Exhibit R-2, RDT&E Budget Item Justification: PB 2017 Defense Health Agency											Date: February 2016	
Appropriation/Budget Activity 0130: *Defense Health Program* / BA 2: *RDT&E*					R-1 Program Element (Number/Name) PE 0602787DHA / *Medical Technology (AFRRI)*							
COST ($ in Millions)	**Prior Years**	**FY 2015**	**FY 2016**	**FY 2017 Base**	**FY 2017 OCO**	**FY 2017 Total**	**FY 2018**	**FY 2019**	**FY 2020**	**FY 2021**	**Cost To Complete**	**Total Cost**
Total Program Element	5.857	1.145	1.222	1.242	-	1.242	1.331	1.356	1.383	1.411	Continuing	Continuing
020: *CSI - Congressional Special Interests*	0.000	0.124	0.000	0.000	-	0.000	0.000	0.000	0.000	0.000	Continuing	Continuing
241A: *Biodosimetry (USUHS)*	1.195	0.208	0.249	0.254	-	0.254	0.272	0.277	0.283	0.289	Continuing	Continuing
241B: *Internal Contamination (USUHS)*	0.621	0.109	0.131	0.133	-	0.133	0.143	0.146	0.149	0.152	Continuing	Continuing
241C: *Radiation Countermeasures (USUHS)*	4.041	0.704	0.842	0.855	-	0.855	0.916	0.933	0.951	0.970	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 2015	FY 2016	FY 2017 Base	FY 2017 OCO	FY 2017 Total
Previous President's Budget	1.117	1.222	1.242	-	1.242
Current President's Budget	1.145	1.222	1.242	-	1.242
Total Adjustments	0.028	0.000	0.000	-	0.000
• Congressional General Reductions	-	-			
• Congressional Directed Reductions	-	-			
• Congressional Rescissions	-	-			
• Congressional Adds	0.124	-			
• Congressional Directed Transfers	-	-			
• Reprogrammings	-	-			
• SBIR/STTR Transfer	-0.096	-			

Congressional Add Details ($ in Millions, and Includes General Reductions)	FY 2015	FY 2016
Project: 020: *CSI - Congressional Special Interests*		
Congressional Add: *472A – Program Increase: Restore Core Research Funding Reduction (USUHS)*	0.124	0.000

Exhibit R-2, RDT&E Budget Item Justification: PB 2017 Defense Health Agency	Date: February 2016
Appropriation/Budget Activity 0130: *Defense Health Program I* BA 2: *RDT&E*	**R-1 Program Element (Number/Name)** PE 0602787DHA *I Medical Technology (AFRRI)*

Congressional Add Details ($ in Millions, and Includes General Reductions)	FY 2015	FY 2016
Congressional Add Subtotals for Project: 020	0.124	0.000
Congressional Add Totals for all Projects	0.124	0.000

Change Summary Explanation

FY 2015: Realignment from Defense Health Program, Research, Development, Test and Evaluation (DHP RDT&E), PE 0602787-Medical Technology (AFRRI) (-$0.096 million) to DHP RDT&E PE 0605502-Small Business Innovation Research (SBIR) / Small Business Technology Transfer (STTR) Program (+$0.096 million).

FY 2015: Restore core research funding to the DHP RDT&E, PE 0602787-Medical Technology (AFRRI) (+$0.124 million).

FY 2016: No Change.

FY 2017: No Change.

Exhibit R-2A, RDT&E Project Justification: PB 2017 Defense Health Agency			Date: February 2016
Appropriation/Budget Activity 0130 / 2	R-1 Program Element (Number/Name) PE 0602787DHA / *Medical Technology (AFRRI)*	Project (Number/Name) 020 / *CSI - Congressional Special Interests*	

COST ($ in Millions)	Prior Years	FY 2015	FY 2016	FY 2017 Base	FY 2017 OCO	FY 2017 Total	FY 2018	FY 2019	FY 2020	FY 2021	Cost To Complete	Total Cost
020: *CSI - Congressional Special Interests*	0.000	0.124	0.000	0.000	-	0.000	0.000	0.000	0.000	0.000	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 2015	FY 2016
Congressional Add: 472A – Program Increase: Restore Core Research Funding Reduction (USUHS)	0.124	0.000
FY 2015 Accomplishments: 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.		
FY 2016 Plans: No Funding Programmed.		
Congressional Adds Subtotals	0.124	0.000

