Integrated Biological and Computational Low-Dose Radiation Research

Summary of BER-supported projects awarded in 2024 under DOE Funding Opportunity Announcement DE-FOA-0003281

GSP Genomic Science Program

genomicscience.energy.gov

Projects

- *Ab Initio* Modeling Capabilities for Simulating DNA Damage Under Ionizing Radiation
- AI-Facilitated Assessment of Exosome-Mediated Bystander Effects of Low-Dose Ionizing Radiation
- Automated High-Throughput Screening of the Effect of Low-Dose Radiation on Breast Using Fibroblast-Incorporated Mammographic Density Models
- Bridging the Gap Between Low-Dose Exposures and Emergent Physiology Using Integrative Modeling and Experimentation from Epigenome to Cell Phenotype
- Combined Dosimetric and Toxicological Contributions to Bone Marrow Response in Mice from Low-Dose Strontium Exposure Using AI-Driven Mouse Model and Digital Twins
- Combined Experimental and AI-Based Deep-Learning Approach to Low-Dose and Low-Dose-Rate Breast Cancer Radiation Risk Prediction
- Evaluation of the Cellular and Molecular Responses of Human Skin Fibroblasts and Neurons Derived from iPSCs with Varying Intrinsic Radiosensitivity to Low-Dose Radiation
- Exploring T-Cell Functional Dynamics Following Low-Dose Radiation Exposure: Insights into Metabolic and Other Regulatory Alterations Using a Multiomics Systems-Biology Approach
- Genetic Diversity of Human Heart Responses to Low-Dose Radiation
- Human Bone Marrow Model for Response Network Studies of Low-Dose/Low-Dose-Rate Radiation Exposures
- Modeling the Circadian Effects of Low-Dose Radiation on Immunometabolism and its Effect on Liver Organoid Physiology
- Quantitative Protein Signatures of Low-Dose Radiation Exposure
- Single-Cell-Level Elemental Signatures of Low-Dose Radiation Exposures in Mammalian Model Systems
- Understanding the Mechanism and Health Consequences of Low-Dose Radiation at a Molecular Level

In August 2024, the U.S. Department of Energy (DOE) Biological and Environmental Research program (BER) funded 14 research projects studying cellular and molecular changes resulting from low-dose radiation exposure.

Projects will leverage the latest advances in biotechnology, artificial intelligence (AI), and machine learning (ML) in an integrated biological and computational approach to understand radiation-induced changes in cellular metabolism and other processes. Research will also develop disease risk prediction capabilities, such as identifying biomarkers and patterns linked with altered cellular function that may signal adverse health outcomes.

Initial projects will develop a series of highly curated experimental datasets across a range of cell types to assess changes in cell function. Model systems to be used in the research range from human-derived cell and organ systems to mouse and computational models.

The resulting datasets will provide training data for a burgeoning AI/ML modeling capability for continuing low-dose radiation research. These efforts will ultimately lay a foundation for informing future radiation protection measures for the public and the workplace.

The 14 awarded projects involve multi-institutional teams employing diverse and innovative experimental and computational approaches. This integrated approach has the potential to produce broader insights into transient and persistent low-dose radiation exposure and its potential human health effects.

Contact

BER Program Manager
Resham Kulkarni
240.687.4785
resham.kulkarni@science.doe.gov

Websites

Integrated Biological and Computational Low-Dose Radiation Research
genomicscience.energy.gov/lowdose

DOE Office of Science
energy.gov/science

DOE Biological and Environmental Research Program
science.osti.gov/ber

BER Genomic Science Program
genomicscience.energy.gov

U.S. Department of ENERGY | Office of Science
Biological and Environmental Research Program

Ab Initio Modeling Capabilities for Simulating DNA Damage Under Ionizing Radiation

Principal Investigator: Yosuke Kanai
(University of North Carolina, Chapel Hill)

This project will advance first-principles computational methods to (1) examine how different ionizing radiations induce varying electronic excitation responses and (2) model how the induced electronic excitations lead to specific DNA damages that impede cellular functions. Over the last few decades, it has become clear that linear energy transfer (or electronic stopping power) itself is not enough to explain experimentally observed differences in ionizing radiation types. Charged-particle radiation (e.g., protons and alpha particles) induces cellular responses that are distinctively different from those of photon-based radiations (e.g., X-rays or gamma rays) or even those of beta particles (i.e., electrons). At a fundamental level, differences arise from the interaction of electrons in DNA/water with ionizing radiation; understanding such a quantum-mechanical process is a great challenge.

Ionizing radiation leads to the generation of highly energetic holes (and some electron–hole pairs) in DNA and water. Experimentally observed macroscopic differences on the cellular scale for different types of ionizing radiation are believed to derive from differing electronic responses induced by the radiation. While such a hypothesis is physically sound, the important scientific questions are how and why the electronic responses are different depending on the ionizing radiation type. How the electronic excitation differences lead to different outcomes in terms of molecular-level DNA damage must also be characterized. Answering these fundamental scientific questions has remained a daunting task in the field. Without overcoming this challenge, the scientific community will not transcend its current crude understanding of experimental observations.

With advanced atomistic modeling based on first-principles theory using high-performance computers, the research team aims to drastically change how this problem is tackled. Instead of using different empirical models with parameters for different types of ionizing radiation, the team will spearhead development of a unifying first-principles computational method that treats different types of ionizing radiation equally. In this first-principles approach, modeling does not rely on experiments but stands as an equal partner to scrutinize experimental observations.

