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MEETING REPORT

Advanced Technologies in Radiation Research

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The U.S. Government is committed to maintaining a robust research program that supports a portfolio of scientific experts who are investigating the biological effects of radiation exposure. On August 17 and 18, 2023, the Radiation and Nuclear Countermeasures Program, within the National Institute of Allergy and Infectious Diseases, National Institutes of Health (NIH), partnered with the National Cancer Institute, NIH, the National Aeronautics and Space Administration, and the Radiation Injury Treatment Network to convene a workshop titled, Advanced Technologies in Radiation Research (ATRR), which focused on the use of advanced technologies under development or in current use to accelerate radiation research. This meeting report provides a comprehensive overview of the research presented at the workshop, which included an assembly of subject matter experts from government, industry, and academia. Topics discussed during the workshop included assessments of acute and delayed effects of radiation exposure using modalities such as clustered regularly interspaced short palindromic repeats (CRISPR) – based gene editing, tissue chips, advanced computing, artificial intelligence, and immersive imaging techniques. These approaches are being applied to develop products to diagnose and treat radiation injury to the bone marrow, skin, lung, and gastrointestinal tract, among other tissues. The overarching goal of the workshop was to provide an opportunity for the radiation research community to come together to assess the technological landscape through sharing of data, methodologies, and challenges, followed by a guided discussion with all participants. Ultimately, the organizers hope that the radiation research community will benefit from the workshop and seek solutions to scientific questions that remain unaddressed. Understanding existing research gaps and harnessing new or re-imagined tools and methods will allow for the design of studies to advance medical products along the critical path to U.S. Food and Drug Administration approval.

INTRODUCTION

The responsibility for developing safe and efficacious products to diagnose and treat radiation injuries relies on strong collaborations that have been formed between multiple U.S. Government agencies and the scientific community. These partners are engaged in a common mission: to accelerate development of technological approaches to accelerate medical product development in the radiation space and beyond. Recently, there have been dramatic advances in the technologies available to researchers across all scientific areas, and many investigators in radiation biology have been at the forefront of capitalizing on cutting-edge modalities such as clustered regularly interspaced short palindromic repeats (CRISPR) – based gene editing, tissue chips, advanced computing, artificial intelligence (AI), and immersive imaging techniques. Because these technologies are just now gaining traction and represent areas for potentially explosive growth over the next few years, four organizations came together to host a meeting to combine in-house expertise in their respective fields with subject matter experts from their communities to participate as presenters at a workshop on Advanced Technologies in Radiation Research (ATRR), held in Rockville, MD August 17–18, 2023. This hybrid meeting offered both an in-person experience and a virtual option. The workshop was well attended, with nearly 100 attendees present in person and more than 250 remote participants.

This meeting report summarizes discussions and key insights from the ATRR workshop. Twenty speakers (Table 1) presented across four scientific sessions that included microphysiological systems and chip technologies, novel imaging modalities, computational methods using AI and machine

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TABLE 1
Workshop Speakers and Areas of Expertise[a]

Name	Affiliation	Areas of expertise
Sally Amundson, PhD	Columbia University, New York, NY	Functional genomics, low-dose radiation biodosimetry, space radiation, heavy ion radiotherapy
Molykutty Aryankalayil, PhD	NCI, Bethesda, MD	Radiation oncology, microfluidics, liver-on-a-chip models, coding/non-coding RNAs
Afshin Beheshti, PhD	NASA Ames Research Center, Moffett Field, CA	Space radiation biology, microRNA cancer immunotherapies, computational biology
David Chen, PhD	OCICB[b], NIAID, Rockville, MD	Computer science, 3D visualization, medical image analysis, virtual and augmented reality (SimpleITK)
David Chou, MD, PhD	Wyss Institute, Boston, MA	Bone marrow chip-model, pharmacokinetics, multilineage hematopoietic development
Shaheen Dewji, PhD	Georgia Institute of Technology, Atlanta, GA	Monte Carlo radiation transport, nuclear material assays, AI, internal dosimetry, high-fidelity 3D CT
Asim Ejaz, PhD	University of Pittsburgh, Pittsburgh, PA	Retrovirus-specific T-cells, soft tissue engineering, adipose tissue biology, radiation-induced fibrosis
Svend Engelholm, MD	Meabco Inc., Copenhagen, Denmark	Therapeutic radiation oncology, stereotactic cranial radiotherapy, cutaneous radiation injuries
Gregory Holmes-Hampton, PhD	AFRRI, Bethesda, MD	Bioinorganic chemistry, cutaneous radiation injury, acute radiation syndrome
Espoir Kyubwa, MD, PhD	ChromoLogic LLC, Monrovia, CA	Biological neural networks, computer vision algorithms, machine learning, in silico DEARE[c]
Philip Low, PhD	Purdue University, West Lafayette, IN	Ligand-targeting, folate receptors, miRNA/siRNA constructs, drug pharmacodynamic optimization
Ceferino Obcemea, PhD	NCI, Bethesda, MD	Medical physics, AI radiotherapy, Big Data analytics, McCulloch-Pitts perceptron neural nets
Ileana Pazos, PhD	NIST, Gaithersburg, MD	High-dose radiation dosimetry, electron paramagnetic resonance
Shahin Rafii, MD	Weill Cornell Medicine, New York, NY	Hematology-oncology, vascular & stem cell biology, endothelial organotypic development
Rahim Rizi, PhD	University of Pennsylvania, Philadelphia, PA	Hyperpolarized gas, liquid MRI, lung metabolism and functionalities, pulmonary disorders
Daniel Naveed Tavakol, PhD	Columbia University, New York, NY	Biomedical engineering, tissue engineering, stem cells, multi organ-on-a-chip systems
Jan-Peter van Pijkeren, PhD	University of Wisconsin, Madison, WI	Genome editing, GI tract studies via *L. reuteri*, recombinant protein engineering
Harris Wang, PhD	Columbia University, New York, NY	Gut microbiome, mammalian host genomics, GI-ARS, CRISPR epigenetics
Gayle Woloschak, PhD	Northwestern University, Chicago, IL	Radiation molecular biology, nanotechnology, elementalomics, X-ray fluorescence
Joseph Wu, MD, PhD	Stanford Cardiovascular Institute, Stanford, CA	Cardiovascular medicine, induced pluripotent stem cells, precision medicine

[a] All speakers had the opportunity to review this meeting report prior to journal submission.
[b] Office of Cyber Infrastructure and Computational Biology.
[c] Delayed effect of acute radiation exposure.

learning (ML), and applications of these approaches (Table 2). This report is intended to serve as a resource for researchers and clinicians interested in radiation biology and medicine, as well as policymakers and other stakeholders in the radiological emergency preparedness and response domain.

BACKGROUND

Missions of the Workshop Planning Organizations

Since 2004, the Radiation and Nuclear Countermeasures Program (RNCP) within the National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH) has spearheaded early through advanced development of radiation medical countermeasures (MCMs) to be employed to diagnose, mitigate, and treat civilian populations during a radiological or nuclear incident. The RNCP accomplishes this goal by supporting a broad portfolio that includes all levels of research – basic to advanced – focused on developing MCMs to diagnose, mitigate, and treat radiation injuries. The RNCP works closely with federal agency partners to stay abreast of research developments and to collaborate in areas common to our mission space. One such partner is the National Cancer Institute (NCI). The NCI, also within NIH, is recognized for leading

TABLE 2
Topics Addressed During the Meeting Discussion Sessions

Session discussion topics
Session I: Microphysiological systems (MPS)/chip technologies
With multiple licensed products for hematopoietic acute radiation syndrome (H-ARS), there remains an unmet need for approved products to address other injuries impacting other organ systems after acute radiation exposure.
What new concepts and/or approaches will need to be considered to develop and expand the field?
What common pathophysiologic mechanisms can be modeled from the clinical/pre-clinical settings to MPS?
What qualifiers should be required to establish that a MPS recapitulates organ or multi-organ physiology and biological responses?
What are the baseline standards that should be implemented in MPS research?
What approaches are considered state of the art in MPS research that are applicable to assessing radiation injury, and what treatment approaches are being tested in MPS?
How can MPS systems be used to generate data relevant to total-body or partial-body irradiation to accurately model exposures to animals/humans? How might information from MPS be incorporated into follow-on animal model studies?
Session II: Novel imaging modalities
There are limitations to studying the natural history of cutaneous radiation injuries (CRI). Current tools for developing the injuries in animal models that are reflective of the human condition and evaluating wound etiology over time are often constrained by human bias.
What approaches show damage and recovery in models of CRI that are reproducible, quantitative, non-biased and translatable to the clinic?
How can we define/distinguish CRI from other ARS sequelae, e.g., H-ARS? How intertwined/interdependent are H-ARS and CRI? Are they sufficiently distinct to be assessed independently, or are they a continuum?
How does this affect practical implementation of CRI medical countermeasures (MCMs) and model development?
Evaluation of radiation injuries are also subject to limitations that include the delayed nature of the injury in some tissues and difficulty in observing temporal changes in tissue from injury onset to resolution.
Are there imaging techniques currently available, or in development, that can be used longitudinally to investigate/quantify the natural history of acute radiation injuries and DEARE?
Are in vitro/in vivo models currently used in studying delayed effects of radiation exposure such as lung, heart, and kidney injury, adequate to support development of advanced imaging technologies?
Are there imaging techniques currently available, or being developed, that can be used in long-duration studies to assess MCM efficacy for injuries in tissues such as lung?
Session III: Computational methods: artificial intelligence and machine learning
Are current computational modeling technologies sufficiently developed to accurately model systemic physiological responses during ARS and/or late effects?
If accurate modeling at the systemic level of physiological response is currently out of reach, can organ-level physiological responses be accurately modeled?
Would such modeling be capable of providing organ-level or organism-level prognostic estimates of radiation exposure acute effects and/or long-term outcomes?
Could empirical data, obtained from organ-centric MPS technologies, be incorporated into computational modeling systems? Would such an approach enhance the robustness and predictive capabilities of computational approaches currently in development?
What other types of wet-lab experimental data would best inform and enhance development of computational approaches for understanding the biological effects of radiation exposure?
What is/are currently the most powerful and productive area(s) for application of computational approaches in radiation biology; physical dosimetry, biodosimetry, physical modeling, signaling pathway response modeling, etc.?
Session IV: Applications of advanced technologies
Are current model systems sufficiently versatile to support continued development of the advanced technologies being presented in this session, and will they be appropriate to support eventual submissions to the U. S. FDA in those cases involving development of MCMs?
What technical challenges need to be overcome to continue to advance development of the technologies presented?
Are there any unique regulatory challenges that may need to be overcome to successfully develop these technologies into MCMs or commercial products?

the nation's research efforts to improve cancer prevention, detection, diagnosis, and treatment. This research includes developing approaches to reduce injuries resulting from radiation therapy, where there is a delicate balance between eliminating tumor cells and protecting normal tissues (*1*).

