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Exhibit R-2, RDT&E Budget Item Justification: PB 2023 Defense Health Agency **Date:** April 2022

Appropriation/Budget Activity 0130: <i>Defense Health Program I BA 2: RDT&E</i>	R-1 Program Element (Number/Name) PE 0602787DHA I <i>Medical Technology (AFRRI)</i>
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COST (\$ in Millions)	Prior Years	FY 2021	FY 2022	FY 2023 Base	FY 2023 OCO	FY 2023 Total	FY 2024	FY 2025	FY 2026	FY 2027	Cost To Complete	Total Cost
Total Program Element	4.070	1.411	1.439	1.468	0.000	1.468	1.497	1.527	1.557	1.587	Continuing	Continuing
241A: <i>Biodosimetry (USUHS)</i>	0.832	0.289	0.295	0.301	0.000	0.301	0.307	0.313	0.319	0.324	Continuing	Continuing
241B: <i>Internal Contamination (USUHS)</i>	0.438	0.152	0.155	0.158	0.000	0.158	0.161	0.164	0.167	0.170	Continuing	Continuing
241C: <i>Radiation Countermeasures (USUHS)</i>	2.800	0.970	0.989	1.009	0.000	1.009	1.029	1.050	1.071	1.093	Continuing	Continuing

A. Mission Description and Budget Item Justification

For the Uniformed Services University of the Health Sciences/Armed Forces Radiobiology Research Institute (USUHS/AFRRI), is a unique Department of Defense asset, responsible for preserving and protecting the health and performance of U.S. military personnel operating in potential radiologically contaminated multi-domain conventional or hybrid battle spaces and urban environments; through research, education, and operational training that advance understanding of the effects of ionizing radiation in line with the 21st century dynamic threat landscape and national security threats posed by non-state actors, hostile state actors, and near-peer adversaries, as well as providing rapidly deployable radiation medicine expertise in response to a radiological or nuclear event domestically or abroad.

The uniqueness of USUHS/AFRRI comes from operating and maintaining state-of-the-art radiation facilities and dosimetry systems to support military relevant radiobiology research. These facilities enable researchers to conduct a wide range of radiobiology experiments in order to investigate militarily-relevant scenarios, and better understand radiation effects and potential mitigation strategies. A team of scientist, physicists, engineers, operators and technicians use proven and traceable dosimetry systems (e.g., ionization chambers, radiochromic film, thermoluminescent dosimeters) and consensus protocols to characterize radiation fields. Due to these facilities our researchers are able to experiment with photons (γ-rays) which are intended to simulate fallout environments and are delivered by two cobalt-60 facilities - the high-level cobalt facility (HLCF), and for lower (chronic) doses and dose rates, the low-level cobalt facility (LLCF). These type of radiation sources are used for acute and chronic studies of materials, biologic specimens, and small and large animals. The LLCF also provides to our scientist low-dose rate gamma rays to simulate chronic exposure to low absorbed doses. Therefore, it also supports research focused on late or delayed radiation effects in biological specimens.

USUHS/AFRRI researchers are also able to use Mixed-radiation fields (photons and neutrons) which are available from USUHS/AFRRI's Training, Research, Isotopes, General Atomics (TRIGA) reactor. The reactor is operated in either steady-state or pulsed mode to simulate a wide range of prompt exposure scenarios on a nuclear battlefield. The USUHS/AFRRI's TRIGA is the only one dedicated to military radiobiology research. The reactor produces a controlled, self-sustaining fission chain reaction in the reactor core which, in addition to the fuel elements and control rods (containing boron carbide), which includes a neutron start-up source (americium/beryllium). It is suspended under 4.9 m of water within a pool (an effective radiation shield) in a carriage assembly that allows movement of the core between two exposure rooms for experimental work with large-animal or other studies. The advantages of such a movable reactor core are that the quantity and character of the radiation that reaches the exposure facilities can be controlled, and more than one exposure facility can be used during reactor operations.

