Exhibit R-2, RDT&E Budget Item Justification Fiscal Year (FY) 2005 Budget Estimates						Date: February 2004	
Appropriation/Budget Activity RDT&E, D BA2				R-1 Item Nomenclature: Medical Technology, PE 0602787D8Z			
Cost ($ in millions)	FY 2003	FY 2004	FY 2005	FY 2006	FY 2007	FY 2008	FY 2009
Total PE Cost	6.100	11.641	10.084	10.266	10.488	10.708	10.929
Medical Technology/P505, Subtotal Cost	6.100	11.641	10.084	10.266	10.488	10.708	10.929

A. Mission Description and Budget Item Justification:

(U) 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 mitigating the health consequences from exposures to ionizing radiation that represent the highest probable threat to US forces under current tactical, humanitarian and counter terrorism 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. Advancements in field-based biological dose assessment systems to measure radiation exposures will enhance triage, treatment decisions and risk assessment. Accurate models to predict casualties will promote effective command decisions and force structure planning to ensure mission success.

(U) The program has three primary goals: (1) rational development of prophylactic and therapeutic strategies based on fundamental knowledge of radiation-induced pathophysiology and on leveraging advances in medicine and biotechnology from industry and academia; (2) development of novel biological markers and delivery platforms for rapid, field-based individual dose assessment; and (3) understanding toxic consequences from chronic exposure to tissue-embedded depleted uranium (DU).

B. Program Change Summary:

	2003	2004	2005
Previous President's Budget	0	0	0
Current FY 2005 President's Budget	6.100	11.641	10.084
Total Adjustments		11.641	10.084
Congressional program reductions			
Congressional rescissions			
Congressional increases			
Reprogrammings			
SBIR/STTR Transfer			
Other			

C. Other Program Funding Summary: Not applicable.

D. Execution:
Armed Forces Radiobiology Research Institute, Bethesda, MD

Exhibit R-2a, RDT&E Project Justification Fiscal Year (FY) 2005 Budget Estimates							Date: February 2004
Appropriation/Budget Activity RDT&E, D BA 2	PROGRAM ELEMENT Medical Technology PE 0602787D8Z		Project Name and Number Medical Technology P505				
Cost ($ in millions)	FY 2003	FY 2004	FY 2005	FY 2006	FY 2007	FY 2008	FY 2009
Project/Thrust Cost	6.100	11.641	10.084	10.266	10.488	10.708	10.929

A. Mission Description and Budget Item Justification:

(U) 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 ionizing radiation.

(U) The program has three primary goals: (1) rational development of prophylactic and therapeutic strategies based on fundamental knowledge of radiation-induced pathophysiology and on leveraging advances in medicine and biotechnology from industry and academia; (2) development of novel biological markers and delivery platforms for rapid, field-based individual dose assessment; (3) understanding toxic consequences from chronic exposure to tissue-embedded depleted uranium (DU).

Cost (in $ Millions)	FY 2003	FY 2004	FY 2005
Mechanisms of AED Radioprotection	1.030	1.353	1.300

FY 2003: To address the FDA requirement for an understanding of the mechanisms responsible for AED's radioprotective actions, demonstrated that AED stimulates phagocytotic activity in circulating granulocytes and the oxidative burst in circulating monocytes in irradiated mice. Demonstrated that AED's protective effects are not due to contamination with endotoxin. Established techniques for measuring AED and other steroids to allow pharmacokinetic analysis of AED.
FY 2004: Initiate experiments on effects of AED on the function of peritoneal macrophages, a critical, non-circulating component of the immune system. Initiate studies on AED's ability in the spleen to induce cytokines, which mediate signals of the immune system.
FY 2005: Complete assays on actions of AED on oxidative burst and phagocytosis of peritoneal macrophages in irradiated and non-irradiated rodents. Provide preliminary measurements of several cytokines in the spleen. Establish correlation of plasma levels of

AED and efficacy of drug actions in the rodent model.