Another agency partner is the National Aeronautics and Space Administration (NASA). Similar to NCI, NASA has included discovery and development of radioprotectors and mitigators in their agency mission, although for protection of astronauts during space missions. Astronauts exposed to low doses of high linear energy transfer (LET) radiation for

extended durations are at risk of biological injury. Potential biological outcomes from this chronic high-LET radiation exposure overlap with the late effects of acute radiation exposure. Understanding the effects of radiation on biological tissue during space flight and developing approaches to mitigate the effects are of particular importance to NASA, as the agency plans extended missions beyond low-Earth orbit to the Moon and Mars.

One non-governmental partner on the workshop organizing team is the Radiation Injury and Treatment Network (RITN). RITN comprises a national network of medical centers, with an expertise in managing acute radiation syndrome (ARS) and its health-related consequences. Established in 2006, RITN is a cooperative effort of the National Marrow Donor Program and the American Society for Transplantation and Cellular Therapy. RITN is recognized for having developed treatment guidelines and standard operating procedures for centers involved in the response to large-scale radiologic incidents, with an aim of translating research from the benchtop into practice in the field.

MEETING OVERVIEW

The content of the talks presented during the workshop and an overview of the moderated discussions that followed each session are summarized below. Many scientific findings within the report include referenced sources; however, in-depth details of any pre-publication information discussed during the meeting are not provided, and when discussed, findings are cited by the presenter's first initial and last name. All speakers had the opportunity to review this manuscript for accuracy before submission.

SESSION I: MICROPHYSIOLOGICAL SYSTEMS AND CHIP TECHNOLOGIES

The U.S. Food and Drug Administration (FDA) Modernization Act 2.0[2], signed into law on December 29, 2022, has ushered in a new era of technological approaches to drug discovery. The bill authorizes use of alternative testing platforms and could reduce the need for some animal testing in preclinical safety and efficacy studies. The bill is timely, but the science that alternative testing platforms are built upon is not new. The ex vivo study of organ physiology began in the 1930s with the design of the first perfusion pump (*2*), which provided a pulsative flow of oxygenated liquid to maintain thyroid viability for up to three weeks (*3*). This research has significantly advanced during the last two decades, from the ability to form organoids from clusters of cells in a three-dimensional (3D) cell culture (*4*) and the creation of tissues-on-a-chip (*5–7*) consisting of a single cell type/tissue to the development of more complex microphysiological systems (MPS). An organ-on-a-chip, a subset of MPS, is a model of a cross-section of a functional unit within an organ. A lung-on-a-chip was one of the first MPS described in the literature (*8*, *9*). Leaps in technology in the ability to re-program adult human somatic cells to produce induced pluripotent stem cells (iPSC) and the establishment of protocols to differentiate the iPSC into different types of cells that form specific organs (*10*, *11*) advanced the development of different types of MPS. MPS, using iPSCs from human patients, further the possibilities of personalized medicine and the development of MCMs to combat exposure to radiation from nuclear/radiological incidents, therapeutic applications, or space travel (*12*). Session I included discussions of single and multi-organs-on-a-chip for the heart, bone marrow, tissue-specific vascular systems, and liver. As described below, speakers discussed using MPS for drug development and assessing radiation toxicities and MCM efficacy (Fig. 1).

Assessing long-term effects of radiation exposure in engineered heart and vascular tissues. Exposure to ionizing radiation is a less understood risk associated with spaceflight in deep space. An increase in planned space missions demands a clearer understanding of the lifetime effects of radiation exposure on susceptible organs such as the heart. Genetics, lifestyle, and environmental stressors, including radiation, are factors associated with cardiovascular disease (CVD) - the leading cause of morbidity and mortality worldwide (*13*, *14*). The risk of CVD due to radiation exposure from spaceflight will become more concerning for astronauts on long-duration missions, a scenario challenging to model in the laboratory setting. This is where engineered 3D tissues can play a critical role. Primary cells, such as human umbilical vein endothelial cells (HUVEC) and human aortic endothelial cells (HAEC), have a limited cell lifespan and are not applicable for long-term disease modeling. Animal models using mice and rats are also limited due to different cardiovascular physiology, such as heart rate and electrophysiology (*15*). There is also a discordant gene response after radiation exposure (*16*). To this end, engineered cellular platforms using soft lithography with microfluidics and controllable functional units are rapidly emerging as promising alternatives to animal models (*17*).

Understanding radiation dose and dose-rate effective factor values for tissue-specific endpoints across different tissue types has long been of interest to the radiation research community. The advent of 3D iPSC-based tissue platforms to monitor long-term effects of radiation exposure will facilitate the deployment of countermeasures in the medical realm. Additionally, the essential structural and functional features needed in an engineered tissue platform can be defined by the question being asked in a particular study. Studies that aim to model subtle physiological changes, such as biomarker discovery (e.g., transcriptome, proteome, epigenome, secretome), may benefit from 3D platforms that recapitulate more mature and complex tissues. Alternatively, a two-dimensional (2D) monolayer may be more suitable for high-throughput, early drug discovery studies; however, merging of 2D throughput and 3D

[2] https://www.govinfo.gov/app/details/BILLS-117s5002cps.

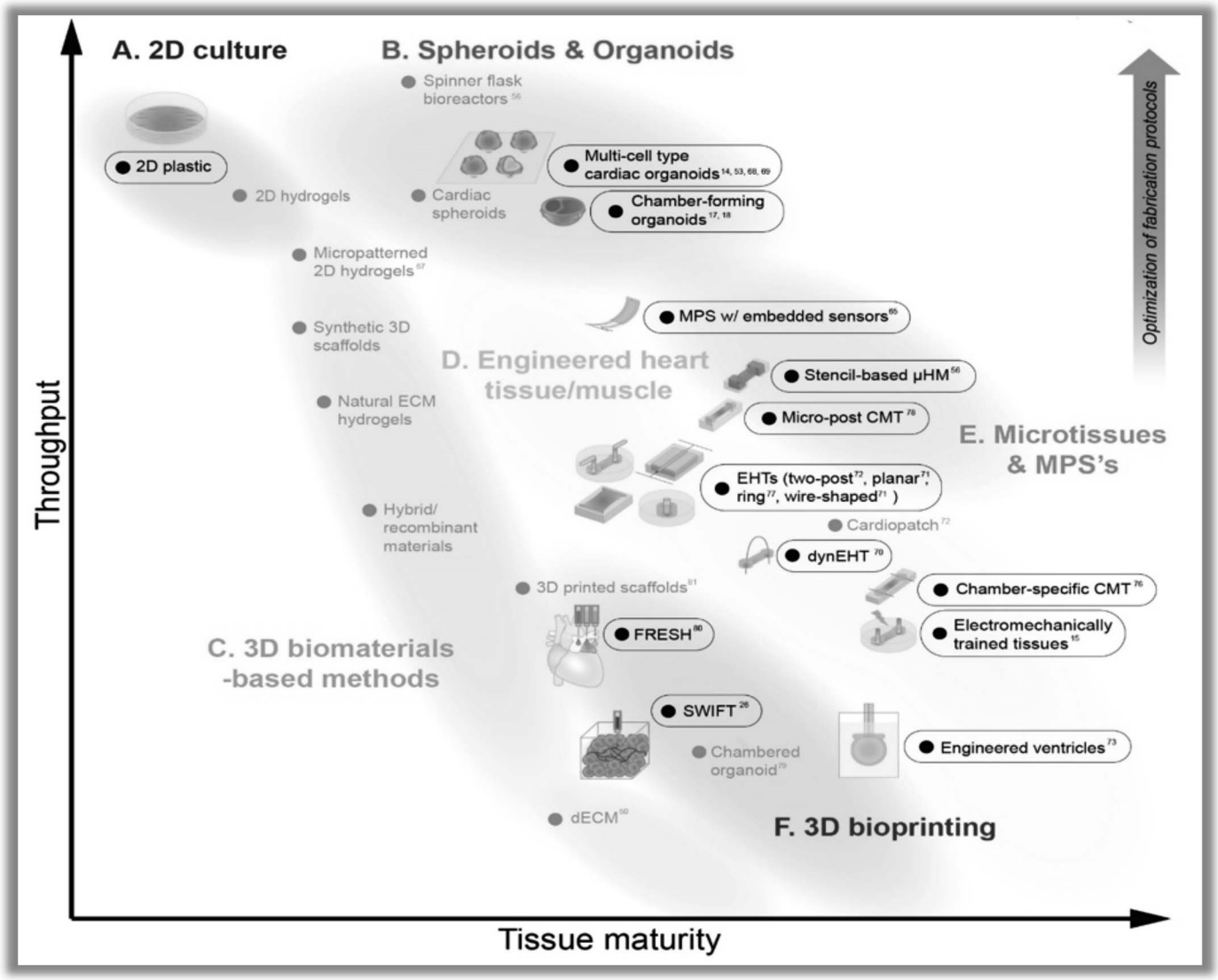

FIG. 1. U.S. FDA Modernization Act 2.0 Paves the Way for "Clinical Trials in a Dish" for Radiation-Induced Injuries. Figure used with permission: Cho, S., Discher, D.E., Leong, K.W. et al. Challenges and opportunities for the next generation of cardiovascular tissue engineering. Nat Methods 19, 1064–1071 (2022).

maturity capabilities will require balancing the necessary quality controls with continual refinement of platform manufacturing (*18*).

Functional 3D heart and vascular tissues can be engineered using iPSC, allowing for a more accurate simulation of the cardiovascular system ex vivo (J. Wu). Using an array of genetically diverse human iPSC lines, the effects of X-ray radiation on tissue integrity and function have been studied in iPSC-engineered heart (cardiomyocytes, endothelial cells, and cardiac fibroblasts) and vascular (endothelial and smooth muscle cells, and macrophages) tissues. These systems allow for real-time and functional analyses such as contractility and vessel integrity under the duress of radiation exposure. Supported by a NIAID/NASA contract, 3D heart and vascular tissues are being studied over 6-months postirradiation. The project includes the construction of cardiac and vascular tissues from genetically diverse iPSC lines after optimization of culture conditions to stabilize long-term cardio- and vasculogenesis. Cell lines pooled from many patients enable the possibility of conducting "clinical trials in a dish" that can recreate natural human variability for drug safety and efficacy testing. Together with the implementation of advanced computational approaches such as AI and ML, complex molecular dynamics can be simulated and interrogated within cellular constructs (J. Wu). In theory, in vitro efficacy data can steer drug development toward a more effective and efficient process that reduces the number of clinical studies needed and expedites the time a drug moves along the critical path to approval. This streamlined drug development process would ultimately enable more clinical indications to be addressed and a larger patient population to be reached.

Astronaut-on-a-chip: Human multi-tissue platform to study effects of space radiation and countermeasures. Space radiation is composed of galactic cosmic rays (GCR), which

originate from outside our solar system, the solar wind, and solar particle events (*19*). On Earth, the magnetosphere protects us from most of the space radiation, and the background radiation from the Earth can vary depending on the composition of the location, but the worldwide average is 2.4 mSv/year. On the International Space Station in low-Earth orbit, the dose rate is 110–180 mSv/year, as there is some protection from the Earth's magnetic fields. However, as astronauts venture further into space, the dose rate will increase to 110–300 mSv/year on the Moon and 130–260 mSv/year on Mars. Since solid tumor induction may occur at effective doses as low as 200 mSv (*20*) and radiation health effects for astronauts include but are not limited to accelerated aging of tissues, an increased risk of cardiovascular disease, cancer, and neurodegeneration (*21*, *22*), radiation countermeasures are needed to protect astronauts.