Our state-of-the-art radiation facilities are also able to provide a wide range of photon and electron irradiations for partial- and whole-body geometries by using a linear accelerator (LINAC) and a small animal radiation research platform (SARRP) providing a range of radiation types, energies, field sizes and dose rates and is extensively

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used to support standard cell configurations (i.e., 6-, 24- and 96-well plates), and targeted partial body irradiations of mice, minipigs, and nonhuman-primates (NHP) animal models. AFRRI's LINAC is used to produce, monitor, control and form photon or electron beams to the specified target. Whole-body irradiations are also possible depending on the animal size and desired dose rate. An Xstrahl SARRP facility is capable of operating at 220 kVp and 13 mA yielding a dose rate at the isocenter of approximately 2.6 Gy/min. Onboard portal camera and cone beam CT imaging systems are used to ensure precise dose delivery. Lung- and gut-only irradiation protocols are approved and have been extensively used to support radiation countermeasure development in the mouse model. Other imaging support is provided by a Philips Brilliance computed tomography (CT) big bore scanner. Some features of the scanner include an 85-cm bore size to accommodate larger research subjects, 60-cm true scan field of view and 16-slices per revolution. The above radiation sources and generators are used to support USUHS/AFRRI's current research focus areas which we will address in the following section.

Our scientific research goals includes maintaining a pool of highly qualified radiation biologists, and basic and applied research in identification and early development of measures to prevent, assess, and treat radiation injury. USUHS/AFRRI scientists conduct and publish research critical to the Department of Defense for force health protection and also contribute to the health and well-being of the population at large. USUHS/AFRRI research thrusts include development of diagnosis of radiation induced injury (biodosimetry), internalized radionuclides (internal contamination) and radiation countermeasures.

Research findings are mainly focused to advance the development and to produce the following: (1) To establish processes to permit rapid assessment of radiation exposed specimens using novel triage protocols; (2) To developed novel technologies to minimized the use of animal models in the study of radiation effects; (3) To investigate the overall radiation effect by internal contamination in the microbiome and anatomical tissue; (4) To find novel biomarkers, late effects and immunosuppression of radiation injury that can quantitate effects on combat performance decrements; (4) To identify novel therapeutic strategies that will support military operations within a nuclear or radiological environment minimizing ground troops short and long term adverse risk.

B. Program Change Summary (\$ in Millions)	<u>FY 2021</u>	<u>FY 2022</u>	<u>FY 2023 Base</u>	<u>FY 2023 OCO</u>	<u>FY 2023 Total</u>
Previous President's Budget	1.411	1.439	1.468	0.000	1.468
Current President's Budget	1.411	1.439	1.468	0.000	1.468
Total Adjustments	0.000	0.000	0.000	0.000	0.000
• Congressional General Reductions	-	-			
• Congressional Directed Reductions	-	-			
• Congressional Rescissions	-	-			
• Congressional Adds	-	-			
• Congressional Directed Transfers	-	-			
• Reprogrammings	-	-			
• SBIR/STTR Transfer	0.000	-			

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Appropriation/Budget Activity 0130 / 2					R-1 Program Element (Number/Name) PE 0602787DHA / Medical Technology (AF RRI)				Project (Number/Name) 241A / Biodosimetry (USUHS)			
COST (\$ in Millions)	Prior Years	FY 2021	FY 2022	FY 2023 Base	FY 2023 OCO	FY 2023 Total	FY 2024	FY 2025	FY 2026	FY 2027	Cost To Complete	Total Cost
241A: <i>Biodosimetry (USUHS)</i>	0.832	0.289	0.295	0.301	0.000	0.301	0.307	0.313	0.319	0.324	Continuing	Continuing

A. Mission Description and Budget Item Justification

For the Uniformed Services University of the Health Sciences/Armed Forces Radiobiology Research Institute (USUHS/AFRRI), the Biodosimetry program address clinical symptoms of radiation exposure, reach back reference capabilities and is strategically poised to host the DoD's advance Radiationbiology clinical (CLIP) certified laboratory, meeting the objective of Senate Report SR 114-63. The Biodosimetry laboratory also received clinical specimens from the Fukushima radiation accident in 2011, showcasing USUHS/AFRRI's capabilities to support the Department of Defense in case of a radiation incident.