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 2017 Defense Health Agency			Date: February 2016
Appropriation/Budget Activity 0130 / 2	R-1 Program Element (Number/Name) PE 0602787DHA / *Medical Technology (AFRRI)*	Project (Number/Name) 241A / *Biodosimetry (USUHS)*	

COST ($ in Millions)	Prior Years	FY 2015	FY 2016	FY 2017 Base	FY 2017 OCO	FY 2017 Total	FY 2018	FY 2019	FY 2020	FY 2021	Cost To Complete	Total Cost
241A: *Biodosimetry (USUHS)*	1.195	0.208	0.249	0.254	-	0.254	0.272	0.277	0.283	0.289	Continuing	Continuing

A. Mission Description and Budget Item Justification

For the Uniformed Services University of the Health Sciences (USU), 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. Accomplishments/Planned Programs ($ in Millions)	FY 2015	FY 2016	FY 2017
Title: Biodosimetry (USUHS)	0.208	0.249	0.254
Description: For the Uniformed Services University of the Health Sciences (USU), 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.			
FY 2015 Accomplishments: - Sustained studies evaluating new radiation-responsive biomarkers in animal models for early-phase and organ-specific bio indicators. - Reported on development of circulating pro-inflammatory factor IL-18 as novel radiation biomarker in mice, mini pigs and nonhuman primates. - Reported mechanisms of microRNA-30 as apoptosis inducer released in mouse serum in radiation dose-dependent manner, useful for radiation biomarker. - Characterized dosimetry and radio response for use of multiple parameter radiation biomarkers in a murine partial-body exposure model. - 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). - Began pilot study using blood samples from mouse and NHP total-body irradiation models to permit testing of measurement of novel tissue- and organ-specific biomarkers in peripheral blood using commercially available antibodies and assays developed at AFRRI.			

Exhibit R-2A, RDT&E Project Justification: PB 2017 Defense Health Agency		Date: February 2016
Appropriation/Budget Activity 0130 / 2	**R-1 Program Element (Number/Name)** PE 0602787DHA / *Medical Technology (AFRRI)*	**Project (Number/Name)** 241A / *Biodosimetry (USUHS)*

B. Accomplishments/Planned Programs ($ in Millions)	FY 2015	FY 2016	FY 2017
- Began to analyze hematology and blood serum chemistry data collected in NHP dose-response study with limited supportive care and in high-dose study with full supportive care (G-CSF, antibiotics, blood transfusions, etc.) to evaluate radiation damage to specific organs. - Began to analyze 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. - Began to compare results/data from NHP dose-response TBI (photon/low-LET) studies with data collected from radiation accident victims and radiation therapy patients - Completed pilot studies to establish 3-D primary mouse intestinal epithelial cell (IEC) organoid culture model and characterized radiation effects on histological and proteomic profile using LC-MS/MS. - Continued studies to evaluate effects of low dose radiation on hematology and leukemia markers and identified specific changes in epigenetic markers. - Initiated study to determine whether epigenetic markers i.e., histone methylation and acetylation, could discriminate between low dose single and low dose repeated exposures. - Initiated study to assess whether epigenetic markers i.e., DNA or histone methylation, or miRNA levels, could discriminate differences in dose rate.			
FY 2016 Plans: - Establish partial-body radiation model using mice involving exposure of abdomen with AFRRI's small animal irradiator to support studies identifying and validating organ (i.e., small intestine, kidney) injury biomarkers. - Continue studies evaluating new radiation-responsive biomarkers in animal models for early-phase and organ-specific damage. - Continue pilot study using blood samples from mouse and NHP total-body irradiation models to permit testing of measurement of novel tissue- and organ-specific biomarkers in peripheral blood using commercially available antibodies and assays developed at AFRRI. - Complete analysis of hematology and blood serum chemistry data collected in NHP dose-response study with limited supportive care and in 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. - Continue comparing results from NHP dose-response TBI (photon/low LET) studies with data collected from radiation accident victims and radiation therapy patients. - Assess whether hematology and leukemia markers during leukemogenesis can be differentially expressed at early and late phases of carcinogenesis. - Determine whether epigenetic changes can be used to discriminate differences in dose rate at low doses.			