Due to the quantum dynamical nature of the interaction between DNA/water and radiation, developing a microscopic understanding of this process has remained elusive. Over the last decade, computational methods for simulating quantum dynamics from first-principles theory have greatly advanced. The research team has played an important role in this endeavor, and its work with proton and alpha-particle radiation has been widely disseminated in the field. Additionally, it is necessary to model atomic nuclear responses to the transient state of nonequilibrium electrons to understand how atomistic DNA damage is induced. In this regard, modeling the quantum dynamical nature of atomic nuclei remains a difficult scientific challenge.

The team will expand development of its new computational method for simulating coupled quantum dynamics of electrons and nuclei in complex heterogeneous environments. Together with the new generation of exascale computers, it is now possible to develop software and perform first-principles modeling of nonequilibrium processes to study DNA damage induced by ionizing radiation without relying on inconvenient empirical parameters.

AI-Facilitated Assessment of Exosome-Mediated Bystander Effects of Low-Dose Ionizing Radiation

Principal Investigator: A. Ray (Keck Graduate Institute)

Co-Principal Investigator: K. Ganguly
(Los Alamos National Laboratory)

Co-Investigators: M. Freeman, P. Mach, and B. McMahon
(Los Alamos National Laboratory)

This study aims to understand how low-dose ionizing radiation (LDIR), such as from low-dose X-rays or gamma rays, affects immune cell function at a distance from the site of irradiation. LDIR comes from natural and artificial sources, including high-altitude airplane flights and space travel, exposure to medical scans and armament dust, and is everywhere in the environment. Studies show it can lead to various health effects, both harmful and protective.

It is challenging to link specific health problems directly to LDIR exposure due to many factors. The chief among these is the lack of understanding of its effects on human tissues. Ionizing radiation does not just affect the cells it directly hits but also nearby and distant organs through the radiation-induced bystander effect (RIBE). RIBE can mimic the biological effects of direct radiation damage, including producing typical signs of radiation damage such as chromosome breaks. However, distinct differences exist between direct radiation effects and RIBE, especially effects related to dose-response characteristics. Bystander effects do not decrease proportionately with dose.

Recent research suggests that part of RIBE involves tiny particles called exosomes or similar extracellular vesicles secreted by human cells. These carry RNA, proteins, and signaling molecules. This study tests the idea that RNA in exosomes released after LDIR exposure affects immune cells, even when these cells are not directly exposed to the radiation. The research team will

use human skin cell cultures exposed to low radiation doses to analyze changes in RNA and proteins carried by exosomes. Unirradiated human immune cells will then be exposed to these exosomes and their responses examined using advanced genetic and biochemical techniques, including cellular assays, single-cell transcriptomics, and proteomics. The measured responses will be integrated using high-dimensional machine learning methods, helping to generate mechanistic explanations of how LDIR RIBE works.

Goals include (1) understanding how exosome contents change in response to different radiation doses; (2) using machine learning to find links between radiation dose, genes, and exosome contents; and (3) examining how exosomes affect immune cells using detailed single-cell genomics and biochemical analysis.

By disrupting identified genes or RNA in immune cells that respond to LDIR RIBE, the team aims to understand how LDIR affects immune responses. This could reveal new radiation exposure biomarkers for future testing through "liquid biopsies." Ultimately, the study seeks to explain how LDIR affects the immune system, potentially influencing health responses across the body.

Automated High-Throughput Screening of the Effects of Low-Dose Radiation on Breast Using Fibroblast-Incorporated Mammographic Density Models

Principal Investigator: Qingsu Cheng
(University of Wisconsin, Milwaukee)

In the United States, one woman in eight will develop breast cancer during her lifetime, with the risk increasing for those with high-density breasts. Radiation is a known additive risk factor for breast cancer, but the effects of low-dose and low-dose-rate radiation remain poorly understood. The U.S. population is exposed to these types of radiation through natural sources, medical procedures, and occupational settings. It is crucial to link low-dose and low-dose-rate radiation exposure to adverse health effects, including cancer susceptibility, progression, and metastasis.

Cancer development is complex and involves interactions with stromal cells, such as fibroblasts, which can inhibit or promote microenvironment tumor growth. This complexity requires a thorough investigation of the phenotypic and molecular responses to low-dose and low-dose-rate radiation at both the cellular and organoid levels, particularly regarding the interplay between cancer cells and stromal cells. To address this need, the research team will utilize its combined expertise in radiation, breast cancer biology, and computational biology to elucidate the adverse health effects of low-dose and low-dose-rate radiation on breast tissues.

The overarching goal of this initiative is to identify (1) phenotypic markers indicating the effects of low-dose and low-dose-rate radiation on breast tissue and (2) a set of abnormal genetic and protein expression patterns associated with various aspects of breast cancer. The team hypothesizes that high mammographic density combined with exposure to low-dose and low-dose-rate radiation within the range of occupational and medical imaging doses increases the likelihood of developing benign conditions, which may subsequently lead to cancer.

To test this hypothesis, the team plans to develop a high-throughput 3D coculture model that mimics mammographic density using multi-head 3D printing. Cultures of 3D-printed primary epithelial cells will be cocultured with primary mammary gland fibroblasts. Interactions between the fibroblasts and epithelial cells under low-dose radiation will then be examined using biological analyses of relevant biomarkers, such as immunofluorescent staining, qPCR, Western blotting, ELISA assays, and multiomics.