Research findings are focused to advance the development and to produce the following: (1) To establish clinically certified processes to permit rapid assessment of radiation exposed specimens; (2) To access radiation exposure by developing and providing biological and biophysical dosimetry capabilities for acute, protracted, and prior radiation exposure; (3) To develop novel triage protocols for rapid assessment of radiation exposure; (4) To establish equipment triage automation to support the ability to manage mass-casualty radiation incidents around the globe.

B. Accomplishments/Planned Programs (\$ in Millions)

	FY 2021	FY 2022	FY 2023 Base	FY 2023 OCO	FY 2023 Total
Title: Biodosimetry (USUHS)	0.289	0.295	0.301	0.000	0.301
Description: Biodosimetry (USUHS/AFRRI): Research findings are focused to advance the development and to produce the following: (1) To establish clinically certified processes to permit rapid assessment of radiation exposed specimens; (2) To access radiation exposure by developing and providing biological and biophysical dosimetry capabilities for acute, protracted, and prior radiation exposure; (3) To develop novel triage protocols for rapid assessment of radiation exposure; (4) To establish equipment triage automation to support the ability to manage mass-casualty radiation incidents around the globe.					
In addition to the primary achievement of research objectives, the program educates Federal employees as a benefit to the public they serve through Federal service, through support to civil authorities, and in non-Federal professional and academic collaborations.					
Description: Biodosimetry (USUHS/AFRRI): Research findings are focused to advance the development and to produce the following: (1) To establish clinically certified processes to permit rapid assessment of radiation exposed specimens; (2) To access radiation exposure by developing and providing biological and biophysical dosimetry capabilities for acute, protracted, and prior radiation exposure; (3) To develop novel triage protocols					

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B. Accomplishments/Planned Programs (\$ in Millions)

	FY 2021	FY 2022	FY 2023 Base	FY 2023 OCO	FY 2023 Total
<p>for rapid assessment of radiation exposure; (4) To establish equipment triage automation to support the ability to manage mass-casualty radiation incidents around the globe.</p> <p>In addition to the primary achievement of research objectives, the program educates Federal employees as a benefit to the public they serve through Federal service, through support to civil authorities, and in non-Federal professional and academic collaborations.</p> <p>FY21 Accomplishments:</p> <p>(1) Validated the HIRI algorithm concept using animals (i.e., baboons, canine, and mice) dose-response databases for an extended time window up to 10 to 14 days after exposure.</p> <p>(2) Established a RICA algorithm for assessment of H-ARS severity using NHP radiation dose-response model based on CBC cell types biomarkers using the METREPOL system.</p> <p>(3) Developed a quantitative inhibition PCR assay of nuclear and mitochondrial DNA using long- and short amplicon PCR.</p> <p>(4) Evaluated radiation-dose and time-course response following exposure initially to photon irradiation.</p> <p>(5) Compared gamma ray v/s neutron mixed field exposures on the inhibition PCR assay.</p> <p>(6) Characterized the utility of hematology biodosimetry algorithms (i.e., HIRI, RICA) to assess radiation injury after radiation exposures.</p> <p>(7) Established a quantitative inhibition PCR of DNA damage using blood lymphocyte models and characterize its utility for assessment of radiation exposure.</p> <p>(8) Performed simulated in vitro partial-body exposure studies and use cytogenetic biomarkers (PCC assay).</p> <p>(9) Evaluated the PCC endpoints (i.e., excess PCC fragments, lengths ratios, rings, and dicentric) for optimum assessment of the fraction of body exposed and dose to the irradiated fraction using in vivo human blood lymphocytes model.</p> <p>(10) Investigated gamma rays v/s mixed field exposures on PCC assay to distinguish whole-body v/s partial body high dose radiation exposures.</p> <p>(11) In 2019/2021, 15 manuscripts were published.</p> <p>FY 2022 Plans:</p> <p>FY 2022 plans are to continue efforts as outlined in FY 2021 and to perform the following studies:</p> <p>(1) To establish processes to permit processing assessments of radiation exposure from specimens by testing the novel cytokinesis-block micronucleus cytome assay (CBMN). The CBMN is a comprehensive system for</p>					