Current pre-clinical models such as 2D cell culture and animals do not recapitulate human tissue complexity and phenotype. Drugs tested in animal models often fail in human clinical trials (*23*, *24*), yet multi-organs-on-a-chip can be used to understand the effect of a single variable in a complex multicellular system. Using iPSCs allows different tissues to be person-specific, and a single chip has been developed to stably emulate liver, bone, heart, and skin (*25*) from a combination of primary and iPSC-derived cells, connected for over four weeks. On the chip, tissues are maintained in a controlled environment providing a specific medium, which was discussed earlier in the session. In this case, tissues are connected through a vascular channel lined with endothelial cells, allowing signals to travel from one type of tissue to another to better mimic the physiological system of a human.

Work has also been performed with a chip containing heart and bone marrow tissues, each in isolation, to examine the effects of neutron and photon radiation (*26*). Neutrons were used to simulate the high-LET component of the GCR spectrum, as they are relevant to space travel, and neutrons are generated as secondary radiation when GCR hits the surface of the Moon or spaceship shielding (*19*). Heart tissue in this chip was engineered from human iPSC-derived cardiomyocytes and primary fibroblasts in fibrin hydrogels, attached to two flexible pillars. Forming tissues were matured by electromechanical stimulation to a more adult-like tissue (*19*). Contractile function of the heart tissue was evaluated by quantitative analysis of videos of the beating tissues. The bone marrow component of the chip was engineered from human iPSC-derived mesenchymal stem/stromal cells (iMSC) infused into decellularized bone structure to first form a bone niche. Human umbilical cord endothelial cells and iMSCs were then added to induce vascularization, and finally, primary $CD34^+$ hematopoietic cells were added. Irradiation of the heart tissue with 4 Gy photons or 1 Gy neutrons decreased the heart beating frequency. The neutron radiation also significantly increased the excitation threshold and the contraction velocity. These findings suggest early hypertrophy of the muscle after irradiation, consistent with increased expression of hypertrophy-related genes (*26*). In bone marrow, there was a dose-dependent decrease in $CD45^+$ blood cell number after exposures to photon and neutron radiation, suggesting reduced cell proliferation, with greater effect of neutron relative to photon radiation. After irradiation, single-cell sequencing revealed skewing towards myeloid cell types. When differentially expressed genes for irradiated and non-irradiated tissues were examined, there were more significant changes in gene expression for bone marrow tissues exposed to neutrons as compared to photons. There were also expected radiation-related changes in gene expression between the two unique tissue types (bone marrow and heart), again to a greater extent in tissues subjected to neutron radiation.

Known radioprotective agents were also tested in this study: amifostine was found to rescue increases in excitation threshold and contraction velocity seen in the heart tissue after neutron treatment, and G-CSF was found to increase $CD45^+$ hematopoietic cells in the bone marrow after photon irradiation to levels higher than those seen in non-irradiated controls. These findings helped validate the chip, given that these compounds are known to be radioprotective in the clinic.

Recent work has used a liver-bone marrow-heart multi-organ chip to compare effects of acute and chronic neutron doses (D. N. Tavakol). Again, there was skewing of the bone marrow cell population towards myeloid lineages, with transcriptomic changes that were different for the acute and protracted treatments and non-radiated controls. Expression of genes involved in oxidative stress were increased, whereas expression of cell cycle, replication, and DNA repair genes was decreased in cells that underwent acute treatment relatively to the 2-week treatment with the same cumulative dose. Ongoing studies are investigating effects of simulated GCR, and use of nanoparticles carrying G-CSF as a potential long-term countermeasure. There are also plans to individualize studies using iPSCs from a single donor to generate all tissues in the chip, allowing the study of differences due to biological sex and genetic backgrounds in radiation responses. Finally, iPSCs generated from astronaut donors may be employed to inform potential detrimental effects of long-term space travel before and after spaceflight.

Mitigation of radiation-induced tissue-specific vasculature damage by transplantation of organotypic endothelial cells. As mentioned above, 2D monolayers suit high-throughput drug screening; however, they do not fully replicate the complexity of a 3D cellular system. Missing from the 2D format are blood vessels, which serve as a critical and dynamic interface between circulation and the organ environment. Blood vessels exert control over tissue homeostasis and its ability to adapt to pathological insults. Differentiated, organ-specific endothelial cells are the building blocks that create a monolayer lining blood vessels. They

can adopt characteristics to instruct organ development and regeneration as well as mediate vascular function (*27*). Work is underway on unconstrained 3D matrices composed of a mixture of laminin, entactin, and type-IV collagen; with reprogrammed adult endothelial cells termed "reset" vascular endothelial cells (R-VECs) (S. Rafii). Human adult endothelium is transiently transduced with a master regulator of fetal development, ETV2, that sets the development of all endothelial cells in motion and is normally switched off in the adult endothelium. R-VECs, unlike cells applied to a bioprinted scaffold, can self-assemble becoming stable, multilayered, tubulogenic perfusable vessels within scalable microfluidic chambers, capable of transporting human blood – termed "Organ-on-VascularNet". R-VECs have been shown to undergo tissue-specific "education" when implanted subcutaneously in mice. When co-cultured with 3D organoids, R-VECs directly interact with the cells, including numerous membrane-bound growth factors. Within 7 days, R-VECs co-cultured with normal colon organoids quickly acquired an intestinal-derived molecular profile (*28*).

Organ vasculature is tissue-specific and differentially sensitive to radiation. In the vascular niche, radiation induces angiocrine growth factor dysfunction, endothelial to mesenchymal transition (EndoMT), fibrosis, and premature senescence, which can be captured using 3D vascularized organoids but not 2D vascular monolayers (S. Rafii). Vast endothelial cells heterogeneity exists across organs, as well as inter- and intra-organ. Factors differentially impacted by radiation include coagulation, vasomotor, metabolic, inflammatory/immune, angiogenic, angiocrine, and permeability functions. Radiation exposure early in development of the endothelium can disrupt angiogenic sprouting, resulting in vessel regression. Interestingly, the R-VEC-perfused vascular network is stable and can maintain its vascular integrity, such as vessel area and tight junction structure, after doses of radiation as high as 160 Gy. However, after perfusion with CellTracker-labeled peripheral blood in the presence of 50% red blood cells, the R-VEC VascularNet exhibited increased inflammation after 40 Gy irradiation. Investigation of the signaling pathways preventing vascular regression and promoting expedited vascular DNA repair in the VascularNet model have pointed to Notch ligand, Delta-like 4 (DLL4), and BMP-TGF-beta (Lrg1). After irradiation (40 Gy), TGF-beta inhibition provokes regression of the vascular network, with decreasing vessel area, tight junctions, and length. Notch inhibition does not have the same detrimental effect. Thus, it was hypothesized that after irradiation TGF-beta activation through DNA repair promotes vascular regeneration. Single-cell analysis showed changes in gene expression 3 days postirradiation (8 and 40 Gy).

The landscape of vascular therapeutics currently in development is rapidly growing. Tissue-specific stem cells within a vascular niche platform can be expanded for a more targeted treatment of endothelial radiation injury. Vascularized, implantable mini-organs can also be used for a wide range of pathologies, including diabetes, heart disease, stroke, and degenerative disorders. Further, intravenous infusion of endothelial cells for multiorgan injury is underway. In C57BL/6J mice transplanted with mouse endothelial cells for four successive days after 7 Gy, hematopoietic and intestinal crypt recovery was restored (*29*). In humans, generic allogeneic endothelial cells infusion is under study in a Phase 3, U.S. multi-center, clinical trial to assess effects in patients with lymphoma autologous hematopoietic cell transplantation (NCT05181540). Approaches taken to understand the intricacies of tissue-specific endothelium are already leading to new paradigms in vascular physiology and radiation and will advance development of organotypic radiation therapeutics in the future.

Radiation injury in the bone marrow chip and ongoing countermeasure discovery efforts. The bone marrow is the primary site of hematopoiesis, which through differentiation of hematopoietic stem cells (HSCs), produces erythrocytes, megakaryocytes, B and T lymphocytes, monocytes, eosinophils, neutrophils, and dendritic cells (*30*). Therefore, bone marrow is critical to immune function and blood cell production. Additionally, HSCs and different types of differentiated cells derived from HSCs have different radiosensitivity (*31*), thus changes to the cells in the bone marrow need to be studied after irradiation. A novel bone marrow, two-compartment microfluidic chip, with a top hematopoietic channel and a lower vascular channel separated by a porous flexible membrane, has been described (*32*). The top hematopoietic channel of the microfluidic chip is filled with a 3D co-culture of human bone marrow stromal cells and $CD34^+$ hematopoietic progenitor cells in a fibrin gel. The lower vascular channel is lined with human umbilical vascular endothelial cells (HUVECs) to simulate a blood vessel. Perfusion of the vascular channel with medium is achieved by specialized hardware (Emulate, Inc) or a peristaltic pump, and the membrane allows communication and the flow of nutrients between the two compartments. The result is an environment similar to the bone marrow where hematopoietic parenchyma, containing growing and differentiating blood cells, is perfused by a rich vascular plexus.

Comparison of cell types in the hematopoietic channel to human bone marrow has been done using flow cytometry to monitor cell surface markers. Detection of CD13 and CD16, markers of neutrophil differentiation (*28*), allowed for identification of different stages of neutrophil maturation, in which similar patterns of maturation were observed in the hematopoietic channel of the bone marrow chip and in ex vivo human bone marrow. Similarly, markers of erythroid cells allowed for tracking of their maturation in the chip (*33*), which also showed the same maturation profile in the chip and ex vivo marrow.

Radiation injury studies using cells from 5 different donors to produce bone marrow chips demonstrated reproducible dose-dependent cell killing from 0 to 2 Gy, with the $CD34^+$ cells being more sensitive than the total cell

population. Dose-dependent cell killing was also seen with the neutrophils and the erythroid cells, and the immature cells were again more radiosensitive than the mature population. To test whether bone marrow chips can be used to measure the efficacy of radiation countermeasures, bone marrow chips were treated with G-CSF for nine days post-irradiation (2 Gy) at GCSF concentrations approximating those observed in human plasma with clinically relevant dosing. Neutrophil recovery was accelerated while $CD34^+$ progenitor toxicity was unaffected, thus validating the chip model for radiomitigator identification. A higher-throughput screening assay based on suspension culture of hematopoietic cells in a 96-well format has also been developed and piloted with a commercially available drug library (D. Chou). The screen has identified several possible radiation mitigating compounds that are undergoing validation testing in the bone marrow chip.

