Planned Programs Subtotals	0.786	0.770	0.842

Exhibit R-2A, RDT&E Project Justification: PB 2016 Defense Health Program		Date: February 2015
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C. Other Program Funding Summary ($ in Millions)

N/A

Remarks

D. Acquisition Strategy

N/A

E. Performance Metrics

By FY14

-Complete evaluation of the therapeutic effects of G-CSF and ALXN4100TPO on survival after radiation combined injury.
-Complete evaluation of the micro-RNA profile in mouse serum after radiation alone and combination with wound trauma.
-Complete evaluation of IL-10 as a countermeasure to radiation combined injury-induced bone loss and effects on immune system.
-Complete evaluation of molecular mechanisms involved in radiation, wounding, hemorrhage, and/or combined injury.
-Complete determination of the role that sclerostin has on radiation and/or combined injury-associated reductions in bone mass and its effects on Wnt/β-catenin signaling in bone.
-Measure methylation and histone changes in radiation-leukemogenic mice.
-Begin analysis of underlying mechanisms of therapeutic effects of G-CSF, TS-mobilized progenitors, and ALXN4100TPO after radiation combined injury.
-Complete studies on CDX-301 mechanism(s) of action.
-Complete DRF studies with filgrastim using our optimized schedule.
-Repeat strain survival studies to determine LD50 in four mouse strains.
-Establish supportive care in rhesus macaque model to include antibiotic treatment, blood transfusions and thereby establish LD50 in primates.
-Complete establishing the combined injury model with radiation followed by hemorrhage.
-Complete evaluation of peg-G-CSF and Alxn4100TPO co-therapy after irradiation-wound combined injury.

By FY15

-Begin determining the potential efficacy of a sclerostin antibody to inhibit combined injury-induced bone loss.
-Evaluate effect of chronic or repeated low dose radiation on neoplastic transformation of bronchial tissue.
-Initiate investigations into mechanisms of mitigation/protection by BB-001. Determine optimum dose and time schedules, followed by DRF studies.
-Characterize mTOR-AKT and MAPK signal mediation of radiation-hemorrhage combined injury.
-Identify dynamic changes in circulatory blood cell counts, bone marrow cellularity and ileum structure morphology after radiation-wound combined injury.
-Determine systemic bacterial infection after radiation-wound combined injury.
-Complete low dose study on bronchial tissues measuring low dose responses in vitro.
-Evaluate effect of chronic or repeated low dose radiation on neoplastic transformation of bronchial tissue.
-Screen 10-15 drugs in a mouse model for their radiation countermeasure potential.

Exhibit R-2A, RDT&E Project Justification: PB 2016 Defense Health Program		Date: February 2015
Appropriation/Budget Activity 0130 / 2	**R-1 Program Element (Number/Name)** PE 0602787HP / *Medical Technology (AFRRI)*	**Project (Number/Name)** 241C / *Radiation Countermeasures (USUHS)*

By FY16
-Correlate mTOR-AKT and MAPK signaling network and ATP production after radiation-hemorrhage combined injury.
-Characterize dynamic changes in cytokine/chemokine concentrations, signal transduction pathways, and miRNA regulation after radiation-wound combined injury.
-Measure the incidence of leukemia development in vivo after chronic or repeated exposure to low dose radiation in a murine model.
-Evaluate effects of Ex-RAD on phosphorylated Akt.
-Elucidate cell survival role of pAkt.
-Study apoptotic pathway targets for identification of biomarkers for Ex-RAD.