Exhibit R-2A, RDT&E Project Justification: PB 2017 Defense Health Agency		Date: February 2016
Appropriation/Budget Activity 0130 / 2	**R-1 Program Element (Number/Name)** PE 0602787DHA / *Medical Technology (AFRRI)*	**Project (Number/Name)** 241A / *Biodosimetry (USUHS)*

B. Accomplishments/Planned Programs ($ in Millions)	FY 2015	FY 2016	FY 2017
- Develop IL-18 and IL-18 binding protein (IL-18BP) as dual biomarkers for assessment of radiation dose, severity and lethality in mice after total body radiation exposure. - Evaluate correlations between level of radiation biomarkers and survival rate in individual mice after radiation. ***FY 2017 Plans:*** - Report on use of multiple parameter biodosimetry for radiation dose assessment using murine partial-body exposure model. Initiate effects to measure chromosomal aberrations in mouse radiation model in support of dose assessment using multiple parameter biodosimetry. - Establish partial-body animal radiation models (mouse and NHP) using animals involving low-LET exposure with AFRRI small-animal irradiator (for mice) and LINAC (for NHPs) to identify organ-specific radiation injury biomarkers evaluated earlier in low-LET TBI studies. - Establish mouse TBI model for combined hematological and proteomic biodosimetry approach following mixed-field (photon and neutron, high-LET) in addition to one already established and evaluated for a pure photon (60Co γ-rays, low-LET) exposure. - Test murine model system to assess specific low dose epigenetic markers. - Establish in vitro and in vivo parameters for AFRRI low level radiation facility for multiple delayed radiation organ effects. - Evaluate effects and mechanisms of proinflammatory cytokine IL-18 and IL-18BP on radiation-induced cell damage and apoptosis pathways. - Develop circulating miRNAs profile in γ-irradiated mouse serum using miRNA microarray and quantitative reverse transcription (RT)-real-time-polymerase chain reaction (PCR). - Evaluate threshold doses of radiation-induced lymphocyte damage.			
Accomplishments/Planned Programs Subtotals	0.208	0.249	0.254

C. Other Program Funding Summary ($ in Millions)

N/A

Remarks

D. Acquisition Strategy

N/A

E. Performance Metrics

By FY 2015

- Begin analyses of blood samples from mouse and NHP total-body irradiation models to identify novel tissue- and organ-specific biomarkers.
- Begin analysis of blood chemistry data collected in NHP dose-response study with limited supportive care and in high-dose study with full supportive care (G-CSF, antibiotics, blood transfusions, etc.) to evaluate radiation damage to specific organs.

Exhibit R-2A, RDT&E Project Justification: PB 2017 Defense Health Agency		Date: February 2016
Appropriation/Budget Activity 0130 / 2	**R-1 Program Element (Number/Name)** PE 0602787DHA / *Medical Technology (AFRRI)*	**Project (Number/Name)** 241A / *Biodosimetry (USUHS)*

- Begin 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.
- 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 necessary FDA approval.
- Prepare preliminary report for FDA on combined utility of hematological and protein biomarkers for biodosimetry applications in two FDA-required animal models.
- Begin to compare results/data from the NHP dose-response TBI (photon/low-LET) studies with data collected from radiation accident victims and radiation therapy patients.
- Report on dosimetry and radioresponse for use of multiple parameter radiation biomarkers in a murine partial-body exposure model.
- Identify proteomic markers from irradiated organoid cultures for validation by enzyme linked immunosorbent assay.
- Initiate studies to evaluate radiation-induced chromosomal damage in murine radiation model.
- Measure epigenetic markers in early, mid, and late carcinogenesis samples after low dose radiation.
- Identify differences in cell growth rate responses to low and high dose rate radiation in cell samples.
- Measure and compare epigenetic markers in low and high dose rate cell samples at single low dose.