Development of an organ-on-a-chip lung and liver model: RNA biomarkers to study normal tissue radiation injury. Alterations to the cell/tissue mRNA identified by transcriptome studies or non-coding RNA (ncRNA) biomarkers can reveal how tissue responds to injury. Since approximately 76% of the human genome is transcribed, and only ~2.5% of the transcribed DNA corresponds to protein-encoding mRNA, ncRNA is abundant in the cell (*34*). ncRNAs have many functions in cells due to the ncRNAs binding to DNA, protein, or mRNA, including cellular stress responses (*35*, *36*). The types of ncRNA relevant for normal tissue injury after exposure to ionizing radiation are miRNAs, long ncRNAs, and circRNAs (*35*). Long ncRNAs are greater than 200 nucleotides in length. Some known functions include altering the epigenome (*37*) and regulating DNA double-strand break repair, apoptosis, glycolysis, and signaling pathways (*38*). circRNAs are a form of long ncRNAs that can be produced during the splicing of transcripts and are covalently closed circles of single-stranded RNA (*38*). miRNAs are less than 22 nucleotides long and are single-stranded. They inhibit translation or decrease mRNA stability and can also alter radiosensitivity by modulating the cell cycle, double-strand break repair, autophagy, apoptosis, glycolysis, and signaling pathways (*38*).

Validation of a liver chip under development involves comparing transcriptome findings from the MPS with results from previous animal/cell culture experiments (M. Aryankalayil). The Emulate liver chip system (Emulate Bio, Boston, MA) was used in these studies, and it is possible to obtain circulating stable RNA in the perfusion solution and perform gene expression analysis and single-cell sequencing from the liver chip. Therefore, it will be a useful tool to perform transcriptome studies and study the circulating RNAs, as can be done in animals and humans. One type of chip studied after irradiation was a co-culture of human liver sinusoidal endothelial cells and hepatocytes, with the cell layers separated by a membrane coated with extracellular matrix (M. Aryankalayil). The liver chips were irradiated with 1, 4, or 10 Gy and RNA isolated from hepatocytes or endothelial cells at various time points after irradiation. For both the hepatocytes and endothelial cells, CDKN1A expression increased at all time points, especially after 10 Gy. CDKN1A encodes p21 and is critical to the down-regulation of cell cycle genes after DNA damage induction and p53 activation (*39*). Other prominent increases in gene expression in the hepatocytes included PCNA, MDM2, and PHLDA3. For the endothelial cells, expression of GDF15, a cytokine in the TGFβ family (*40*), increased. Interestingly, the expression of 28 members of the histone family also decreased. Transcriptome studies from the liver chip were compared to previous radiation studies of 2D cell culture of human coronary artery endothelial (HCAE) cells, and the transcriptome of the liver from total-body irradiated (TBI) mice and similarities in cell cycle-associated and other genes were noted. GD15 has also been found to be upregulated in a study of partial body irradiation of nonhuman primates (NHPs). GD15 is, therefore, a good marker for radiation treatment across species. Pathway analysis links the common gene expression changes with cell death, survival, senescence, and liver fibrosis when considering the hepatocytes in the chip and the mouse liver. Cell cycle arrest, senescence, impaired endothelial repair, and vascular leakage pathways are highlighted for the changes in comparing endothelial cells on the chip and mouse liver.

Recent studies have used a quad-culture model where the endothelial channel of the liver chip also contains immune cells (*41*) and stellate cells. The liver chips were irradiated with 0 or 8 Gy and followed for up to 7 days; cell death was noted. Albumin, produced by hepatocytes, was measured in the effluent medium of the chip. A reduction in albumin occurred by day 7 in the irradiated group compared to the untreated control group, demonstrating that hepatocyte function is decreased by radiation. Recommendations were proposed for baseline standards for the chip models that included consideration of the contents of the medium used to perfuse the chip and using primary cells or differentiated iPSCs, if possible, from multiple donors so that studies can account for the heterogeneity in the population for treatments such as radiation (M. Aryankalayil). Current studies include using these chip models to examine the effects of radiomitigators. As previously suggested by other speakers, the 2D culture system could be used to determine the setup of the conditions for the mitigators due to the high cost of the chip models. The chips are also being explored to help identify RNA markers to differentiate between high and low doses of radiation. The plan is to be able to use the chips to identify targets for radiation oncology, to investigate organ-specific markers due to radiation-combined modality injury, and to use the information about RNA markers at high and low doses to establish a mass casualty triage to determine how people are treated after a radiation incident.

SESSION I: DISCUSSION

Questions posed ranged from optimization of the systems, their utility, and ability to be used in the development of MCM for radiation injuries. The discussion began with whether the culture medium used for the chips would need to be optimized for each tissue type being developed and how multiple organs present on a single chip are treated. The panel responded that the complexity of an organ and, hence, the multiple types of cells needed to mimic an organ must be considered. Multiple organs on a single chip are maintained in separate compartments with specialized media since there is a negative impact on cells exposed to suboptimal media. An ideal system would include a "common" media that would benefit all organ types, such as human plasma. Having tissue-specific functional assays is also necessary. There is more to be done, but these needs are being addressed by those working with these systems through optimization and standardization of methods.

When asked how drug developers should think about the correlations and predictions being made using these alternative chip systems before moving into clinical trials, the comment was made that we must use all of the "tools in the toolbox;" these systems alone will not be fully predictive. Instead, a combination of 2D, 3D, and in vivo studies will be needed to better understand a product in terms of pharmacokinetics (PK), pharmacodynamics (PD), and efficacy. Due to the expense and lack of high throughput capabilities, it is advisable to start drug testing in a 2D cell culture system before moving into a 3D system/organ-on-a-chip. Furthermore, comparing animal and human organ-on-a-chip models can aid in validating the human organ-on-a-chip and the translatability of the data to humans.

A question was asked about how MPS/chip technologies can assist in providing information throughout a patient's treatment that clinicians can use to determine any needed changes in treatment strategy, and the use of bioinformatic analyses was stressed. For example, published data from long-term studies can be included in ML systems that could identify potential downstream challenges in a model. This is particularly important in short-term organ chip models such as the liver-on-chip, where long-term toxicity data could be applied. Another strategy is the addition of post-treatment patient blood to a vascularized organ-on-chip to investigate the effect of circulating hematopoietic cells on the tissue under study. These cells are the gateway to understanding what is happening in the patient and can inform how other tissues, such as the endothelium, will behave under their influence. For example, adding pro-fibrotic signaling in the chip system could alert a clinician that anti-fibrotic mitigation is needed and the best timing for the treatment.

Understanding how the microbiome could be integrated into chip systems was also a major challenge. The difficulty of capturing sensitive organ systems on a chip, such as the gastrointestinal (GI) tract and bone marrow, within a single system was also discussed. A gut-on-chip, for example, could be combined with other organs-on-chip, as some in the field are developing. Multi-plex chip networks will be a critical part of accurate organ system modeling. Also mentioned was the importance of testing in both animal and human organs-on-chip so that data can be bridged across species. Thus, animal chip models are a critical component in the stepwise process essential to drug development that can span all research areas, such as radiation biology, infectious disease, and organ transplant.

Known cellular diversity can be represented on chip platforms, and lineage diversity can be captured with chip systems noted by the phenotypic localization of proteins observed. Capturing heterogeneity on the population level is also possible. Ideally, one could develop many chips from many people with one person-on-a-chip, or a cell "village" idea could be adopted with organs developed from a mixed population of iPSC. In summary, although the study of human and animal tissues using MPS has been around for quite some time, the goal of having these systems faithfully recapitulate expected human responses so that they can be used in toxicity and drug development is now becoming a reality.

SESSION II NOVEL IMAGING MODALITIES

In Session II, four diverse imaging technologies that explore a range of high-impact applications to radiation science were presented. These technologies are used to examine multiple approaches to investigate, characterize, and quantifiably evaluate cutaneous radiation injuries (CRI), longitudinally assess the injury and physiological recovery of radiation-induced lung injury (RILI), as well as elemental identification and injury assessment for inhaled radioisotopes using X-ray fluorescence microscopy.

Porcine CRI model. Study designs, wound assessment approaches and findings. The CRI condition describes radiation injury to the skin after doses $\geq$2 Gy, and over the course of 2 weeks to 1 year, can present as reactions ranging from transient erythema, epilation, moist desquamation, edema, acute ulceration, and dermal atrophy, to induration and necrosis (*42*). One compound under investigation as a potential mitigator of CRI is BP-C2 (S. Engelholm). BP-C2 is an orally administered molybdenum-complexed, polyphenolic ligand with immunomodulatory and anti-inflammatory activity that also exhibits a stimulatory effect on hematopoietic and intestinal progenitor cells. When administered subcutaneously as a prophylactic treatment or orally as a mitigator in irradiated mice (30 Gy to one thigh), animals were protected from CRI compared to untreated control animals that experienced dermatitis, edema, and severe skin ulceration (*43*). Based on these results, plans were implemented to investigate the potential of BP-C2 as a CRI mitigator in Yorkshire swine skin. In this model, 8 equidistant locations, each $\sim$6 cm in diameter, were irradiated on the dorsal skin (4 on either side of

the spine) of 10–11-week-old male and female animals with either 15kVp Grenz X rays (80 Gy), or Sr-90 beta rays (47 Gy). Wound development, progression, and recovery were followed for 120 days postirradiation. The pig model was chosen because swine skin is a close biological comparator to human skin.

In humans, there are primarily two clinical CRI scoring schemes currently in use: the Kumar scale (*44*) and the Radiation Therapy Oncology Group (RTOG) scoring criteria (*45*). Both wound scoring schemes are subjective and based on the skin's physical appearance at the wound site. However, the subjective nature of these CRI scoring systems does not translate well to unbiased quantitative measurements that can reliably predict injury outcomes or accurately assess MCM efficacy for CRI wound treatment. Therefore, it was considered important to develop new quantitative imaging methods and metrics to definitively characterize the natural history of CRI in the swine model and, in turn, apply reproducible quantitative evaluation to MCM efficacy evaluation for CRI treatments.

To address this need, several imaging systems with different capabilities were employed. In addition to standard 2D digital single lens reflex (DLSR) photography, 3D optical imaging using laser planimetry to obtain wound depth/height and area was used, as well as dermatoscopy to gather additional wound topography and vascular structure data. Finally, near-infrared imaging (NIR) was used to assess wound oxygenation, and magnetic resonance imaging (MRI) was employed to assess subcutaneous changes and structures. Optical (2D, 3D, dermatoscopy) and NIR data were acquired every 3 days for each wound on each animal. For comparison to data obtained by imaging, all wounds were also assessed using the modified Kumar and RTOG scoring systems mentioned above. These techniques have provided vast data for which evaluation and integration are ongoing. Integrating the imaging data from these various systems as quantitative metrics within the context of the existing subjective scoring systems is challenging. For example, it is not immediately obvious how data such as vascular structure obtained with the dermatoscope might be related to the current observational wound characteristics that are scored using Kumar or RTOG. To accomplish such a complex task and extract unbiased predictive evaluations, advanced computational methods (AI and ML) may be needed to compile and objectively interrogate the data in toto to obtain differentiable outcome parameters. To further complicate the challenges of making these measurements, the pigs used in these studies were young and growing throughout the observation period, which introduces the complication of increasing skin area during wound observation.