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B. Accomplishments/Planned Programs (\$ in Millions)

	FY 2021	FY 2022	FY 2023 Base	FY 2023 OCO	FY 2023 Total
<p>measuring DNA damage, cytostasis and cytotoxicity. DNA damage events are scored specifically in once-divided binucleated (BN) cells and include (a) micronuclei (MNI), a biomarker of chromosome breakage and/or whole chromosome loss, (b) nucleoplasmic bridges (NPBs), a biomarker of DNA misrepair and/or telomere end-fusions, and (c) nuclear buds (NBUDs), a biomarker of amplified DNA and/or DNA repair complexes. Cytostatic effects are measured via the proportion of mono-, bi- and multinucleated cells and cytotoxicity via necrotic and/or apoptotic cell ratios. Further information regarding mechanisms leading to MNI, NPBs and NBUDs formation is obtained using centromere and/or telomere probes. The assay have the probability to be applied successfully for biomonitoring of in vivo genotoxic radiation exposure, in vitro radiation genotoxicity testing and in diverse research fields such as nutrigenomics and pharmacogenomics as well as a predictor of normal tissue and tumor radiation sensitivity and cancer risk.</p> <p>(2) To test the CBMN assay for triage automation and multivariable linear regression analysis to compare with already proven and globally accepted assays.</p> <p>(3) To establish a surge request procedure for cytogenetic analysis by developing sex and age-dependent CBMN dose-response calibrations curves and validate specimens cryopreservation protocols for delayed analysis using the metaphase-spread chromosome aberrations (i.e., DCA, PCC) assays.</p> <p>(4) To evaluate blood biomarkers to monitor radiation injury of radiation countermeasures.</p> <p>(5) To established the Department of Defense CLIP/CLIA Clinical Biodosimetry laboratory with automated clinical specimen testing to manage mass-casualty radiation incidents around the globe.</p> <p>(6) To publish manuscripts on research findings.</p> <p>FY 2023 Base Plans:</p> <p>(1) To setup sex and age dependent donors in order to establish radiation dose response CBMN assay using optimized processing and staining procedures.</p> <p>(2) To establish dual staining using two different fluoresce probes and to implement those in the automated assay.</p> <p>(3) To produce a suite of protocols for clinical approval and testing validations.</p> <p>(4) To implement quality control and quality assurance processes in order to preserve and ensure specimen testing and integrity.</p> <p>(5) To sustain laboratory clinical accreditation and competency in the cytogenetic biodosimetry service capability.</p> <p>(6) To participate in CBRNE/WMD NATO and military operations exercises.</p> <p>(7) To continue providing Department of Defense radiobiology expert reach back support.</p> <p>FY 2023 OCO Plans:</p>					

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B. Accomplishments/Planned Programs (\$ in Millions)	FY 2021	FY 2022	FY 2023 Base	FY 2023 OCO	FY 2023 Total
N/A					
<i>FY 2022 to FY 2023 Increase/Decrease Statement:</i> Pricing adjustment for inflation.					
Accomplishments/Planned Programs Subtotals	0.289	0.295	0.301	0.000	0.301

C. Other Program Funding Summary (\$ in Millions)
N/A

Remarks
The program element 0602787DHA for AFRRRI in addition to the three program elements: 0601115HPPE, 0602115HPPE, and 0603115HP are coordinated and integrated into the portfolio management by the Joint Program Committee-7/ Radiation Health Effects Research Program (RHERP).