Additionally, the team will develop an unsupervised deep-learning framework for high-throughput profiling of phenotypic and molecular changes using a CustomNet customization method. CustomNet will synergistically combine customized convolutional blocks with advanced axial attention mechanisms to provide a robust and nuanced understanding of image features. To ensure rigor, all lab materials will be authenticated, and the team will collaborate closely with a biostatistician throughout experimental design to implement appropriate statistical methods.

Project innovations include modulating stiffness within the range of mammographic density in 3D coculture assays for high-throughput imaging using primary cells, specifically in the context of low-dose and low-dose-rate radiation. The team aims to deliver quantifiable metrics on colony organization and heterogeneity using deep-learning algorithms to decipher fibroblast–epithelium interactions. This approach will pave the way for developing targeted interventions and personalized treatment strategies using generative AI. Furthermore, efforts will lay the groundwork for creating digital twins of the experimental models, enabling the exploration of the nuanced relationships between low-dose and low-dose-rate radiation exposure and breast cancer, providing valuable insights that could inform public health policies and clinical practices and facilitating the design and administration of reagents to reduce individual risks.

Bridging the Gap Between Low-Dose Exposures and Emergent Physiology Using Integrative Modeling and Experimentation from Epigenome to Cell Phenotype

Principal Investigator: Rachel McCord
(University of Tennessee, Knoxville)

Extended low-dose radiation is encountered in the natural environment (e.g., radon exposure) through frequent exposure to medical diagnostics (e.g., X-rays), and in occupational situations. However, the effects of these exposures on human cell and tissue types and the molecular pathways through which such effects occur require further investigation.

Numerous sources of evidence indicate that low-dose radiation can cause epigenetic changes. However, systematic data for different cell types at the same doses is lacking, preventing the connection of chromatin-level changes to changes in gene transcription, protein expression, and cell phenotype. This project will examine the impacts of low-dose gamma radiation on human lung cell types, including fibroblasts, epithelials, and endothelials.

The overarching project objective is to connect observable changes in cell function (i.e., phenotypes) with the molecular alterations causing the changes. This goal will be accomplished by first monitoring changes in cellular function. As cells are exposed to radiation, do fibroblasts become reactive and fibrotic? Do endothelial and epithelial cells lose the ability to form barriers or undergo epithelial-to-mesenchymal transition? Does DNA damage occur and cell proliferation change? Next, genome-wide information will be collected on which genes are transcribed (i.e., transcriptomics), which proteins are made (i.e., proteomics), and how epigenetic marks along DNA change in response to low-dose radiation. The team will use mechanistic and data-driven computational models to connect molecular-level changes to observed cell phenotypes. Based on prior data that radiation can change the structure of chromosomes, the team will test the hypothesis that extended low-dose radiation changes epigenetic marks along DNA, which can encode a memory of the cellular exposure and lead to long-term changes in gene and protein expression and, therefore, cell function.

Humans can be impacted by several different types of low-dose radiation and these different radiation types may have different biological effects. Therefore, the study will also compare the effects of low-dose X-rays and gamma rays to the effects of low-dose alpha particle exposure (e.g., chronic radon exposure in natural settings). To better represent a natural tissue or organ context, the results for single cell types cultured in a dish will be compared to the impacts of low-dose radiation on cells grown in spheroids and cocultured in lung-like organoids.

Research results will help identify biomarkers for tracking whether a tissue is experiencing negative consequences of radiation exposure. Further, the developed models will enable prediction of the effects of low-dose radiation in other scenarios.

Combined Dosimetric and Toxicological Contributions to Bone Marrow Response in Mice from Low-Dose Strontium Exposure Using AI-Driven Mouse Model and Digital Twins

Principal Investigator: Wesley Bolch
(University of Florida, Gainesville)

This research collaboration between the University of Florida, the Georgia Institute of Technology, and Lawrence Berkeley National Laboratory (LBNL) will investigate molecular markers for bone marrow dose response to low linear energy transfer (low-LET) and low-dose and low-dose-rate irradiation of marrow tissues in a mouse model for intravenous intratracheal installation of stable and radioactive strontium.

Internalized radionuclides constitute a significant pathway by which humans are exposed to low-LET ionizing radiation at low doses and at low dose rates. These exposures include environmental and occupational radionuclide scenarios and use diagnostic nuclear medicine for cancer detection and treatment monitoring. In most radiation-response modeling studies, the laboratory mouse is exposed to external beams of radiation whether whole or partial body. As such, tissue dosimetry in these study populations may be readily assessed using beam energy and intensity characterization coupled to standardized depth-dose profiles. For internalized radionuclide exposures, tissue dosimetry is much more complex. It must invoke anatomic computational mouse models for organ self-dose and cross-dose radiation transport simulations coupled with time-dependent biokinetic models of radionuclide airway, blood, and tissue radionuclide parent and progeny transport.

This series of studies will significantly enhance computational tools for internal radionuclide dosimetry in the laboratory mouse, including the use of artificial intelligence across all tasks. This enhanced computational tool will be made available throughout the low-dose radiation research community. The enhanced anatomic and physiological mouse computational phantom will be utilized in this study to further enhance dose-response modeling to low-dose bone marrow irradiation via radiostrontium inhalation studies at LBNL to determine the biomarker expression attributable to the toxicological versus radiological component of internal emitters.

The team further notes that the 2022 National Academies report, "Leveraging Advances in Modern Science to Revitalize Low-Dose Radiation Research in the United States," recommends elucidation of (1) biological localization of internalized radionuclides, (2) measurements of radiation-induced damage and response mechanisms, (3) development of high-fidelity anatomically and physiologically based dosimetry, and (4) development of modern statistical and computational methods for dose reconstruction. This research will explicitly address each report recommendation in the project scope.