In summary, it is possible to use Kumar and RTOG scoring systems to assess CRI wounds, but both systems are subjective, and they don't provide reliable quantitative and predictive output values. In contrast, 3D imaging provides precise wound measurements and images, including surface curvatures that are not captured by 2D photography. It is currently unclear how to incorporate dermatoscopy data as informative measures of CRI wound scoring or assessment, and while NIR imaging provides valuable information regarding tissue perfusion, it can be confounded by skin hyperpigmentation that can develop in response to radiation exposure. Integration and evaluation of the vast amount of complex imaging data obtained from these studies may be amenable to analysis using advanced computational approaches such as ML and AI, and work in this area is ongoing.

Monitoring radiation-induced organ injury in animal models with imaging. The second presentation focused on the power of MRI to assess physiological function in healthy and radiation-injured lungs. Assessing lung function requires detailed structural, physiological, and metabolic information (R. Rizi). Acquiring sufficient information to assess these three lung characteristics accurately permits a thorough assessment of function. Imaging at the structural level allows the gathering of alveolar and small airway data, such as alveolar surface area per unit volume, septal wall thickness, and capillary transit time. Imaging data can also be used to obtain physiological parameters, such as tidal volume and air transit time (i.e., how long it takes for air to move from the trachea to the lung parenchyma, and how much oxygen there is in each region of the lung), as well as blood oxygenation rates and partial pressures. Equally important is metabolic information, such as conversion of pyruvate to lactate, since normal lung tissue does not have high lactate levels, and this endpoint can provide insights into underlying pathology. Local pH is also important and is a factor in fibrosis. These parameters can be obtained using the most recent MRI imaging technologies.

Quantitative assessment of healthy lung function allows applying this information to the investigation of RILI. The early effects of RILI may occur from a couple of weeks up to 12 or more weeks postirradiation. Early RILI is characterized by tissue injury, macrophage activation, inflammation, and radiation pneumonitis, all of which might be amenable to therapeutic intervention. However, in the later stages of RILI (~6–24 months postirradiation), irreversible effects such as tissue remodeling and fibrosis occur (*46*). Therefore, the ability to assess lung function and identify manifestations of RILI early in the injury process may be critical for effective application of MCMs and managing the injury toward a beneficial outcome. This approach is similar to that currently used for chronic obstructive pulmonary disease (*47*).

A powerful technique for measuring many of the parameters discussed above is the combination of hyperpolarization of a breathable gas, such as Xe-129 and MRI. In this technique, a patient breathes a small volume of hyperpolarized xenon gas while an MRI machine is acquiring images, and exchange of xenon atoms from the gas-phase to the dissolved-phase compartments (tissue and blood cells) can be directly measured (*48*). Further, the dynamic distribution

of xenon between the three compartments (gas, tissue, and blood cells) can be spectrally resolved, and simultaneous longitudinal measurements can be conducted over multiple breaths during an imaging sequence (*49*). This approach permits the extraction of the structural and physiological parameters discussed above that are required to assess lung function. Using this technique to investigate the natural history of RILI after acute radiation exposure, a rat model has been developed employing unilateral conformal irradiation of the right lung while leaving the left lung as an unirradiated control in the same animal (*50*). In this model, alveolar septal wall thickness and differential dynamic gas uptake in the tissue and blood cells between the irradiated and non-irradiated lungs can be measured. Blood volume shunting to the unirradiated left lung is observed due to capillary collapse in the irradiated right lung. Consequently, regional ventilation, gas exchange, and lung function are decreased in the irradiated lung due to increased septal wall thickness, which is also observable in dynamic spectral and imaging sequences. Among the parameters derived from this data for the irradiated lung vs. the unirradiated lung are decreased tidal volume, lower functional residual capacity, later arrival time, earlier departure time, and a longer exhalation time constant.

A similar imaging technique employing hyperpolarization of carbon-13 can be used to obtain real-time metabolic data for tissue lactate/pyruvate ratios, pH mapping, and other metabolites underlying molecular pathways. This technique has also been applied to the rat lung model to differentially measure pH between the injured and uninjured lungs. Although trends toward lower pH in the injured lung occur at early timepoints post-injury, this work is ongoing, and more data is needed to draw significant conclusions (R. Rizi). In summary, these powerful imaging techniques can be applied longitudinally in animal models to quantitatively assess lung function and physiology to inform the natural history of RILI induction and progression. These technologies could be a powerful tool for identifying the onset of early radiation-induced lung injury and the subsequent evaluation of MCM therapeutic efficacy for the treatment of RILI, as well as potentially applicable to the evaluation of other delayed effects of acute radiation exposure (DEARE).

Application of X-ray fluorescence for elementalomics. The next speaker described the application of X-ray fluorescence microscopy (XFM) to help answer biological questions (G. Woloschak). This technique provides distinctive imaging capabilities permitting localization of specific elements in biological samples. The XFM method relies on the photoelectric effect and the principle that each element gives off a characteristic fluorescent signal when irradiated. These signals can be measured with an appropriate detector. Almost every element on the periodic table can be detected by varying imaging parameters, leading to the practice of "elementalomics." One of the main reasons this technique does not see widespread use is the requirement for an X-ray microscope, which necessitates specialized facilities. However, certain U.S. government national laboratories provide these capabilities, which can be accessed without cost upon application.

A major strength of XFM is its flexibility in resolution. While traditional microscopy relies on multiple techniques to image samples over a range of distances, XFM can be tuned to image the same sample from the tissue level down to $\sim$10 nm resolution. Furthermore, this technique incorporates spectroscopy, which can inform on the chemical state of the observed element, such as plutonium. As with all scientific techniques, this microscopy method has some inherent limitations and considerations that must be recognized. Beyond the specialized equipment required, sample preparation needs to be carefully considered because there may be a loss of information during this pre-processing step. For example, free calcium may be washed out during sample preparation, while bound calcium will remain.

Additionally, some loss of information can occur due to signal self-absorption during imaging, especially in 3D studies. Even with these considerations, XFM provides powerful element localization capabilities for introduced and native chemical elements. In one study, lung samples from canines dosed with Y-90 were imaged at high and low resolutions using XFM (*51*). The Y-90 had been packaged in silicon microbeads, so while the Y-90 had all decayed to zirconium-90, the silicon and zirconium signals could still be detected. It was found that the signals were still highly colocalized, indicating the encapsulation of the Y-90 was stable for the duration of the experiment. Interestingly, the native iron signal in these samples was also colocalized with the silicon signal. Combining this finding with immunohistochemistry revealed that the microparticles had been taken up by macrophages, which generally contain high iron levels. A wide array of biomedical studies have also relied on the unique capabilities of XFM. This technique has provided insight into several areas of biology and medicine (*52–54*).

Many technical modifications and adaptations can be applied to XFM to improve functionality and data collection. For example, a free ion beam can be employed to precisely remove material that is blocking regions of interest in samples. The free ion beam modification can also be used to remove the coating of ice that covers cryogenically preserved specimens, which can obscure sample features. Additionally, tissue microarrays can accelerate sample imaging times through batch imaging. Finally, tomography can be performed using XFM to create 3D images of element localization in biological samples, such as single cells. Overall, this powerful imaging technique enables elementalomics in multiple dimensions and across a span of resolution ranges unheard of in traditional microscopy.

SESSION II: DISCUSSION

The discussion largely centered around how the advanced imaging technologies introduced by the speakers might be applied to animal models of radiation injury and

if these approaches can be used to improve efficacy assessments for therapeutic interventions. The discussion was initiated with a question about how the imaging technologies presented might be used to identify damage and recovery in animal models of radiation injury, such as CRI, in ways that are non-biased, reproducible, quantitative, and potentially translatable to the clinic. In the case of XFM, it was pointed out that while the technique is unsurpassed in resolution, it requires a biopsy or tissue sample. So, although XFM can achieve atomic resolution of a radionuclide and/or its daughters and permit a targeted means of assessing proximal intracellular, cellular, and tissue damage depending on the markers used, it is currently most applicable to model systems and not as clinically translatable as some of the other imaging methods presented.

Regarding the MRI methods presented, while repeatable experiments are achievable, reproducibility among different performance sites presents inherent challenges due to variability between scanners and the metrics needed to ensure MRIs at different locations yield equivalent, reproducible results. This outcome is possible, and the field is addressing this issue by working to increase coordination between sites. For the work presented, robust methods and parameters ensure that outcomes and endpoints are repeatable. Concerning the imaging approaches presented to assess CRI and recovery, a large amount of seemingly disparate data is generated for each wound over a 120-day study, and the method by which that data should best be integrated and analyzed is not immediately apparent. Also, although the same radiation dose is applied to each of the eight irradiated areas, the wound development trajectory and resolution can have notable variability. Using AI and ML to interrogate the data was proposed as a potential approach for deconvoluting the problem. However, employing AI may also be a challenge since it requires a result with which to correlate the data. The dataset for a typical porcine experiment is large. However, it still needs to provide clearly defined outcomes that can inform outcomes for humans in a radiation therapy setting. Thus, scoring with AI may also have inherent challenges, although additional work is ongoing in this area.

MRI-based methods for lung function assessment in animal models, such as the hyperpolarized Xe-129 technique, which is very well developed and provides a powerful means of identifying early changes to the gas exchange machinery of the lung after radiation injury, were discussed. Hence, the effectiveness of an MCM in counteracting the early effects of radiation injury could be measured using hyperpolarized xenon MRI imaging. A drawback is that only 30–40 sites nationwide currently possess the expensive broadband scanners needed to read the three-compartment spectra assessed in these experiments. In contrast, less expensive hyperpolarization devices are now commercially available.

While MRI may be effective for an external beam exposure, XFM is likely the best approach for evaluating the effects of, and mitigation for, internal radionuclide exposures. With XFM, one can detect the radionuclide (or daughters as appropriate) and assess damage to the surrounding tissues caused by its decay. Also, XFM can be modified to detect specific structures and/or molecular damage by using labeled tracers such as nanoparticles and antibodies. XFM could also be used to evaluate the effectiveness of radionuclide decorporating agents. In conclusion, session speakers expressed enthusiasm for continued efforts to advance these imaging technologies to assess injuries in animal models and improve the natural history of acute high-dose radiation injury and their utility to assess MCM efficacy in these models.

Immediately following the Session II discussion, meeting participants were taken to NIAID's Biovisualization (BioViz) Laboratory for a presentation on using imaging tools to evaluate a CRI animal model. The BioViz Laboratory was designed and is managed by the Bioinformatics and Computational Biosciences Branch's (BCBB) imaging team at NIAID.[3] This session included exploring the laboratory's advanced imaging and virtual reality capabilities.

Immersive visualization in an animal model of cutaneous radiation injury. Since 2005, NIAID has funded the Armed Forces Radiobiology Research Institute (AFRRI) to develop animal models and to screen MCMs. One of these models uses Göttingen minipigs to study CRI. While initial investigation into the mechanisms of CRI often begins in small animal models such as rodents, porcine skin better reflects the physiology of human skin and thus is more suitable for understanding the biological impact of CRI and the efficacy of drugs being tested (*42*). Historical documentation of cutaneous radiation injuries goes back to the late 1800s. Within the first few years after Röntgen's discovery of X rays in 1895, hair loss and skin damage after X-ray exposure were observed. The renowned physicist and Nobel Laureate Marie Curie, documented skin burns on her hands from handling radium in 1901. As interest grew in the novelty and potential therapeutic benefits of radiation, others who used and handled radiation devices manifested both acute and long-term injuries spanning from dermatitis, painful erythema, moist desquamation, ulceration, amputation, and squamous cell carcinoma (*55*).