D. Acquisition Strategy
N/A

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COST (\$ in Millions)	Prior Years	FY 2021	FY 2022	FY 2023 Base	FY 2023 OCO	FY 2023 Total	FY 2024	FY 2025	FY 2026	FY 2027	Cost To Complete	Total Cost
241B: <i>Internal Contamination (USUHS)</i>	0.438	0.152	0.155	0.158	0.000	0.158	0.161	0.164	0.167	0.170	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 2021	FY 2022	FY 2023 Base	FY 2023 OCO	FY 2023 Total
Title: Internal Contamination (USUHS)	0.152	0.155	0.158	0.000	0.158
Description: Internal Contamination (USU): Radioactive material can enter the body by a variety of pathways including ingestion, inhalation, and wound contamination. While some internalized isotopes will be naturally eliminated from the body, many others are not. They remain immobile or are transported and deposited to other organs where they continually irradiate the surrounding tissue. This chronic internal radiation exposure can cause unrepairable cellular damage eventually leading to death. This Program uses innovative approaches to address this pressing health concern.					
FY21 Accomplishments:					
(1) Determined a chemical synthesis route containing a magnetic core.					
(2) Tested the ability of non-magnetic dendrimers to bind uranium and cesium.					
(3) Completed the synthesis of uranium and cesium-templated dendrimers for high-specific metal binding imprinted polymers.					
(4) Completed the preparation of dendrimers with standard metal chelators attached to their terminal ends.					
(5) Assessed the ability of dendrimer containing metal chelators using a novel in vitro system.					
(6) Initiated cytotoxicity assessments of the novel chemically synthesized imprinted polymers.					
(7) Determined the efficacy of molecular imprinted polymers on reducing the body burden of internalized radionuclides using the novel in vitro system.					
(8) Received IACUC approval.					
(9) Animal specimens were submitted for histopathological evaluation and are being evaluated by a board certified pathologist.					

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B. Accomplishments/Planned Programs (\$ in Millions)	FY 2021	FY 2022	FY 2023 Base	FY 2023 OCO	FY 2023 Total
<p>(10) Sternal sections were evaluated for Megakaryocytes, indicative of proliferation.</p> <p>(11) Bone marrow was assayed for colony forming units, indicative of proliferation effects and complete blood cell counts were analyzed.</p> <p>(12) Fecal pellets were collected from male and female C57BL/6 mice one and six month's post-TBI were submitted to WRAIR for 16S microbiome sequencing.</p> <p>(13) Serum samples were collected and submitted to Georgetown University for metabolic and lipidomics analysis.</p> <p>(14) Fabricated of gut organ-on-chip model and quality control evaluation.</p> <p>(15) Identified and evaluated small molecules for gut organ-on-chip model.</p> <p>(16) In 2019/2021, five manuscripts were published.</p> <p>FY 2022 Plans:</p> <p>(1) FY2022 plans continue efforts as outlined in FY 2021 in addition to the following: The Department of Defense and Department of Veterans Affairs recognized the need for a better understanding of the health effects of embedded metal fragments and enhanced health surveillance of personnel suffering from such injuries. In response, the Department of Defense Health Affairs issued a directive instructing surgeons to save any excised fragments for further analysis so that the metals could be identified. In addition, the directive compiled a list of "metals of concern" to enhance patient follow-up with the establishment of the Toxic Embedded Fragment Center at the Baltimore VA Medical Center in order to follow-up with service members. These developments led to further collaborations between USUHS/AFRRI and the Baltimore DVA, University of Maryland School of Medicine, U.S. FDA, and the University of Kentucky resulting in receiving support by a Congressionally Directed Medical Research Program (CDMRP) funded project.</p> <p>(2) To validate signaling pathways by western blot and compare protein expression with age matched control minipigs tissues.</p> <p>(3) Perform ELISA for protein markers for gut leakage/intestinal permeability to support disruption of gut microflora to confirm the data from microbiome analysis.</p> <p>(4) Validation of small molecules for gut organ-on-chip model in murine model.</p> <p>FY 2023 Base Plans:</p> <p>FY2023 plans continue efforts as outlined in FY 2022.</p> <p>FY 2023 OCO Plans:</p>					

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B. Accomplishments/Planned Programs (\$ in Millions)	FY 2021	FY 2022	FY 2023 Base	FY 2023 OCO	FY 2023 Total
N/A					
<i>FY 2022 to FY 2023 Increase/Decrease Statement:</i> Pricing adjustment for inflation.					
Accomplishments/Planned Programs Subtotals	0.152	0.155	0.158	0.000	0.158

C. Other Program Funding Summary (\$ in Millions)
N/A

Remarks
The program element 0602787DHA for AF RRI in addition to the three program elements: 0601115HPPE, 0602115HPPE, and 0603115HP are coordinated and integrated into the portfolio management by the Joint Program Committee-7/ Radiation Health Effects Research Program (RHERP).