Combined Experimental and AI-Based Deep-Learning Approach to Low-Dose and Low-Dose-Rate Breast Cancer Radiation Risk Prediction

Principal Investigator: Francis A. Cucinotta
(University of Nevada, Las Vegas)

Co-Investigators: Mingon Kang and Janice M. Pluth
(University of Nevada, Las Vegas)

Cancer risk following chronic low-dose and low-dose-rate (LDLDR) irradiation is a major concern for the public; radiation workers in the medical, nuclear energy, and aviation fields; and recipients of diagnostic radiation such as mammography and computed tomography scans. Low doses are often considered tissue doses below 100 milligrays (mGy). Radiation cancer risk has been described by a linear non-threshold dose-response model based on epidemiology analysis of cohorts exposed to medium- to high-dose acute or fractionated radiation. Radiation breast cancer risk is found to be one of the highest risks in epidemiology findings. However, epidemiology findings are severely limited at low doses (<100 mGy) and low-dose rates (<5 mGy/h), including chronic irradiation. Alternate hypotheses cannot be ruled out, including a dose threshold or a supra-linear dose response. A dose threshold would indicate no risk or a risk so small as to be undetectable. A supra-linear dose response would indicate a risk larger than a linear dose-response model predicts.

A variety of molecular and cellular marker studies, including assorted omics data, have been reported in humans with breast cancer and in mouse or cellular models exposed to medium to high radiation doses. Single-cell RNA is a more recent and powerful technology enabling studies of variation in many genes across a cell population. In addition, limited genomics information exists on subjects with breast cancer amongst survivors of the atomic bomb in Japan and the accident at the Chernobyl Nuclear Power Plant. However, these studies are severely limited at low doses and have used a variety of methodologies.

The goal of this study is to develop biophysics and artificial intelligence (AI)-based deep-learning models that combine information from high- and low-dose irradiation in humans and experimental models to inform breast radiation predictions for low-dose radiation exposures. The primary hypothesis is that low-dose and low-dose-rate radiation will induce transcriptional changes in a human mammary 3D acini model system, and that stochastic effects will occur due to fluctuations in signaling processes that impact transcriptional changes. Furthermore, these data and biophysics approaches can be used in a deep-learning model that combines them with existing data from mice and humans exposed to radiation at higher doses and human mammary cancer databases to discern LDLDR changes associated with breast cancer risk. This research will lead to a significantly innovative, biologically interpretable, deep-learning risk score model to provide breast cancer risk assessment for chronic exposures to low-dose (<100 mGy) radiation.

The specific aims in support of these goals described in the research plan are:

Aim 1: Perform single-cell RNA sequencing (scRNA-Seq) on cells from LDLDR-exposed human mammary 3D acini cultures to provide insights into cellular heterogeneity; identify rare changes induced by LDLDR irradiation; and shed light on stochastic gene expression, regulatory mechanisms, and functional diversity within exposed cell populations.

Aim 2: Develop a stochastic biochemical approach to relate DNA damage and oxidative stress signaling after LDLDR irradiation that precedes transcriptional changes. The team's previous model of DNA damage repair, ATM protein kinase, and transforming growth factor-beta (TGFβ) signaling pathways will be extended and recast using a stochastic formalism and studied in relation to the experimental transcription changes in Aim 1.

Aim 3: Develop an AI deep-learning approach using Pathway Graph Convolution Networks (PathGCN) that characterizes cell type–specific transcriptional and pathway-based mechanisms using gene expression (e.g., RNA-Seq and scRNA-Seq) data from human and mouse studies to predict breast cancer risks from low- to high-dose radiation.

This project comes from a multidisciplinary research team with expertise in computational modeling in radiation biophysics, AI and deep-learning approaches, and experimental radiobiology. The research uses an innovative experimental model in Aim 1, while Aim 2 focuses on modeling radiation effects leading to observed transcriptional changes. Aim 3 will investigate methods to combine the results from Aims 1 and 2 with experimental data from other radiation studies and breast cancer patient data. This research will establish a highly innovative LDLDR radiation model for breast cancer risk predictions.

Evaluation of the Cellular and Molecular Responses of Human Skin Fibroblasts and Neurons Derived from iPSCs with Varying Intrinsic Radiosensitivity to Low-Dose Radiation

Principal Investigator: Anthony Davis
(University of Texas Southwestern Medical Center)

Exposure to low-dose ionizing radiation is increasing due to medical procedures (e.g., CT scans, X-rays, and mammograms), natural sources (e.g., radon, soil radionuclides, and cosmic rays), and occupational sources affecting radiation workers. An average individual receives about 2.4 milligrays (mGy) per year, but this can vary based on occupation and geographic location. The health impact of chronic low-dose radiation exposure remains largely unknown. The response to ionizing radiation varies among individuals. Studies show that individuals with deleterious mutations in DNA damage response (DDR) genes exhibit radiosensitivity and increased cancer risk. However, variability in intrinsic radioresponse is also observed in individuals without gross DDR gene

defects, suggesting other factors—genetics, epigenetics, metabolic pathways, and subtle DDR perturbations—govern radioresponse. A key question is whether a relationship exists between intrinsic radiosensitivity and the risk of adverse events from low-dose radiation exposure. Conversely, are radioresistant individuals less prone to radiation-induced adverse events?