While CRI is similar to thermal burns, they differ in that the onset is usually delayed with CRI (from days to months) and can continue for years due to persistent waves of inflammation. The time course and severity of CRI wounds depend on the dose and type of radiation received and the location and size of the exposed area (*42*). The development of the CRI model at AFRRI was based on a documented human radiation accident involving cutaneous injuries, which occurred at an industrial facility in Maryland in 1991 (*56*). The Göttingen minipig was selected as the porcine strain to study at AFRRI due to its response to cutaneous radiation and its manageable size and demeanor,

[3] https://www.niaid.nih.gov/research/3d-printing-biovisualization-bioinformatics-computational-biosciences-branch.

among other attributes. Radiation is delivered dorsally to six localized (6 × 6 cm) sites on the animals at 50 Gy/site via linear accelerator (LINAC, Elekta), with 6 MeV electrons at ~3.7 Gy/min and radiation doses verified by thermoluminescent dosimetry (G. Holmes-Hampton).[4]

Before irradiation, computed tomography (CT) scans are taken, and the skin surface is tattooed to demarcate the skin wounding area. After irradiation, animals are observed for 120 days with clinical evaluations, including skin scoring, complete blood counts, serum chemistry, body weight, heart rate, body temperature, and oxygen saturation. Skin wound evaluations include transepidermal water loss, skin moisture measurement imaging (2D, 3D, and infrared), as well as histology at study termination. A visual overlay of the CT and white light images along with postirradiation images of wounds are converted to digital images, along with other metadata, for application into an immersive visualization platform. The outcome of this method was featured during the above-mentioned BioViz Laboratory presentation, which allowed meeting attendants to wear virtual reality goggles and partake in a 3D experience, in which they were able to "walk inside" the radiation injuries in the minipigs (Fig 2) (D. Chen).

SESSION III: COMPUTATIONAL METHODS: ARTIFICIAL INTELLIGENCE (AI) AND MACHINE LEARNING (ML)

In this session subject matter experts were invited to explain the tenets of AI and ML at a level that could be understood by audiences with scientific training with the final talk addressing the development of digital twins. The progression of the talks allowed the participants to understand potential uses of AI/ML in the radiation space. It provided diverse examples of how it could be applied to understand the complexities of the kinetics of inhalation of radionuclides, to explore existing databases to recognize the potential risks to astronauts during spaceflight and to evaluate complex images of radiation-induced injuries to bridge preclinical findings to expected human scenarios.

Artificial intelligence/machine learning (ML)/deep learning (DL) techniques in radiation research. ML and deep learning, which are subfields of AI, are concepts critical to the future of the radiation research field, and several key concepts were summarized in the first talk of the session in a manner that enhanced and synergized with the three following talks (C. Obcemea). The first key concept is that ML and DL are, essentially, an interpolation scheme between data points. This definition is a critical point that sheds light on what is traditionally felt by many to be a relatively impenetrable area of computer science and research. It is critical to understand because if data are fit to a matrix of what is expected and understood, then a complex code can be generated to analyze huge amounts of data. It will tend to produce things that are recognized, and in this way, appear "intelligent" and "insightful," when it is simply trying to fit in (interpolating) the missing points into the known (labelled) data set. The algorithms of ML and DL ML are complex and highly nonlinear, but this critical interpolation theme underlies both approaches and makes the science more transparent and approachable.

The second critical area explored was a consideration of the terminology of deep learning. Supervised and unsupervised learning methods were formally defined and explained in a memorable fashion using an analogy that focused on the creation of marinara sauce based on only baseline knowledge of the ingredients and the idea of sauce (unsupervised), versus having in addition to these items a successful comparator in the form of a sample of an excellent sauce to consider (supervised). Computational neural network architecture was explained, as was the use of encoder-decoder methods to detect not only what an image is but where in the overall image a component sits. This discussion was placed in the context of biomedical image segmentation, and the concept of the U-Net, a neural network conceived and developed in Germany in 2014, was reviewed[5] (*57*). Convolution filters were reviewed in the context of feature extraction from images. Finally, transformer architecture was explained in the context of ChatGPT (Chat Generative Pretrained Transformer; OpenAI, San Francisco, CA)[6] and similar tools. A logical progression from feed-forward neural networks to recurrent neural networks, gated recurrent units, and transformer networks was defined and explored. The third and final major point made in the presentation is the downsides and caveats that apply to AI applications, which include performance failures in critical-safety domains, reproducibility issues, the need to open the black box of the code AI typically presents, the need for uncertainty quantification, and the fact that measurement of the area under the curve for accuracy of ML/DL is not a sufficient metric. The last item was developed a bit more than the others in the context of curve fitting of the data overall. As noted, an interpolation scheme of assembled data points is the basic operational paradigm of ML and DL. Data quality was formally noted to be hypercritical, as it directly impacts prediction quality. In addition, optimization is important, but most critical is opening the "black box" of the process to allow people to understand the operations of ML/DL and to see it as a mathematical tool, and the change agent it promises to be. ChatGPT was noted to be a curve-fitting exercise with approximately 570 gigabytes of data used to train the model,[7] which allows it to fit output well, in accordance with human expectations.

[4] Valenzia K, Wuddie K, Aschenake Z, Kumar VP, Ghosh SP, Holmes-Hampton GP. PS5-67: The development of a porcine cutaneous radiation injury model to understand the mechanism of injury and develop countermeasures. 17th International Congress for Radiation Research, Montreal, Quebec, Canada (2023).

[5] https://arxiv.org/abs/1505.04597.

[6] https://help.openai.com/en/collections/3742473-chatgpt.

[7] https://analyticsindiamag.com/behind-chatgpts-wisdom-300-bn-words-570-gb-data/.

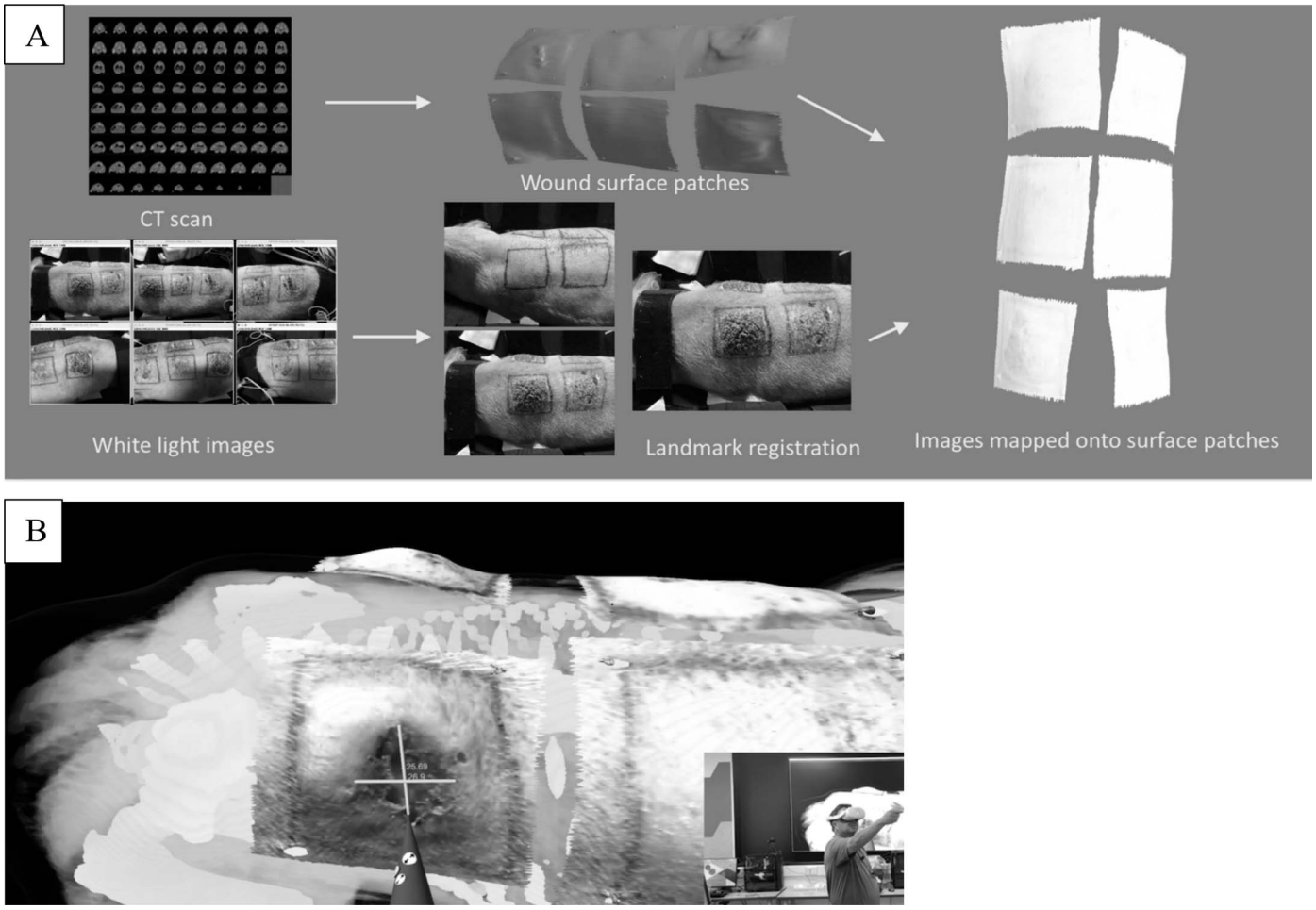

FIG. 2. A "Walk Inside" cutaneous radiation injuries in the Göttingen minipig. Panel A: Workflow for creation of the "walk inside" model. In the top left, contrast markers are applied at the skin surface and a CT scan obtained to map the locations of the wounds relative to skeletal structure. These scans are stitched together to produce a 3D model and the wound locations are mapped on the 3D model (top center). Over the course of the 120-day study, the wound sites are photographed to track injury progression (bottom left) and the corners of the wound sites are registered (bottom center). Finally, landmark registered photographs are referenced to the wound sites (right). Panel B: Real-time demonstration provided by D. Chen, demonstrating the 3D model and highlighting measurement tools that can aid in the evaluation of the wound sites. Used with permission from G. Holmes-Hampton.