By FY 2016
- Evaluate new early-phase and organ-specific damage radiation-responsive biomarkers in animal models.
- Compare/correlate hematology, blood serum chemistry, protein biomarkers and necropsy results in NHP dose-response study to evaluate radiation damage to specific organs.
- Compare results/data from NHP dose-response TBI (photon/low LET) studies with data collected from radiation accident victims and radiation therapy patients.
- Continue to refine combination of radiation biomarkers in blood with best balance of discrimination, sensitivity and specificity.
- Evaluate predictive radiation-responsive biomarkers in animal models for ARS outcome.
- Continue partial-body exposure study to characterize organ specific injury biomarkers using abdomen exposures of mice.
- Identify specific gene pathways that differ in early, mid, and late carcinogenesis samples after low dose radiation.
- Characterize dose rate effects on cell growth to identify gene pathway differences between low and high dose.
- Evaluate role of miR30 on regulation of radiation-induced apoptosis and apoptotic protector Mcl-1 activation in cells and in mitochondria.

By FY 2017
- Establish partial-body animal radiation models (mouse and NHP) using animals involving low-LET exposure with AFRRI small-animal irradiator (for mice) and LINAC (for NHPs) to identify organ-specific radiation injury biomarkers evaluated earlier in low-LET TBI studies.
- Establish mouse TBI model for combined hematological and proteomic biodosimetry approach following mixed-field (photon and neutron, high-LET) in addition to one already established and evaluated for a pure photon (60Co γ-rays, low-LET) exposure.
- Report on use of multiple parameter biodosimetry to characterize partial-body exposures using murine model.
- Measure leukemia development in vivo after chronic low dose radiation, and identify specific genes silenced in early, mid, and late leukemogenesis
- Identify network of miRNAs and their targeting mRNAs in radiation-induced apoptotic signal pathways.
- Evaluate mechanisms of radiation-induced lymphocyte damage.

Exhibit R-2A, RDT&E Project Justification: PB 2017 Defense Health Agency		Date: February 2016
Appropriation/Budget Activity 0130 / 2	**R-1 Program Element (Number/Name)** PE 0602787DHA / *Medical Technology (AFRRI)*	**Project (Number/Name)** 241A / *Biodosimetry (USUHS)*

- Develop biomarkers which can identify “treatment-point” after radiation injury.

Exhibit R-2A, RDT&E Project Justification: PB 2017 Defense Health Agency			Date: February 2016
Appropriation/Budget Activity 0130 / 2	R-1 Program Element (Number/Name) PE 0602787DHA / *Medical Technology (AFRRI)*	Project (Number/Name) 241B / *Internal Contamination (USUHS)*	

COST ($ in Millions)	Prior Years	FY 2015	FY 2016	FY 2017 Base	FY 2017 OCO	FY 2017 Total	FY 2018	FY 2019	FY 2020	FY 2021	Cost To Complete	Total Cost
241B: *Internal Contamination (USUHS)*	0.621	0.109	0.131	0.133	-	0.133	0.143	0.146	0.149	0.152	Continuing	Continuing

A. Mission Description and Budget Item Justification

Internal Contamination (USU): For the Uniformed Services University of the Health Sciences (USU), 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 2015	FY 2016	FY 2017
Title: Internal Contamination (USUHS)	0.109	0.131	0.133

Description: Internal Contamination (USU): For the Uniformed Services University of the Health Sciences (USU), 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.

FY 2015 Accomplishments:
- Initiated feasibility study to determine if non-radioactive metals can substitute as template molecules for high-specific activity radionuclides in synthesis of molecularly imprinted polymers.
- Identified specific epigenetic changes associated with depleted uranium damage in vivo.
- Measured genes associated with chromatin regulation in depleted uranium leukemia in vivo.

FY 2016 Plans:
- Evaluate kidney gene pathway changes induced by depleted uranium 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 2017 Plans:
- Design feasibility study to assess chelating potential of molecularly imprinted polymers linked to magnetic nanoparticles.

Exhibit R-2A, RDT&E Project Justification: PB 2017 Defense Health Agency		Date: February 2016
Appropriation/Budget Activity 0130 / 2	**R-1 Program Element (Number/Name)** PE 0602787DHA / *Medical Technology (AFRRI)*	**Project (Number/Name)** 241B / *Internal Contamination (USUHS)*

B. Accomplishments/Planned Programs ($ in Millions)	FY 2015	FY 2016	FY 2017
- Initiate study to determine if depleted uranium and low dose radiation induced changes in chromatin remodeling can be reversed by countermeasures.			
Accomplishments/Planned Programs Subtotals	0.109	0.131	0.133

C. Other Program Funding Summary ($ in Millions)

N/A

Remarks

D. Acquisition Strategy

N/A

E. Performance Metrics

By FY15
- Initiate feasibility study to determine if non-radioactive metals can substitute as template molecules for high-specific activity radionuclides in synthesis of molecularly imprinted polymers.
- Complete in vivo study on the mechanism of depleted uranium-induced leukemia.