To address these questions, the research team has collected primary human skin fibroblasts (PHSFs) from diverse individuals to assess genetic diversity in the development of adverse late normal tissue effects following low-dose radiation exposure. This panel includes cells from different sexes, ethnic groups, and ages (i.e., childhood to late adulthood), enabling exploration of heterogeneity in the cellular response to low-dose radiation. The team hypothesizes that intrinsic radioresponse influences the cellular response to low-dose radiation. To test this, induced pluripotent stem cells (iPSCs) and iPSC-derived neurons will be generated from PHSFs to characterize neuronal responses to low-dose radiation, as the brain is at risk from such exposure. Transcriptomics, genomics, metabolomics, and proteomics will be used to analyze responses in "radionormal," "radioresistant," and "radiosensitive" cohorts (10 per year) compared to untreated controls. This hypothesis will be tested by pursuing the following aims: (1) identify consequential transcriptomic and genomic features of intrinsic radioresponse that are associated with the response to low-dose radiation; (2) identify consequential metabolomics features of intrinsic radioresponse that are associated with the response to low-dose radiation; and (3) assess functional proteomics and examine radiation-induced DDR, genomic instability, and mutation frequency associated with the response to low-dose radiation. Collectively, the team believes the data generated during this study will enable DOE to better model the understanding of an individual's response to low-dose radiation.

Exploring T-Cell Functional Dynamics Following Low-Dose Radiation Exposure: Insights into Metabolic and Other Regulatory Alterations Using a Multiomics Systems Biology Approach

Principal Investigator: Albert Fornace
(Georgetown University)

Low-dose radiation (LDR) emanating from natural sources, medical procedures, or workplace environments can affect the general population. Importantly, LDR can significantly impact the immune system by changing its balance, metabolism, and overall function. Immune cells have critical roles in combating infections, as well as cancer prevention, and disturbances can result in autoimmune diseases. While many studies focus on high radiation doses, even low doses can alter immune health, as seen in various global reports. Understanding how LDR affects immune responses, especially in radiosensitive immune cells like T lymphocytes (T cells), is crucial for accurate health risk assessments.

T cells are vital for the adaptive immune response and are highly sensitive to radiation. Though changes in T cells are well-documented at medium or high radiation doses, a knowledge gap exists about how LDR affects these cells and immune responses. Therefore, this project's aim is to address gaps in understanding LDR-induced immune alterations. Research will focus on T-cell metabolic dysregulation through a combination of well-controlled humanized and wildtype mouse models; immunologic studies; and integrative analyses of multiomic datasets that measure amounts and properties of genes, proteins, and metabolites.

The project will focus on radiation doses of 10 centigrays (cGy) or less using a multiomics approach, which includes studying metabolites, gene expression, proteins, and epigenetic changes. This team's previous research and preliminary data show that LDR can cause lasting molecular changes. Notably, LDR has measurable impacts on T-cell metabolism and gene expression related to energy metabolism. Animal models will be used to examine impacts on T-cell function in response to antigen challenge and the thymus, an organ that plays a pivotal role in orchestrating T-cell maturation.

This in-depth multiomics approach will analyze changes in signaling pathways after LDR exposure. Metabolomics, which studies small molecules (e.g., metabolites within cells), will be central to this research as it provides dynamic insights into how T-cell function can be disturbed in response to LDR. The approach will complement other omics data and help understand how genetic and transcriptomic changes lead to altered metabolic phenotypes and T-cell immune responses. A network map showing interactions between genes, proteins, and metabolites will be created, serving as a resource for further hypothesis generation and integration with ongoing studies at DOE laboratories.

This research has three main aims, each designed to provide a comprehensive understanding of how LDR impacts T-cell function and overall immune system health:

Aim 1: Decipher thymus microenvironment responses to LDR in T-cell maturation. This includes studying alterations in cellular composition, multiomic profiles, and molecular interactions within the thymus, an organ crucial for T-cell development and maturation.

Aim 2: Examine LDR effects on mature T-cell responses and assess age-related impacts. This aim focuses on the effects of LDR on mature T cells, which are fully developed and actively participate in immune responses. Researchers will assess how LDR influences T-cell functionality, including the cells' ability to respond to SARS-CoV-2 mRNA vaccination, which serves as antigens that trigger an immune response. Additionally, researchers will explore how these effects vary with age, given that aging can alter immune responses and increase susceptibility to radiation.

Aim 3: Perform multiomic data management, integration, and interpretation. The team will integrate findings from metabolomics, transcriptomics, proteomics, and epigenomics. Using advanced artificial intelligence and machine learning techniques, detailed maps of the molecular regulatory networks affected by LDR will be created. These maps will illustrate the interactions between genes, proteins, and metabolites, providing a

comprehensive view of how LDR impacts immune function. The integration of these multiomic datasets will enable identification of key molecular drivers of LDR-induced changes, offering new insights into the mechanisms underlying these effects.

Studying LDR is challenging because molecular responses can be complex yet subtle. Therefore, choosing the right model systems is crucial. The planned studies on T cells are significant, as clear responses within DOE's preferred low-dose range have already been observed. Both short- and long-term immune effects of LDR are relevant to human health, as shown in population studies. This research will provide a detailed understanding of how LDR influences T-cell development, functionality, and the broader immune system. This knowledge is crucial for developing accurate health risk assessments and for creating strategies to mitigate the adverse effects of LDR exposure on human health.