Integrating mathematical, multiphysics, and AI approaches for enhanced physiological modeling of internalized radiation. Bridging the divide between the vast amounts of data generated at the bench, and their incorporation into computer models, the Radiological Engineering, Detection, and Dosimetry (RED^2) Laboratory at Georgia Institute of Technology is engaged in research that spans multiple areas of radiation research including biology, chemistry, and physics (S. Dewji).[8] Through Monte Carlo modeling (a technique using mathematical algorithms to calculate event outcomes), high-tech computing and wet lab experimentation, the group leverages collaborations with other academic institutions, national laboratories, and U.S. Government agencies to advance programs in computational dosimetry, radiation detection, radiation shielding, and nuclear safety. The RED^2 lab utilizes neural networks (an ML approach where models are used to make high level decisions) and platforms such as the TensorFlow Playground[9] to achieve advanced Monte Carlo simulations and metabolic and computational fluid-particle dynamics modeling. Both supervised and unsupervised approaches (defined above) can assist in the analysis of complex biology, and the RED^2 group is using these methods to simulate human scenarios of internal radionuclide contamination. To start, the dynamics of the system are understood using mathematical models that use assumptions, hypotheses, mathematical formulas and known facts to arrive at a solution. For example, in the study of how a decorporating agent can bind to a radionuclide, the challenge lies in the complexity of the chemical species surfaces to be bound, and uncertainties in the chelation process. Further, it is possible to incorporate a large range of biological information to further improve the simulation. These datasets could include "-omics" information

[8] https://sites.gatech.edu/dewji/.

[9] http://playground.tensorflow.org.

(e.g., proteomics, metabolomics, transcriptomics, genomics, etc.) to enable simulations very similar to anticipated exposure scenarios.

To create an effective human model to study internal contamination, there must be a convergence of AI, computational fluid dynamics, and advanced mathematics, which allows the neural network to process a large number of inputs (e.g., age, sex, weight, time, radionuclide activity, etc.) to define a nonlinear relationship between the variables, and then derive a highly predictive output. This method involves identifying common features across all the information provided, and then using those connections to uncover similar attributes in new data. Through supervised learning, it is possible to reconstruct inhalation of a radionuclide using a network architecture that considers multiple interactions between, and weighting of, discrete variables in "hidden layers" made up of several nodes, and then resolves them back to a single output. For example, in considering an inhaled, internal exposure to Sr-90, it is possible to generate several models with varying levels of complexity to predict the biokinetics of the particles in the body. In this example, the estimated dose to an individual allows for a reconstruction of reduced body burden from contamination.

Although there are several approaches to solving these problems, one that has been shown to accommodate inputs consistent with those generated scientifically in this area of radiation research (e.g., lung imaging) is a convolutional neural network (*58*). This network can distinguish patterns in video and image inputs, through a process that involves 1. weighting an image by importance of its most prominent features; 2. capturing higher level patterns; 3. extracting specific relationships in the input; 4. carrying out a deeper identification of nonlinear patterns; 5. normalizing outputs; and 6. staking predictions to enhance inputs for future calculations. With these tools, the RED^2 team can use CT scans to produce a realistic model of the human lung. However, because a complete understanding of the kinetics of an inhaled particle does not entirely depend on lung morphology, it is also important to consider the geometry of the nasal cavities, through which the radionuclides will pass en route to the lung, coupled with high-fidelity particle characteristics (morphology, solubility, etc.) of the source term. This modeling requires other tools, which allow image refinements to accomplish accurate capture of the sinuses. Principal component analysis can then be used to extract dimensions in the outputs by seeking the direction of the spread of data. This analysis can allow for the mining of specific information, for example, parameters associated with the human population (sex, age, etc.), to inform any anticipated differences in airway structure.

Several different supervised and unsupervised learning algorithms used to interpret data generated by a neural network exist. For example, a supervised, Random Forest Regressor, which can be trained on the lung inputs to extract important features, determined that the diameter of the trachea was an important consideration in creating the branching in the models. An example of an unsupervised learning approach, Kernel K-Means Clustering, allows for the clustering of data and their relative distances to label points with the highest number of commonalities. Using the latter algorithm, both the diameter of the trachea and the angles of the bronchi were found to be critical features needed to appropriately model particle inhalation. Other methods, such as the Reynold Adveraged Navier-Stokes finite volume approach, used to consider fluids numerically, and Lagrangian Particle Tracking, which is a numerical method of simulating the path particles take in the airway, can be used to assess dust particles and enhance the reliability of the lung models. All these techniques can help generate robust simulations that effectively consider many variables and provide accurate representations of the movement of radionuclides and dust particles in the respiratory tract.

With these models in hand, it is possible to evaluate how these elements may be retained in and excreted from the different bodily compartments by coupling known biokinetic models and more advanced fluid dynamic designs. The significance of this work is the ability to combine data generated in biological models with the mathematics needed to enable ML, leading to a dramatic increase in the volume of data that can be considered, and a reduction in the time it takes to obtain meaningful results. These capabilities will continue to expand and are also being used in other areas of medicine, such as bone remodeling, evaluation of endoscopic images, and blood vessel abnormalities. The use and standardization of these approaches have enhanced research capabilities and specifically improved understanding of airway function allowing improved testing of chelating agents to decorporate damaging inhaled radionuclides (Fig. 3).

Computational and systems biology approach utilizing multiple public databases/platforms to address key biological changes associated with health risks occurring during spaceflight. Taking the understanding of the basics of AI and ML laid out in the first two talks of the session, another possible use of these advanced technologies in the field of radiation research was considered, with a focus on possible radiation risks to astronauts during space missions (A. Beheshti). Using a systems biology approach, which looks at the whole of the organism, instead of collecting subcomponents, it is possible to further synthesize varied "omics" data to create multi-omics datasets. This is a challenging prospect, given the need to integrate data from disparate platforms, the requirement of extensive computer resources, the prevalence of incomplete datasets (e.g., lacking controls, insufficient replicates, poor experimental design, etc.), and the fact that analytics may be biased and have no uniform processing. Nonetheless, developing advanced software tools, supercomputers, guidance on future research experimentation, and creating a universal pipeline for varied data analyses have paved the way for exciting findings.

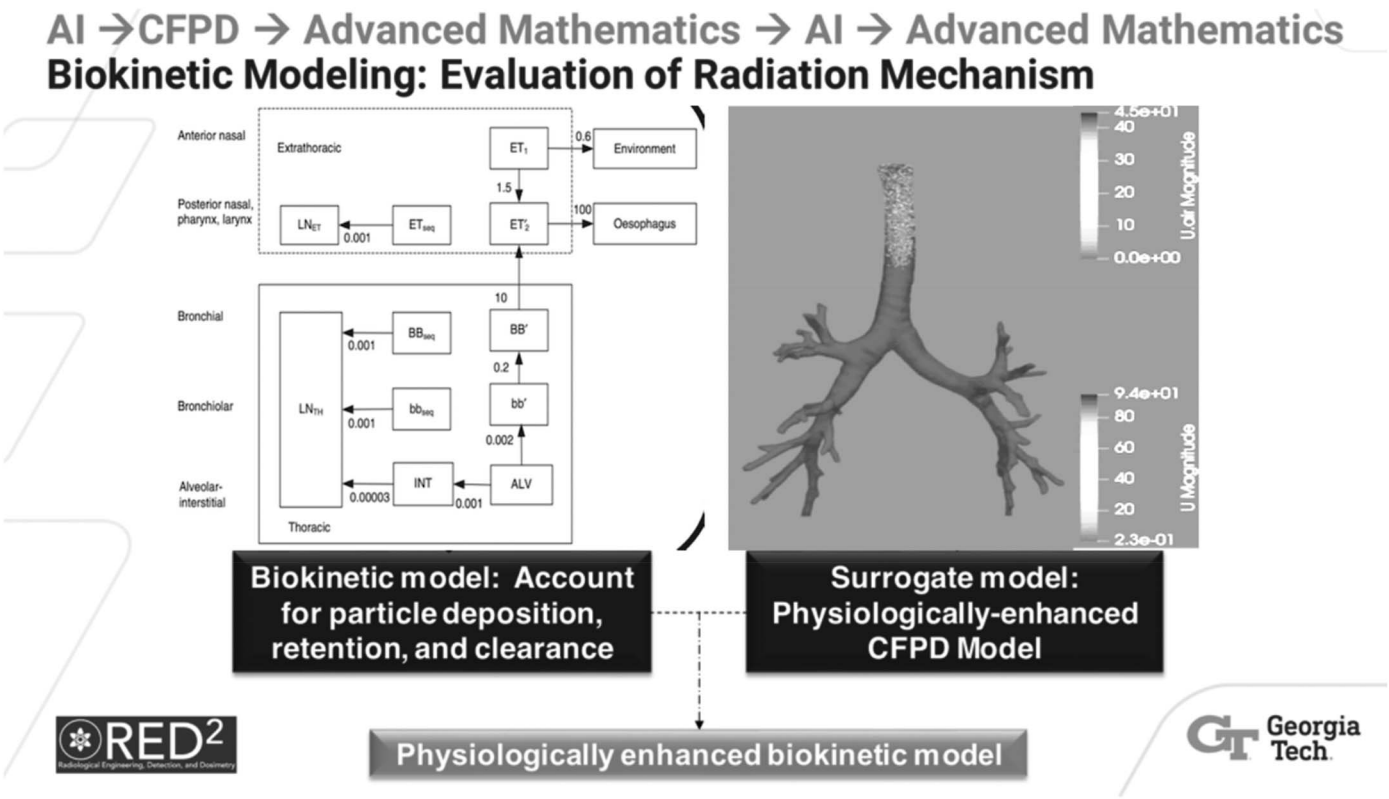

FIG. 3. A model of internalized radioactive particle deposition. Physiological modeling of internalized radioactive particle deposition in the human respiratory tract informs pharmacokinetic models of chelating agents employing mathematical compartment-based biokinetic models (left) and multi-physics computational fluid and particle dynamics. Used with permission from S. Dewji.

With long-term accommodations on the International Space Station, planned deep space explorations, and the advent of commercial spaceflight, NASA has a keen interest in the biological impacts of space travel on individuals who journey outside the Earth's protective atmosphere. The identified hazards of spaceflight include distance, confinement, hostile and closed environments, gravity, and radiation (*59*). Specifically, GCR has a high LET, meaning that there can be a large swath of destruction along a GCR particle's path. The relative composition of these forms of radiation has been investigated (*60*). Data surrounding space biology are generated through a number of different kinds of experiments, including those carried out in space, terrestrial-based studies using simulated space radiation (e.g., at Brookhaven National Laboratory), and microgravity models employing hindlimb unloading of rodents. Experiments in model organisms and resulting "omics" findings generated by NASA-supported work are made available to the greater research community through the utilization of the GeneLab data repository platform.[10] Recently, there have been efforts to mine and leverage information contained in other U.S. Government-funded data repositories, including the ImmPort database hosted by the NIAID.[11]

To explore the possibility of connecting data available through these two U.S. Government-supported repository resources, a data mining study was undertaken to explore possible connections between spaceflight (experienced by female astronauts) and any possible increase in the incidence of birth defects experienced later after returning to Earth. Using information on a study in ImmPort looking at plasma samples taken from pregnant women to determine if there were plasma miRNAs that might predict the propensity to deliver a low birthweight infant, researchers compared those findings with study data generated from pre- and post-flight astronaut samples. Specifically, both sets of samples were assessed for miRNAs, which are known to play an important role in human health and can regulate protein expression at multiple steps. They have also been extensively studied relative to space biology (*61–63*), and these data were deposited into GeneLab.[12] Additional efforts to relate low-birthweight findings to data from space mission samples included leveraging several studies uploaded to GeneLab to detect miRNA signatures and ascertain any changes in immune responses (*62*). It is known that a number of miRNAs are conserved between mice and humans (*64*), and there are also identified health risks and biological functions associated with certain common miRNAs (*65*). These overlaps include pathways relating to mitochondria, oxidative phosphorylation, and metabolism. Furthermore, the identified overlapping pathways are

[10] https://genelab.nasa.gov/.
[11] www.immport.org.
[12] https://three.jsc.nasa.gov/articles/miRNA_Beheshti.pdf.

consistent with earlier reports of mitochondrial stress as a mediator of the biological effects of spaceflight (*66*), and are also shared with maternal small birthweight infant miRNA profiles. The finding that there may be similar miRNA profiles between samples taken from female astronauts and women delivering small birthweight infants subsequently led to the search for MCMs that may be able to mitigate these adverse outcomes, both in the clinic and among postmission females.