By FY 16
- Conclude feasibility assessment studies on possibility of using non-radioactive templates for the synthesis of molecularly imprinted polymers designed to bind radioactive metals.
- Continue study to assess novel countermeasure to low dose radiation that targeted specific chromatin remodeling.

By FY 2017
- Initiate study to assess applicability of nanoparticle-linked molecularly imprinted polymers for radionuclide de-corporation.
- Measure specific chromatin changes that are associated with low dose radiation or depleted uranium exposure in vivo.

Exhibit R-2A, RDT&E Project Justification: PB 2017 Defense Health Agency			Date: February 2016
Appropriation/Budget Activity 0130 / 2	R-1 Program Element (Number/Name) PE 0602787DHA / *Medical Technology (AFRRI)*	Project (Number/Name) 241C / *Radiation Countermeasures (USUHS)*	

COST ($ in Millions)	Prior Years	FY 2015	FY 2016	FY 2017 Base	FY 2017 OCO	FY 2017 Total	FY 2018	FY 2019	FY 2020	FY 2021	Cost To Complete	Total Cost
241C: *Radiation Countermeasures (USUHS)*	4.041	0.704	0.842	0.855	-	0.855	0.916	0.933	0.951	0.970	Continuing	Continuing

A. Mission Description and Budget Item Justification

Radiation Countermeasures (USU): For the Uniformed Services University of the Health Sciences (USU), 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), termed combined injury (CI). 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 preventing and 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 2015	FY 2016	FY 2017
Title: Radiation Countermeasures (USUHS)	0.704	0.842	0.855

Description: Radiation Countermeasures (USU): For the Uniformed Services University of the Health Sciences (USU), 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), termed combined injury (CI). 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 preventing and 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.

FY 2015 Accomplishments:
- Completed strain comparison studies to establish the efficacy of 3 doses of filgrastim in mice exposed to LD70/30 dose of Co-60 radiation.
- Completed strain comparison studies to establish the efficacy of 3 doses of filgrastim in accelerated recovery from radiation-induced pancytopenia in mice exposed to sub-lethal dose of Co-60 radiation.
- Identified micro-RNAs involved in the GT3 mediated recovery from radiation-induced damage in spleen.
- Studied efficacy of TPOm administered subcutaneously 24 h before exposure to radiation.
- Performed comparative study of GT3, DT3 and DG70 administered subcutaneously. Formulations supplied by American River Nutrition.

Exhibit R-2A, RDT&E Project Justification: PB 2017 Defense Health Agency		Date: February 2016
Appropriation/Budget Activity 0130 / 2	**R-1 Program Element (Number/Name)** PE 0602787DHA / *Medical Technology (AFRRI)*	**Project (Number/Name)** 241C / *Radiation Countermeasures (USUHS)*