To maximize data utility, the multiomic datasets will be made publicly available, enabling integration with other research programs like the Low-dose Understanding, Cellular Insights, and Molecular Discoveries (LUCID) program. All data and code will be publicly accessible to ensure reproducibility and to benefit the broader radiation research community.

Genetic Diversity of Human Heart Responses to Low-Dose Radiation

Principal Investigator: Joseph Wu (Stanford University)

Co-Principal Investigators: Adam J. Chicco and Michael M. Weil (Colorado State University)

Prediction and intervention of radiation-induced heart disease (RIHD) pose formidable challenges due to the multitude of individual-specific risk factors leading to diverse presentations. This study endeavors to overcome these challenges by generating comprehensive datasets aimed at unraveling underlying genetic factors and identifying biomarkers associated with cardiac injury resulting from low-dose and low-dose-rate radiation exposures. Informed by previous investigations utilizing human induced pluripotent stem cell-derived engineered heart tissues (iPSC-EHTs) and animal models, this research focuses on oxidative stress, DNA damage, and mitochondrial dysfunction as pivotal contributors to radiogenic cardiac injury. The approach entails the development of a "cell village" using an innovative computational methodology for parallel transcriptomic profiling in diverse patient populations of iPSC-derived cardiomyocytes (iPSC-CMs) following low-dose/low-dose-rate irradiation. Collectively, this study aims to establish a novel model system for systematic investigations into how human genetic diversity influences cellular responses to radiation exposure. Furthermore, the generated datasets and functional outcomes will contribute to elucidating the mechanisms of radiogenic cardiac injury and will be valuable for artificial intelligence and machine learning analyses and sharing within the scientific community.

Human Bone Marrow Model for Response Network Studies of Low-Dose/Low-Dose-Rate Radiation Exposures

Principal Investigator: Sally A. Amundson (Columbia University)

Co-Investigators: Guy Garty, Robert Ullrich, and Aaron Viny (Columbia University)

Consultant: Kellie Machlus (Harvard Medical School and Boston Children's Hospital)

This project focuses on the radiation response of bone marrow stem cells, the target cell type for radiation-induced acute myeloid leukemia (AML). The research team will use flow cytometry to measure changes in cell type composition and differentiation, as well as generation of reactive oxygen species (ROS) after exposure to low-dose [0, 10, 100 milligrays (mGy)] and low-dose-rate (100 mGy at 5 mGy/h) radiation. The team will compare the responses in 2-dimensionally (2D) cultured bone marrow stem cells and in a 3-dimensional (3D) organoid model that reproduces key elements of the bone marrow niche to support the growth and function of human blood-forming stem cells. Changes in gene expression after low-dose and low-dose-rate exposures will also be measured. Standard RNA sequencing (RNA-Seq) will be used in the 2D bone marrow cells. Single-cell RNA-Seq will be used in the bone marrow organoids to assess changes in a cell-type-specific manner and to investigate interactions between cell types. Comparing responses in the 2D and 3D models will help define the contributions of the bone marrow niche to radiation response.

The organoid model can support the engraftment, growth, and function of donor bone marrow stem cells, thereby enabling study of conditions that may increase an individual's sensitivity to radiation-induced AML. The team will engraft the organoids with bone marrow stem cells from healthy donors or donors with a defined pre-leukemic condition, clonal hematopoiesis. Flow cytometry will again be used to measure changes in cell type composition and generation of ROS in the engrafted organoids after low-dose and low-dose-rate radiation exposure.

This project will provide:

- An organoid model that enables low-dose and low-dose-rate radiation experiments on the human bone marrow niche and target cells relevant for development of AML.
- Data on changes in cell types, ROS, and gene expression in bone marrow organoids.
- A model for the study of individual radiosensitivity.
- Insight into the pathway by which low-dose radiation exposure can lead to the development of AML, to better understand radiation risks.

Modeling the Circadian Effects of Low-Dose Radiation on Immunometabolism and its Effect on Liver Organoid Physiology

Principal Investigator: J. Hurley
(Rensselaer Polytechnic Institute)

Co-Principal Investigator: E. Blaber
(Rensselaer Polytechnic Institute)

Co-Investigator: S. Baker (Rensselaer Polytechnic Institute)

Low-dose radiation (LDR) affects multiple physiological systems, including the human immune response and metabolism. Several hallmarks of the immune system are affected after exposure to LDR, leading to an alteration in immunity and an increase in oxidative stress and inflammation. This affects both the innate immune system, such as monocyte-derived macrophages, and the adaptive immune system, including T-cell populations. Furthermore, research has shown that non-alcoholic fatty liver disease, a chronic condition affecting more than 25% of adults globally, develops in response to chronic exposure to environmental hazards like oxidative stress and radiation exposure. Although the liver has some regenerative capacity to combat damage from environmental hazards, this ability is dependent on a delicate balance and specifically timed immune response including both pro-inflammatory and reparative immune populations. This suggests that the effects of LDR on the immune system are acutely detrimental to the liver's regenerative capability.