D. Acquisition Strategy
N/A

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COST (\$ in Millions)	Prior Years	FY 2021	FY 2022	FY 2023 Base	FY 2023 OCO	FY 2023 Total	FY 2024	FY 2025	FY 2026	FY 2027	Cost To Complete	Total Cost
241C: <i>Radiation Countermeasures (USUHS)</i>	2.800	0.970	0.989	1.009	0.000	1.009	1.029	1.050	1.071	1.093	Continuing	Continuing

A. Mission Description and Budget Item Justification

Radiation Countermeasures (USUHS/AFRRRI): For the Uniformed Services University of the Health Sciences/Armed Forces Radiobiology Research Institute (USUHS/AFRRRI), 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, microbiome, gastrointestinal damage, neurobehavioral deficits, bone marrow damage), termed radiation combined injury (RCI). RCI's were observed at Hiroshima and Nagasaki, Japan, where 60-70% of victims received thermal burns concurrent with radiation injury. At the Chernobyl reactor meltdown, 10% of 237 victims exposed to radiation received thermal burns as well. In animal models of RCI including rat, guinea pig, dog, and swine, burns and wounds usually increase mortality after otherwise non-lethal radiation exposures. Consequences of RCI include acute myelosuppression, immune system inhibition, fluid imbalance, macro/microcirculation failure, massive cellular damage, and disruption of vital organ functions, which can lead to multiple organ dysfunction syndrome. There are different syndromes based on the time of manifestation in relation to radiation exposure; acute, delayed, late, and chronic syndromes. Acute radiation syndrome (ARS) is characterized by the differential response of the important organs to different doses of radiation. The ARS sub-syndromes include three major clinically-relevant pathologies; hematopoietic sub-syndrome (H-ARS), gastrointestinal sub-syndrome (GI-ARS), and neurovascular sub-syndrome (NV-ARS or CNS-ARS). Radiation countermeasures have been categorized as radioprotectors, radiomitigators, and therapeutics, based on the time of administration in relation to radiation exposure. The majority of countermeasures developed are for specific tissue injuries or specific syndromes. ARS is receiving the most attention, though other syndromes also need equal consideration.

Currently, treatments for ARS are limited: only the H-ARS has viable therapeutic options and even those are limited; Neupogen, Neulasta, Leukine, and Nplate. USUHS/AFRRRI researchers made significant contributions in the initial development of the first three agents. These H-ARS treatments are genetically engineered recombinant growth factors or cytokines that were developed for other indications and recently repurposed for H-ARS. All U.S. Food and Drug Administration (FDA) -approved agents for H-ARS are radiomitigators. No radioprotector, either for H-ARS or GI-ARS has yet been approved for human use.

Due to the increasing risk of nuclear and radiological terrorist attacks or accidents has renewed interest in developing radiation medical countermeasures. Our Radiation Countermeasures goals 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/or combination of FDA approved treatments 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.

Research findings are focused to advance the understanding and to produce the following: (1) To identify new therapeutics candidates that show promising advancement to further development; (2) To developed novel technologies to minimized the use of animal models in the study of radiation countermeasure

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effects; (3) To investigate the overall radiation effect by countermeasures in the microbiome and anatomical tissue; (4) To find novel biomarkers, late effects and immunosuppression of radiation injury that can quantitate effects on combat performance decrements; (4) To identify novel therapeutic strategies that will support military operations within a nuclear or radiological environment minimizing ground troops short and long term adverse risk.