Cost (in $ Millions)	FY 2003	FY 2004	FY 2005
Radioprotective effects of isoflavones and vitamin derivatives	.698	1.110	1.480

FY 2003: Demonstrated that the soybean derived isoflavone genistein has radioprotective effects if administered subcutaneous 24 hour prior to radiation exposure in the mouse. Preliminary experiments show that the related compound daidzein also provides radiation protection. Demonstrated that alpha-tocopherol increased the erythrocyte levels in irradiated mice.
FY 2004: Determine the dose response curve for radioprotection by genistein in both male and female rodents. Determine the optimal time for administration of genistein for radioprotection. Complete screening of delta- and gamma-tocopherol in comparison to alpha-tocopherol for radioprotection in mice.
FY 2005: Determine the optimal time for administration of daidzein for radioprotection. Begin to evaluate combinations of genistein and daidzein to determine the optimal ratio. Determine the dose-reduction factor of the most effective isomer of tocopherol. Compare pharmacokinetics of this isomer given subcutaneously in irradiated and non-irradiated mice.

Cost (in $ Millions)	FY 2003	FY 2004	FY 2005
Dual-action Drug Delivery Strategy	.480	.873	.500

FY 2003: The initial design of a sustained-release, lipid-encapsulated (liposomal) delivery system for aminothiol in combination with Vitamin E was improved to provide approximately 35% drug loading.
FY 2004: Continue to perfect the liposomal delivery system. Assess the distribution of the drug achieved with this delivery system and assess the effects on cytokine gene expression as an indicator of drug action.
FY 2005: Establish optimum conditions for radioprotection with the liposomal preparation in rodents using survival following radiation exposure and blood profiles as endpoints.

Cost (in $ Millions)	FY 2003	FY 2004	FY 2005
Radioprotectants/Therapeutics Survey	1.001	1.247	1.376

FY 2003: Continued systematic survey of potential radioprotectant and therapeutic compounds under a drug screen protocol. Included this year were several non-androgenic steroids related to 5-AED,captopril, a cell cycling modulator, and several analogs of Vitamin E. Continued to refine, test, and analyze preventive treatment strategies based on fundamental mechanisms of cellular and molecular injury and repair of blood-forming and gastrointestinal organ systems.
FY 2004: Among the drugs slated to be tested in FY2004 with drug screening protocol are promising products from various pharmaceutical companies. Most of the agents are proprietary; they include a DHEA derivative and statins. Drugs that show potential will be targeted for further development.
FY 2005: New drugs continue to come to the attention of the Institute for assessment. These agents will be evaluated for their ability to prevent and/or treat radiation injury. Approaches to screening new agents will be improved.

Cost (in $ Millions)	FY 2003	FY 2004	FY 2005
PCC Cytogenetic Assay	.240	.240	.340

FY 2003: Patent application filed on the novel premature chromosome condensation (PCC) aberration assay that permits rapid analysis of radiation exposure across a broad dose range from interphase lymphocytes of peripheral blood. Demonstrated that PCC can be induced in a single cell.
FY 2004: Continue to improve sample preparation by promoting signal transduction mechanisms for inducing PCC in peripheral blood lymphocytes. Optimize the color pigment technique that will be used for fluorescent in situ hybridization (FISH) method for detecting chromosome aberrations in multiple chromosomes.
FY 2005: Optimize the multicolor FISH protocol that will allow detection and quantification of radiation-induced chromosome aberrations in multiple chromosomes. This approach will increase the sensitivity of the assay and permit detection and quantification of partial-body exposures.

Cost (in $ Millions)	FY 2003	FY 2004	FY 2005

Molecular Biomarkers- DNA mutations	.466	.466	.566
FY 2003: Developed real-time PCR for detection of DNA mutations (common mitochondria DNA deletion) in genomic DNA samples providing a significant advance in quantitative assessment of target sequences. Initiated studies to optimize the real-time and cytological DNA mutation bioassay to detect low-frequency DNA mutations. **FY 2004:** Develop and evaluate modified deletion primers for quantitative fluid phase PCR bioassay in Human Peripheral Blood Lymphocytes (HPBL). Begin evaluation of low level multiplex detection. **FY 2005:** Develop cytological assay using PCR to measure mtDNA deletions in HPBL. Evaluate the effect of inter-individual variation for this assay. Perform in vitro dose-response studies for fluid-phase PCR assay in HPBL.			
Cost (in $ Millions)	FY 2003	FY 2004	FY 2005
Blood-Based Cell and Protein Markers	.297	.397	.510
FY 2003: Optimized the microassay to determine protein concentration in human blood samples. Developed biotinylated detection antibody cocktails that allow detection of proteins in serum. Determined the level of one important protein (GADD45) in irradiated human blood. **FY 2004:** Continue studies to develop microsphere flow cytometry system for measurement of multiple radiation-responsive protein biomarkers. Optimize sample processing of protein biomarkers for field-based blood analysis. Provide GADD45 and human albumin or beta-actin bead sets for radiation-responsive blood protein biomarker determinations. **FY 2005:** Complete initial phase of in vitro studies evaluating radiation-responsive blood protein biomarkers involving other protein targets measured by the microsphere flow cytometry-based system. Initiate protein biomarker studies to evaluate inter-individual and stress agent effects.			
Cost (in $ Millions)	FY 2003	FY 2004	FY 2005
Toxicity of DU and Tungsten	.160	.433	.050