A key regulator of the immune response is the circadian clock, the 24-hour mechanism that coordinates biology to the persistent day–night cycle. The clock times metabolism and oxidative stress to regulate immune pathways so that the immune response is primed to deal with environmental stresses differently depending on the time of day of exposure. In fact, the clock can temporally regulate the release of some immune molecules in response to LDR. However, despite the known interconnection between the immune system, LDR, and the clock, little is known mechanistically about how time-of-day of LDR exposure affects an organism's immune response and the consequent effects on highly metabolic organs such as the liver. Therefore, this project aims to address this specific knowledge gap by investigating how the timing of both acute and chronic exposure to LDR will affect time-of-day–specific differences in both immune populations and the liver. Time-of-day exposure to LDR is expected to generate different responses in immune cell function that affect the regulation and repair of liver cells. This project will investigate the cellular behavior and metabolic phenotypes of peripheral macrophages, regulatory T cells, and hepatic organoids to determine time-of-day–specific changes induced by LDR. Specifically, artificial intelligence and machine learning predictive algorithms with multiomics methods will be used to forecast key target molecules inducing cellular dysfunction in each cell population. Custom microfluidic microphysiological systems will also be used to identify time-of-day effects of immune cell exposure to LDR on liver organoid physiology. Together, this research will significantly advance understanding of how circadian rhythms affect the physiological response to both acute and chronic LDR exposure. Furthermore, it will provide multiomic mechanistic insight into the pathways and cellular responses that cause both immune dysregulation and progression of fibrotic liver disease in response to LDR exposure.

Quantitative Protein Signatures of Low-Dose Radiation Exposure

Principal Investigator: Michael MacCoss
(University of Washington)

This project's overarching goal is to develop capabilities to assess individual tissue-specific response from low-dose radiation using quantitative proteomics data from plasma extracellular vesicles (EVs). The team will generate a quantitative proteomics dataset that will measure individual tissue responses to low-dose radiation in a mouse model. Using a custom-modified Xstrahl Small Animal Radiation Research Platform (SARRP) capable of delivering a 5 milligray per hour (mGy/hr) dose rate, mouse cohorts will be exposed to varying low doses of X-ray irradiation (0-100 mGy). Untargeted proteomic datasets will be generated from multiple mouse tissues and plasma EVs using data-independent acquisition on an Orbitrap Astral mass spectrometer. Additionally, low abundance DNA damage response proteins and their modifications will be targeted using the new Stellar linear ion trap mass spectrometer, specifically designed for highly multiplexed, ultrasensitive targeted analysis. The team will analyze the proteome of plasma EVs as a "liquid biopsy" and train a machine learning model using the systemic EV measurement to predict X-ray dose and tissue-specific protein perturbations, including DNA damage response.

Single-Cell-Level Elemental Signatures of Low-Dose Radiation Exposures in Mammalian Model Systems

Principal Investigator: Gayle Woloschak
(Northwestern University)

Co-Investigators: Olga Antipova, Si Chen, Mathew Cherukara, Francesco De Carlo, Barry Lai, and Stefan Vogt (Argonne National Laboratory); Rebecca Abergel (Lawrence Berkeley National Laboratory); Ahtesham Ullah Khan (Northwestern Medical Hospital); Demirkan Gürsel, Craig Horbinski, Qiaoling Jin, Priyam Patel, Tatjana Paunesku, Lori Snyder, Stuart Stock, Zequn Sun, Ching Man Wai (Northwestern University); Dorthe Schaue (University of California, Los Angeles)

Objectives: This project will test the hypothesis that low-dose radiation exposures lead to changes in vascularization of normal tissues, a health outcome that may not shorten lifespan but may cause changes in disease incidence. The best way to study vascularization changes is to work with low-dose–exposed model

organisms that have been allowed to live out their entire lifespans. The Woloschak laboratory maintains and curates an archive of data and tissues from beagle dogs exposed to low radiation doses. Among the animals that received low linear energy transfer beta emitters are beagles from the University of California, Davis, that ingested radioactive strontium-90 (Sr-90). No significant life shortening was found in the lowest dose groups; archival samples from these animals will be used for these studies.

Description and Methods: Because strontium accumulates in bones, especially the skull, the research team has selected the brain to study microvasculature. Paunesku will identify and extract archival formalin-fixed paraffin-embedded (FFPE) beagle brain tissues from the Northwestern University Radiobiology Archives exposed to Sr-90 in doses that constitute chronic low-dose radiation exposures. Abergel will inject 40 mice with low doses of Sr-90 and isolate brain tissues 1 month post-exposure; this experiment will include 25 control brains. Khan will perform Monte Carlo simulations to provide detailed dose calculations for animal brains. Stock will use a laboratory-based X-ray computed tomography (LXCT) instrument to evaluate the vasculature in beagle FFPE brain tissues. De Carlo will use the synchrotron-based X-ray computed tomography (sXCT) instrument developed by his team for 3D phase contrast X-ray imaging of rodent brains with 1 μm volume elements. Beagle brain vasculature 3D maps from LXCT and sXCT will be examined by De Carlo and Stock with support from Iruela-Arispe (Northwestern University), who is an expert on vessel abnormalities.

The focus will be on detecting "sprouting angiogenesis" to select subsections of FFPE samples of interest for further studies. Next steps of sample processing will include optical imaging and pathology evaluation by Paunesku, Gürsel, and Horbinski, who have worked together on elemental imaging of human brain samples in the past (Kumthekar et al. 2021); Jin will assist in this effort as well. Schaue will assess these samples from a radiobiology–immunology perspective, focusing on presence and distribution of macrophages and their possible roles in new angiogenesis.