B. Accomplishments/Planned Programs (\$ in Millions)

	FY 2021	FY 2022	FY 2023 Base	FY 2023 OCO	FY 2023 Total
<p>Title: Radiation Countermeasures (USUHS)</p> <p>Description: For the Uniformed Services University of the Health Sciences/Armed Forces Radiobiology Research Institute (USUHS/AFRRI), 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, microbiome, gastrointestinal damage, neurobehavioral deficits, bone marrow damage), termed radiation combined injury. Research findings are focused to advance the understanding and to produce the following: (1) To identify new therapeutics candidates that show promising advancement to further development; (2) To developed novel technologies to minimized the use of animal models in the study of radiation countermeasure effects; (3) To investigate the overall radiation effect by countermeasures in the microbiome and anatomical tissue; (4) To find novel biomarkers, late effects and immunosuppression of radiation injury that can quantitate effects on combat performance decrements; (4) To identify novel therapeutic strategies that will support military operations within a nuclear or radiological environment minimizing ground troops short and long term adverse risk.</p> <p>In addition to the primary achievement of research objectives, the program educates Federal employees as a benefit to the public they serve through Federal service, through support to civil authorities, and in non-Federal professional and academic collaborations.</p> <p>Description: For the Uniformed Services University of the Health Sciences/Armed Forces Radiobiology Research Institute (USUHS/AFRRI), 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, microbiome, gastrointestinal damage, neurobehavioral deficits, bone marrow damage), termed radiation combined injury. Research findings are focused to advance the understanding and to produce the following: (1) To identify new therapeutics candidates that show promising advancement to further development; (2) To developed novel technologies to minimized the use of animal models in the study of radiation countermeasure effects; (3) To investigate the overall radiation effect by countermeasures</p>	0.970	0.989	1.009	0.000	1.009

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B. Accomplishments/Planned Programs (\$ in Millions)

	FY 2021	FY 2022	FY 2023 Base	FY 2023 OCO	FY 2023 Total
<p>in the microbiome and anatomical tissue; (4) To find novel biomarkers, late effects and immunosuppression of radiation injury that can quantitate effects on combat performance decrements; (4) To identify novel therapeutic strategies that will support military operations within a nuclear or radiological environment minimizing ground troops short and long term adverse risk.</p> <p>In addition to the primary achievement of research objectives, the program educates Federal employees as a benefit to the public they serve through Federal service, through support to civil authorities, and in non-Federal professional and academic collaborations.</p> <p>FY21 Accomplishments:</p> <ul style="list-style-type: none"> (1) Completed methylome and proteome studies with hematopoietic progenitor cells using murine model. (2) Characterized and correlated the dose and dose rate effect of sub-lethal neutron radiation on genetic and epigenomic perturbations in hematopoietic progenitor cells in male mice. (3) Determined transcriptomic signatures that are correlated with radiation injury, using whole blood transcriptome analysis. (4) Established the gut organ-on-chip model. (5) Identified and tested small molecule countermeasure following Lipinski's rules. (6) Selected countermeasure therapeutic to test using the gut-organ-on-chip model for radioprotective and radiomitigative potential. (7) Tested long term effect in bone marrow irradiated with 2.5% mice. (8) Characterized injury to lungs, heart, and brain by analyzing biomarkers specific to this organs and vascular endothelial tissue at different radiation doses. (9) Monitored up to six months mice exposed to BPI to study delayed effects of radiation exposure. (10) Screened potential prophylactic countermeasures in PBI with 2.5% model. (11) Established growth conditions for BM endothelium and vascular endothelium in 3D cell culture environment. (12) Established optimal conditions for endothelial/immune cell contact and non/contact co-culture in 3D cell culture environment. (13) Performed gamma radiations with single cultures in 3D cell culture system. (14) Conducted cellular experiments (DNA damage, survival, functions) (15) Performed gamma radiations with endothelium/immune cell 3D cultures. 					