FY 2003: Determined that DU and Tungsten Alloys induce mutations in a marker gene (HPRT) in vitro. Completed studies assessing the effects of the heavy metals on gene expression in liver carcinoma cells. Initiated studies on genomic instability and human-derived (HOS) cell neoplastic transformation.
FY 2004: Continue transformation, genotoxicity, and genomic instability studies on HOS cells. Initiate studies on macrophage cell lines that are important in the toxicity of inhaled DU and other heavy metals. (The related Defense Technology Objective completes in FY 2004.)
FY 2005: Evaluate effects of heavy metals on viability of pulmonary macrophages and initiate evaluation of cell function.

Cost (in $ Millions)	FY 2003	FY 2004	FY 2005
Late-Arising Radiation Injuries	.264	.205	.414

FY 2003: Evaluated in vitro the effects of the pharmacological agents AED, epigallocatechin (EGCG), and phenylacetate on expression of radiation-induced biomarkers that correlate with carcinogenicity. Developed a leukemogenesis mouse model that can be used to study the pre-leukemic phase, identify oncogenic changes, define factors that contribute to the development of leukemia, and test the efficacy of the drugs.
FY 2004: Assess the ability of phenyl acetate to inhibit human cell transformation in vitro (i.e, to block development of pre-cancerous cells). Initiate transformation studies with EGCG.
FY 2005: Complete transformation studies with EGCG. Initiate radiation leukemogenesis studies with phenylacetate and EGCG.

Cost (in $ Millions)	FY 2003	FY 2004	FY 2005
Radiation Injury and Bacterial Sepsis	.700	1.366	1.700

FY 2003: Established animal model to assess antibiotics and biological response modifiers for radiation-related infection and sepsis.
FY 2004: Determine the ability of the non-specific biological response modifier (insoluble beta-1,3-glucan) against infection with K. pneumoniae in sublethally irradiated mice.
FY 2005: Determine the efficacy of the non-specific biological response modifier against a polymicrobial infection from endogenous pathogens with lethal doses of radiation. Determine the effects of the quinolones against a polymicrobial infection from endogenous pathogens with lethal doses of radiation.

Cost (in $ Millions)	FY 2003	FY 2004	FY 2005

Probiotics	.387	0.613	0.924
FY 2003: Developed the experimental mouse model to assess the effectiveness of Lactobacillus reuteri to protect against radiation-induced enteric infections. Established that L. reuteri is not indigenous to the mouse colony. Demonstrated feasibility of model system. **FY 2004:** Evaluate the effectiveness of L. reuteri as a probiotic protective agent when mice are challenged with S. sonnei and radiation exposure **FY 2005:** Compare the effectiveness of L. reuteri and VSL#3 (a commercially available combination of multiple bacteria) in response to lethal radiation at doses that cause gastrointestinal damage and diarrhea.			
Cost (in $ Millions)	FY 2003	FY 2004	FY 2005
Noninvasive "biomodulation" system (Congressional add)	0	2.400	0
FY 2004: Assess the use of hair proteins in the hair follicle as a non-invasive biomarker for exposure to radiation. Develop the methodology for a biological dosimetry tool to allow triage of potential radiological victims using this approach.			
Cost (in $ Millions)	FY 2003	FY 2004	FY 2005
Host-Defense Mechanisms	.377	.938	0.924
FY 2003: In macrophage cell lines, identified patterns of host-defense modulation at the molecular level following sublethal irradiation and viral (influenza) infection. NFkB was found to be one of the primary chemicals regulating macrophage response to virus. **FY 2004:** Determine the activation state of NFkB during virus infection and radiation exposure. Assess cell survival, apoptotic markers, and cytokine production as endpoints. **FY 2005:** Evaluate the effect of antioxidants and radioprotectants including genistein on changes induced by virus infection and radiation exposure.			