B. Accomplishments/Planned Programs ($ in Millions)	FY 2015	FY 2016	FY 2017
- Determine efficacy of CDX-301 in gastrointestinal recovery after radiation exposure. - Demonstrated efficacy of TPOm in hematopoietic recovery after radiation exposure. - Completed micro-RNA profiles in serum and kidney of mice exposed to radiation followed by hemorrhage. - Determined ionizing radiation weakly activates production of inflammatory cytokines and chemokines in macrophage cell lines and ex vivo bone marrow derived macrophages. - Combined exposure to ionizing radiation and virus infection increased inflammatory response in macrophages above single pathophysiological exposures. - Discovered that, unexpectedly, inflammatory response in macrophages controlled primarily by Mitogen Activated Kinases (MAPK), not the expected Nuclear Factor kappa B (NF-kB) gene transcription factors. - Determined phenylbutyrate induced suppression of x-ray induced neoplastic transformation of bronchial tissue. - Measured DNA methylation changes in neoplastic bronchial cells that demonstrated radiation quality effect. - Discovered use of MAPK inhibitors can decrease radiation induced cytokine production by macrophages when added pre- and post- exposure, and for selected exposure/treatment combinations up to 72 hours. - Established that cells from multiple cell linages with stable gene constructs that report gene promoter activity can be used to evaluate effects of ionizing radiation, infectious disease agent agonists and effects of response modulators. - Developed two different types of nanoparticles sensitive to oxidative stress and activation by UV light, respectively. - Discovered nanoparticle encapsulated anti-oxidants that modulate macrophage response to infectious disease agent agonists are equally or more effective than when modulator is in free solution. - Screened 10 radiation countermeasure candidates administered before irradiation to mice, to parallel externally funded program (NIAID) that screened countermeasures given after irradiation. (Civilian agencies do not fund pre-irradiation countermeasures.) - Established coculture model comprising human bone marrow endothelial cells (EC) (hematopoietic microenvironment cells) and human hematopoietic stem and progenitor cells (HSPC). - Showed EC and radiation affect expression of differentiation markers on HSPC: HSPC remain undifferentiated after radiation, and EC accentuate this process. - Discovered subpopulations of HSPC affected differently by EC and radiation. - Discovered radiation-responsive genes in EC, some of which are also modulated by the presence of HSPC. These genes include DNA repair genes and Angiopoietin-1 (Ang-1). - Discovered Ang-1 modulated interactions between EC and HSPC. - Initiated informatics analysis of gene array data from irradiated, co-cultured EC and HSPC. - Reported effects and mechanisms of delta-tocotrienol on radioprotection are through suppression of radiation-induced microRNA-30, protecting mice and human CD34+ cells from radiation injury. - Studied radioprotective efficacy of Ex-RAD in two different strains of mice (CD2F1 and C57BL/6) to demonstrate that countermeasure effective across various mice strains.			

Exhibit R-2A, RDT&E Project Justification: PB 2017 Defense Health Agency		Date: February 2016
Appropriation/Budget Activity 0130 / 2	**R-1 Program Element (Number/Name)** PE 0602787DHA / *Medical Technology (AFRRI)*	**Project (Number/Name)** 241C / *Radiation Countermeasures (USUHS)*

B. Accomplishments/Planned Programs ($ in Millions)	FY 2015	FY 2016	FY 2017
- Evaluated efficacy biomarkers of Ex-RAD using in-vitro models (different cell lines) and several target proteins of various pathways. - Initiated study of efficacy biomarkers for Ex-RAD in mouse model. ***FY 2016 Plans:*** - Perform dose and time optimization and DRF of TPOm. - Determine efficacy of DG70 and nano-GT3 administered orally. - Determine role of micro-RNA in regulating recovery of hematopoietic system by CDX-301 after radiation injury. - Determine effects of citrulline in vitro on human hematopoietic progenitors and other cell lines. - Evaluate mTOR-AKT signaling and MAPK signaling in bone marrow cells after exposure to gamma-radiation combined with hemorrhage. - Assess modulation and correlation of cytokine profiles in serum and ileum after ghrelin therapy in order to find key cytokines associated with ileal recovery after radiation CI. - Determine which specific MAPK pathway intermediates activated in macrophages by ionizing radiation and virus exposure singly and in combination. - Determine effects of ionizing radiation on production of Type I interferon by macrophages. - Determine how reporter cells containing more than one transcription factor can be utilized to gain simultaneous information from dual reporter system. - Develop new interferon detection assay utilizing reporter gene cell lines. - Complete development of oxidation-sensitive drug delivery system tuned to degrade at a rate corresponding to level of oxidants present within microenvironment of the cell. - Complete development multi-photon-responsive nanocarrier designed to respond to UV light, near infrared (NIR) light and ionizing radiation (IR). - Improve low dose risk assessment knowledge base by determining whether chronic or repeated low dose exposure in murine model induces leukemia in comparison to high dose radiation exposure. - Screen 10 radiation countermeasure candidates administered before irradiation to mice, to parallel externally funded program (NIAID) that screens countermeasures given after irradiation. (Civilian agencies do not fund pre-irradiation countermeasures.) - Screen 3 radiation countermeasure candidates originating from AFRRI in mouse survival assay. - Optimize dose and administration timing of promising new radiation countermeasures in mice. - Complete informatics analysis of irradiated, co-cultured EC and HSPC. - Determine effects of ionizing radiation on mitochondrial remodeling and mitophagy. - Compare radioprotective and mitigative effects of γ-tocotrienol (GT3), δ-tocotrienol (DT3) and DeltaGold® (DG) on mouse hematopoietic system and human hematopoietic progenitor cells. - Continue study of efficacy biomarkers for Ex-RAD in mouse model.			