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Exhibit R-2A, RDT&E Project Justification: PB 2023 Defense Health Agency		Date: April 2022
Appropriation/Budget Activity 0130 / 2	R-1 Program Element (Number/Name) PE 0602787DHA / <i>Medical Technology (AF RRI)</i>	Project (Number/Name) 241C / <i>Radiation Countermeasures (USUHS)</i>

B. Accomplishments/Planned Programs (\$ in Millions)

	FY 2021	FY 2022	FY 2023 Base	FY 2023 OCO	FY 2023 Total
<p>(16) Tested bone marrow and ileu of male and female mice to access levels of cytokine, AKT, MAPK and caspase-3 for organ injury.</p> <p>(17) Tested tissue lysates of bone marrow, ileum and spleen of male and female mice for cytokine and complement component 3.</p> <p>(18) Completed IL-18 studies indicating that IL-18 binding protein (IL-18BP) can be tested as potential drug target. Mice treatment with IL-18BP indicates inhibition of downstream signaling, protecting the mice tissue from radiation by decreasing apoptosis after total body radiation.</p> <p>(19) In 2019/2021, 44 manuscripts were published.</p> <p>FY 2022 Plans: FY2022 plans continue efforts as outlined in FY 2021 in addition to the following: (1) To complete methylome and proteome studies and identify early epigenomic steps post-radiation caused by LDR/LDR neutron exposure to murine stem cells populations as potential low dose exposure markers using multiple analytical bioinformatics programs. (2) To down-select potential gut-organ-on-chip small molecule and test for efficacy in murine model. (3) To screen one potential prophylactic countermeasure in the partial body irradiation model with 2.5% sparing of bone marrow. (4) To perform neutron/gamma radiation with single 3D cell culture. (5) To perform neutron/gamma radiations with endothelium/immune cell 3D cultures. (6) To determine DRF for promising candidates. (7) To determine hematological end points to assess recovery from H-ARS. (8) To analyze specimens of the jejunum after lethal irradiation in mice treated with FDA-approved therapeutics. (9) To identify other animal models where various anatomical sites (e.g. intestinal, oral, cutaneous, pulmonary, and urinary, etc) can be interrogated for microbiome alterations. (10) To develop an in vitro Caco2 IL-18 receptor knock out cell line using the CRISPR technology and 3D cell culture to test IL-18BP efficacy prior to animal testing. (11) To optimize the gastro-intestinal organ-on-chip model using intestinal cell lines to mimic the 3D architecture of the intestinal physiology. (12) To define biomarkers of neurobehavioral deficits following low-dose exposure. (13) To identify circulating miRNAs at different time points following low-dose irradiation. (14) To determine the relationship between circulating miRNAs and neurobehavioral deficits. (15) To identify miRNA in exosomes from radiation exposed human primary cell lines that target CXCR4 receptor in recipient cells that facilitate proliferation or neutrophil progenitors using high-throughput methods.</p>					

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Exhibit R-2A, RDT&E Project Justification: PB 2023 Defense Health Agency		Date: April 2022
Appropriation/Budget Activity 0130 / 2	R-1 Program Element (Number/Name) PE 0602787DHA / <i>Medical Technology (AF RRI)</i>	Project (Number/Name) 241C / <i>Radiation Countermeasures (USUHS)</i>

B. Accomplishments/Planned Programs (\$ in Millions)	FY 2021	FY 2022	FY 2023 Base	FY 2023 OCO	FY 2023 Total
(16) To determine the effect of exosome-packed selected miRNA on the release of neutrophils from BM cells using in vitro BM model, and their interactions with G-CSF and GM-CSF, with gamma radiation. FY 2023 Base Plans: FY2023 plans continue efforts as outlined in FY 2022. FY 2023 OCO Plans: N/A FY 2022 to FY 2023 Increase/Decrease Statement: Pricing adjustment for inflation.					
Accomplishments/Planned Programs Subtotals	0.970	0.989	1.009	0.000	1.009

C. Other Program Funding Summary (\$ in Millions)

N/A

Remarks

The program element 0602787DHA for AFRRRI in addition to the three program elements: 0601115HPPE, 0602115HPPE, and 0603115HP are coordinated and integrated into the portfolio management by the Joint Program Committee-7/ Radiation Health Effects Research Program (RHERP).

D. Acquisition Strategy

USU optimizes these research funds to achieve its research objectives, often in partnership and collaboration with funding received from other DoD and interagency sources through Interagency Agreements and inter-service Support Agreements, which may be executed via Federal assistance agreements or contracts.