The samples and sample portions with the most promising pathology features will be "reformatted" to fit different X-ray spectroscopy instruments at the Advanced Photon Source (APS). These samples will be subjected to elementalomic evaluation with support from and in collaboration with APS investigators (Lai, Chen, Antipova, and Jin) from Sectors 8 and 2 beamlines (8-BM, 2-ID-D, 2-ID-E, and BioNanoprobe). Elementalomic changes in brain samples will be evaluated with special attention to copper—an element critically involved in angiogenesis as shown in earlier 2-dimensional (2D) elemental studies by team members (e.g., Finney et al. 2007; Qin et al. 2011; Heuberger et al. 2019). MicroRNAs (miRNAs) in archival tissues will be studied using spatial transcriptomics resources at Northwestern University in collaboration with Wai. The Xenium *In Situ* System from 10x Genomics will be used to evaluate archival samples, while new mouse samples will be run on both Visium and Xenium *in situ* systems. Patel and Sun will conduct statistical data analyses for this project, including miRNA transcriptomics data. Spatial transcriptomic data from archival and fresh tissues will be analyzed with the primary focus on miRNAs and gene expression pathways that correlate with angiogenesis, vascular endothelial cell activity, and copper utilization, as well as macrophage activity.

Artificial intelligence and machine learning work led by Cherukara will primarily support the extensive computational demands of data acquisition, 2D and 3-dimensional (3D) image reconstruction, and image correlations. In addition, this team and the Northwestern University bioinformatics and statistics team will collaborate to analyze and correlate the complete data, including spatial miRNA expression, elementalomic information in 2D and 3D, micro-computed tomography information, and histopathology information. This work will provide a roadmap for characterizing vascular sprouting anomalies in the context of radiation-induced brain complications found in archival canine samples.

Potential Impact: New computational tools will be developed to support high-dimensional data acquisition and processing, enabling linkage of X-ray–generated 2D and 3D images with data from visible light imaging and spatial transcriptomics at the level of single cells. This work will deepen understanding of the effects of low-dose radiation exposures, inspire development of new research pipelines, and generate a wealth of data for future analyses.

Understanding the Mechanism and Health Consequences of Low-Dose Radiation at a Molecular Level

Principal Investigator: Edward Snell
(Hauptman Woodward Medical Research Institute, Inc.)

Low-dose and low-dose-rate radiation effects on human health outcomes and the biological mechanisms of these effects are not fully understood, but concerns exist that such exposure could affect human health. Structural biology techniques—primarily X-ray crystallography and, more recently, cryo-electron microscopy—have overwhelmingly impacted key molecular-level discoveries in health. However, these techniques use ionizing radiation or particles which produce high-dose radiation chemistry processes that effectively mask any zero-dose state. This study aims to model molecular-level low-dose radiation chemistry, offering insights into its systemic biological impacts. Leveraging a combination of classical machine learning (ML) and recent advances in generative artificial intelligence (AI), the research team will analyze biological model data across varying dose extremes, integrating additional data and theoretical findings related to low-dose impacts from radiation chemistry literature. Experimental phases will employ engineered proteins to illuminate radiation's structural impacts at low doses, enabling refinement of ML models and validation of computational predictions.

The objective is innovative, using ML to develop an AI model to understand and predict low-dose radiation health effects at the molecular level and an experimental testing and validation approach to assess confidence in that model.

Existing but disparate data will be combined with both experimental and computational techniques to build the necessary database. Using ML to aid development of an AI model, the team will obtain an understanding and prediction of the health-related impacts of low-dose (10 to 100 milligrays) radiation. This understanding of absorbed dose will then be extended to dose-rate studies. The team will incorporate an experimental feedback loop for both dose and dose-rate analysis to test, refine, and validate predictions, providing a measure of confidence in understanding. The output will be structural models predicting low-dose damage at residue and, potentially, atom-specific levels.

The team will build a training set incorporating experimentally derived structural information from the Protein Data Bank and link it to an X-ray dose or dose-estimate derived from perturbations observed in the information. Simultaneously, expert literature associated with amino acid and whole protein radiation chemistry across a range of X-ray doses will be selected and built into the training set.

The team will then apply ML and AI to make predictions of low-dose impact. The training set will be used to develop a computational system that can predict low-dose radiation impact on proteins of interest at a molecular resolution sufficient to provide mechanistic understanding.

Low-dose effects will be probed experimentally, and the computational model validated. Two approaches will be pursued: (1) engineer proteins that undergo large structural changes with radical attack on specific residues and (2) in parallel, produce samples of proteins that are computationally predicted to be easily damaged to provide more experimental knowledge for the computational system. Low-dose structural approaches will be used to study these samples to improve and validate the model's predictive capability. This will be coupled with zero-dose neutron diffraction and high-dose X-ray diffraction to help understand structural consequences at the residue level.

Key findings of the National Academies' 2022 consensus study report, "Leveraging Advances in Modern Science to Revitalize Low-Dose Radiation Research in the United States," were that "radiation biology studies have contributed to the mechanistic understanding of the effects of radiation on molecular pathways and intra- and extracellular processes," and that "the application of novel and developing technologies will enable more precise definition of the cellular and molecular processes that are affected by low-dose and low-dose-rate exposures." This definition will be achieved by understanding these processes at the molecular level.

The benefits and outcomes of this project include a training set allowing ML and AI to be applied to understanding low-dose health impacts, development of a tested and validated AI model, and prediction of human proteins likely to impact health when exposed to low-dose radiation. Outcomes are expected to be predictable at the residue level, presented in a manner readable by common display software, and interpretable by a wide scientific community. The results will create experimentally testable hypotheses and provide a mechanistic understanding of the effects of low-dose radiation on molecular pathways that can then be related to clinical observations.

This interdisciplinary approach promises to uncover previously hidden health outcomes of low-dose radiation exposure, guiding future research to enhance health outcomes in this critical area.