Exhibit R-2A, RDT&E Project Justification: PB 2017 Defense Health Agency		Date: February 2016
Appropriation/Budget Activity 0130 / 2	**R-1 Program Element (Number/Name)** PE 0602787DHA / *Medical Technology (AFRRI)*	**Project (Number/Name)** 241C / *Radiation Countermeasures (USUHS)*

B. Accomplishments/Planned Programs ($ in Millions)	FY 2015	FY 2016	FY 2017
- Initiate study of efficacy biomarkers for Ex-RAD in nonhuman primate (NHP) model using biosample sharing arrangement with an extramural project. ***FY 2017 Plans:*** - Determine protective and mitigative effects of citrulline in vitro on human hematopoietic progenitors and other cell lines exposed to radiation. - Determine whether oral citrulline before and after radiation enhances survival in mice. - Determine effects of citrulline on GI injury in irradiated mice. - Evaluate mTOR-AKT signaling and MAPK signaling in ex vitro bone marrow mesenchymal cells and in vitro small intestine cells after exposure to gamma-radiation combined with hypoxia. - Evaluate whether ghrelin therapy reduces tissue injury and improves tissue recovery. - Determine whether modulation of radiation-virus induced inflammatory response is best inhibited by use of broad MAPK inhibitors or ones selective for specific targeted pathway intermediates. - Determine MAPK and IRF pathway in human ex vivo macrophages and response during combined exposure to ionizing radiation and FLUA. - Validate pathways resulting in activation of the reporter genes in stably transfected cell lines. - Determine effects of anti-oxidants and other response modifiers of radiation injury, infectious disease inflammatory stimulation and combined injury which result in activation of stable transcription factor reporters. - Measure incidence of leukemia development in vivo after chronic low dose rate radiation or repeated exposure to high dose rate radiation. - Screen 10 radiation countermeasure candidates administered before irradiation to mice, to parallel externally funded program (NIAID) that screens countermeasures given after irradiation. (Civilian agencies do not fund pre-irradiation countermeasures.) - Screen 3 radiation countermeasure candidates originating from AFRRI in mouse survival assay. - Optimize dose and administration timing of promising new radiation countermeasures in mice. - Initiate studies on role of radiation-induced mitochondrial DNA (mtDNA) damage and mitochondrial dysfunction in acute radiation syndrome. - Compare radioprotective effects of DG/DT3/GT3 on mouse gastrointestinal (GI) tract. - Determine and compare mechanisms by which DG/DT3/GT3 mediate survival signaling after radiation. - Evaluate mechanisms of radiation-induced mitochondria DNA damage and apoptosis pathways. - Evaluate effects of DG/DT3/GT3 on protection and/or mitigation of radiation-induced mitochondrial DNA damage. - Study radioprotective efficacy of two drug combination acting through two different mechanisms of action: gamma-tocotrienol (GT3) and amifostine. - Continue study of efficacy biomarkers for Ex-RAD in NHP model.			
Accomplishments/Planned Programs Subtotals	0.704	0.842	0.855

Exhibit R-2A, RDT&E Project Justification: PB 2017 Defense Health Agency		Date: February 2016
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C. Other Program Funding Summary ($ in Millions)

N/A

Remarks

D. Acquisition Strategy

N/A

E. Performance Metrics

By FY 2015

- Complete toxicity and survival efficacy of DG70, DT3 and GT3 administered subcutaneously.
- Complete preliminary survival efficacy of TPOm.
- Complete study to demonstrate that CDX-301 protects gastrointestinal system in mice exposed to lethal dose of radiation.
- Screen 10 radiation countermeasure candidates administered before irradiation to mice, to parallel externally funded program (NIAID) that screens countermeasures given after irradiation. (Civilian agencies do not fund pre-irradiation countermeasures.)
- Optimize dose and administration timing of promising new radiation countermeasures in mice.
- Establish coculture model comprising human bone marrow endothelial cells (EC) (hematopoietic microenvironment cells) and human hematopoietic stem and progenitor cells (HSPC).
- Show EC and radiation affect expression of differentiation markers on HSPC.
- Assess whether there are subpopulations of HSPC affected differently by EC and radiation.
- Assess radiation-responsive genes in EC; determine whether some are modulated by presence of HSPC.
- Analyze role of Ang-1 in interactions between EC and HSPC.
- Initiate informatics analysis of gene array data from irradiated, co-cultured EC and HSPC.
- Identify additional biomarkers which can be used as efficacy biomarkers for radiation countermeasures.

By FY 2016

- Complete time and dose optimization and DRF of TPOm.
- Complete study to demonstrate the efficacy of TPOm in hematopoietic recovery.
- Complete toxicity and survival efficacy of DG70 and nano-GT3 administered orally.
- Analyze signaling pathways in mouse organs after exposure to radiation using qRT-PCR, Western blots and informatics.
- Complete evaluation of mTOR-AKT signaling and MAPK signaling in bone marrow cells after exposure to gamma-radiation combined with hemorrhage.
- Complete assessment of modulation and correlation of cytokine profiles in serum and ileum after ghrelin therapy in order to find the key cytokine(s) that is/are associated with ileal recovery after CI.
- Complete identification and kinetics of MAPK signaling pathway molecules which are activated by ionizing radiation-virus combined injury.
- Complete evaluation of gene activation reporter cells as new and novel Type I interferon assay.
- Complete identification of MAPK intermediates activated by ionizing radiation and combined injury.

Exhibit R-2A, RDT&E Project Justification: PB 2017 Defense Health Agency		Date: February 2016
Appropriation/Budget Activity 0130 / 2	**R-1 Program Element (Number/Name)** PE 0602787DHA / *Medical Technology (AFRRI)*	**Project (Number/Name)** 241C / *Radiation Countermeasures (USUHS)*

- Complete assessment of nanoparticle constructs ability to modulate macrophage inflammatory responses to ionizing radiation and combined radiation-microbial agonist exposures.
- Screen 10 radiation countermeasure candidates administered before irradiation to mice, to parallel externally funded program (NIAID) that screens countermeasures given after irradiation. (Civilian agencies do not fund pre-irradiation countermeasures.)
- Screen 3 radiation countermeasure candidates originating from AFRRI in mouse survival assay.
- Optimize dose and administration timing of promising new radiation countermeasures in mice.
- Complete informatics analysis of irradiated, co-cultured EC and HSPC.
- Determine effects of ionizing radiation on mitochondrial remodeling and mitophagy.
- Continuation of biomarker identification for radiation countermeasure efficacy.
- Identify additional biomarkers for radiation injury.

By FY 2017
- Analyze ERK/MAPK signaling and mRNA responses in endothelial cells to radiation.
- Analyze miRNA and mRNA responses in mice to radiation and radiation countermeasure CDX-301.
- Correlate mTOR-AKT and MAPK signaling network and ATP production after radiation-hemorrhage CI.
- Evaluate mTOR-AKT signaling and MAPK signaling in ex vitro bone marrow mesenchymal cells and in vitro small intestine cells after exposure to gamma-radiation combined with hypoxia.
- Determine whether ghrelin therapy reduces tissue injury and improves tissue recovery.
- Complete assessment of timing and duration of response to MAPK pathway inhibitors to alter inflammatory macrophages exposed to radiation.
- Complete assessment of ex vivo human macrophage response to ionizing radiation, viral infection and combined injury.
- Complete assessment of transcription factor reporter cells to test biological response modulators of gene activation induced by ionizing radiation, microbial agonists and combined exposures.
- Measure incidence of leukemia development in vivo after chronic low dose rate radiation or repeated exposure to high dose rate radiation.
- Screen 10 radiation countermeasure candidates administered before irradiation to mice, to parallel externally funded program (NIAID) that screens countermeasures given after irradiation. (Civilian agencies do not fund pre-irradiation countermeasures.)
- Screen 3 radiation countermeasure candidates originating from AFRRI in mouse survival assay.
- Optimize dose and administration timing of promising new radiation countermeasures in mice.
- Initiate studies on role of radiation-induced mitochondrial DNA (mtDNA) damage and mitochondrial dysfunction in acute radiation syndrome.
- Transcriptomic analysis after irradiation and treatment with various radiation countermeasures.