Using a deep learning machine network capable of predicting entities that will interact with specific miRNAs based on chemical structures, a few candidates were identified, including several small molecules that bind to at least five miRNAs in common between the astronaut and low-birthweight, maternal plasma samples. These leads include a U.S. FDA-approved generic topical steroid already in use to address dermatologic inflammatory complications, and a compound known to accelerate wound healing and improve gas exchange in the lungs of animal models and preterm infants. In summary, systems biology combined with advanced computational methods will continue to accelerate future exploration of biomarkers, and the information gained by accessing multiple, freely-accessible databases creates an opportunity for synergies between clinical and spaceflight-focused research areas.

A generative AI for 3D slicer to detect radiation-induced lung injury in multiple species: Advances in segmentation and integrating multimodal data. The final speaker summarized the prior three talks, giving context and reinforcement to key ideas as noted above, before moving to advanced data methods his company, Chromologic LLC, is developing to better understand radiation-induced lung injury (E. Kyubwa). One goal of the group is to develop MCMs to mitigate the progression of lung fibrosis. This process is hindered by the need for lengthy animal studies, variations between species, challenges in understanding data obtained by multiple groups, and several irradiation models, that have resulted in a tremendous volume of data to be considered to move forward in development. It is the hope that AI/ML models can be effectively used help characterize the late pulmonary complications that can result from acute or chronic radiation exposure.

Critical to the project is a large language model that is being developed to create a reference "digital twin" (DT) that will help characterize the long-term side effects of radiation. These DTs depend on multiscale data integration and have been applied to evaluating findings in an NHP model using whole thorax lung irradiation. Evaluation of CT data with 3D Slicer[13] and injury quantification was demonstrated to workshop participants using a GPT LangChain – a form of data ingestion and model development pipeline.[14] The NHP data on lung fibrosis at 60 days was reviewed (*67*, *68*). The gap in currently available CT radiomics tools was then discussed, in that they require multiple iterations of manual segmentation. This need can lead to misclassifications of certain lung structures, such as trachea, bronchioles, and vasculature. A further challenge is that image platforms that have been trained using human data are not necessarily optimized for monkeys. These concerns can be addressed through the use of an open-source, AI-based, front-end to 3D Slicer called the "Segment Anything Model" (*69*), which can improve the quantification of CT scans and allow human input into the model. Relying on these AI tools enabled researchers to measure changes better in lung volume in irradiated NHPs scanned at several time points in the months after their exposure, revealing the progression of disease (pneumonitis to fibrosis) and vascular changes in the animals (*70*). These findings were then merged with a human-guided retrieval model to integrate multiscale data as described in a recent publication (*71*). Although the work was noted to be in an early stage, a video of the process illustrated interesting analyses of NHP fibrosis for the workshop audience in real-time. In summary, the challenge of identifying biomarkers of radiation injury to allow for bridging between preclinical animal models and humans, which is critical for the regulatory approval of products to address radiation-induced injuries, can be made less daunting using AI/ML methods.

[13] https://www.slicer.org.

[14] https://python.langchain.com/docs/.

SESSION III: DISCUSSION

Participants expressed fears about bias in data generated using AI/ML methods. The potential to add further prejudice as even more information becomes available was also discussed. The speakers offered several responses to this concern starting with the heterogeneity of data. Although the loss of data heterogeneity can certainly lead to bias, if one stores data from different institutions, it is possible to parse out how institutional variation in therapy may affect outcome. Therefore, aggregation of large amounts of data from many institutions is likely beneficial. All data sets, including failed experiments, will be precious.

New data repository resources being created by the U.S. Government were also discussed. The example of NHP studies with partial bone marrow shielding data being put into ImmPort, "omics," blood parameters, lung injury, imaging, and more were noted. One thing brought up was the need to develop tools to link these new data between the NIAID database and NASA's GeneLab repository. Biomarkers were also noted to be a common goal for the data's utility in this context. The speakers discussed opportunities to use tools like Random Forest to look for associations not observed before and felt that the key to the successful use of these methods would be data quality. The discussion's next question focused on how to use and assess the value of these models, predictions, and the overall AI/ML/DL process. The speakers focused on how the methods can save time and resources. However, the science still needs to be

rigorous. The risks to the established world by AI were then discussed. One speaker summarized a constructive way of thinking about AI via linking to the approach used by the Radiological Society of North America, in regard to how they advise clinical radiologists concerned about AI: it won't replace radiologists rather it will render those who don't know AI unemployed and will improve the field to the benefit of everyone. The speakers also emphasized that it is important not to allow AI to be misapplied, resulting in a loss of trust and major setbacks to science.

Drug development has as an end goal of MCM applications, being able to model a mechanism of action for a product translates into the human condition – this is a difficult requirement of the U.S. FDA Animal Rule.[15] It is important to find promising biomarkers to be convinced the drugs will work in humans, and these AI/ML techniques can help us get there faster. AI/ML tools can be used to aggregate large datasets and allow for meaning reconstruction and capture of historical data that might otherwise be lost. For MCMs, mathematical modeling has allowed researchers to better understand how DTPA works. One can use AI to enhance future experiments, ask other questions, and validate methods. For example, it has been used to look at how DTPA might behave differently in mice with intra-tracheal instillation of radionuclides as opposed to inhalation. AI/ML techniques can also be used to identify approved drugs for repurposing, further accelerating the drug discovery process.

Concerns were raised if there is a way to detect papers/grant apps written with ChatGPT. Could the general availability of this kind of AI weaken the capacity of scientists to be elegant and creative? Would these algorithmic models have been able to predict, for example, a penicillin molecule if it had not previously been discovered? There are algorithm "sniffers" that can determine another algorithm generated text, but more importantly how can AI be used to effectively augment the ability to conduct innovative, robust, and reproducible science? These ML processes are, in some ways, analogous to the printing press – a dramatic step forward, but things will normalize in the knowledge base over time. To further make this point, there have been predictive molecular simulations that have identified unique structures and unexpected ligand binding sites for molecules, and in 2023, a group reported using ML to generate an unknown anti-bacterial compound with activity against a multi-drug resistant strain of bacteria (*72*).

In response to questions surrounding how much validation is done with AI/ML algorithms, the speakers stressed the human element, and the need for computational experts to work with experimentalists to address false positives. Also stressed was the need for a large dataset (e.g., 500 CT images) and a good assessment of the data quality to be able to partition between training and testing. Researchers generally train on 80% of the data and validate with 20%. The final consideration of all the talks presented during the session involved acknowledgment of the challenges and opportunities to utilizing advanced AI/ML methods to innovate the field of radiation research, enable new ways to re-examine data from existing resources, and accelerate the pace of research, if used properly.

[15] https://www.fda.gov/regulatory-information/search-fda-guidance-documents/product-development-under-animal-rule.

SESSION IV: APPLICATIONS OF ADVANCED TECHNOLOGIES

Session IV explored applications of advanced technologies to the radiation research space, emphasizing acute radiation exposure and potential new avenues for MCM development. New model systems were discussed, as were novel approaches for dosimetry in mass casualty scenarios and targeted MCM delivery. Presenters also considered what regulatory and technical challenges need to be addressed to move these technologies forward and expand their applications to additional areas of interest in the radiation space.

GI exfoliome and CRISPR-based epigenetic modulators against ARS. The need to consider next-generation biomedical technologies to treat ARS was presented in the context of MCMs that can act through mechanisms of programable gene expression modulation (H. Wang). Currently, all six U.S. FDA-approved therapeutics are biologics approved to treat only H-ARS. To date, no MCMs are approved to treat GI-ARS or any of the DEARE sequelae that may occur in ARS survivors. To address this challenge of identifying a broadly applicable MCM to treat ARS based on programable gene modulation, the potential for use of CRISPR-based therapeutics as an approach to enhance radiation resiliency has been studied.

The goal of the current effort is the creation of orally administered nanoparticles to deliver CRISPR-Cas9 radioprotective gene modulators to key organs to treat H-ARS and GI-ARS. To accomplish this, radioprotective genes need to be identified and validated via CRISPR screening, and targeted CRISPR-Cas9 effectors need to be developed along with highly specific multi-organ targeting nanoparticles. Rather than relying on the CRISPR endonuclease activity, the approach described here exploits the CRISPR-Cas9 ability to target a specific DNA sequence as a function of its Cas-associated sequence-specific guide RNA (gRNA). This approach allows catalytically dead Cas9-engineered protein complexes containing gene activators or repressors to specific genes of interest (*73*, *74*). This method permits target genes of interest to be specifically activated for expression (CRISPRa: dCas9-VPR) or repressed (CRISPRi: dCas9-MeCP2). The conceptual ARS MCM would then incorporate these gene-modulatory CRISPR-Cas9 constructs in orally delivered nanoparticles, where gut-specific constructs would be taken up by GI epithelial cells and hematopoietic-specific constructs would transcytose to the blood and be taken up by the liver. After

uptake, the respective GI-ARS- or H-ARS-targeted CRISPR-Cas9 constructs would modulate the expression of their respective radioprotective target genes (e.g., alpha defensins, G-CSF, etc.).

The first step for this work was an evaluation of potential radioprotective target genes. Radioprotective GI targets were identified based on a large-scale systematic screen using irradiation and co-infection of the HCT116 human colorectal cell line with lentiviruses expressing CRISPRa/i and gRNA genome-wide and targeted libraries (*75*, *76*). Downregulation identified genes involved in transcription, B6 vitamin metabolism, and Hippo signaling as examples of genes exhibiting radioprotection, whereas upregulation identified many genes involved in DNA repair. Some of the highlighted targets of interest were *P53*, which can provide GI radioprotection when transiently activated, and *MEIS2*, a tumor suppressor gene that works cooperatively with other tumor suppressors to reduce proliferation (*77–79*).

The next step in moving this technology forward was to expand the available set of CRISPRa/i effector/modulator protein complex combinations. This was done by computationally mining the UniProt database[16] for promising constructs for specific genes, assembling uniquely barcoded combinations, and testing them using a high-throughput approach for gene modulation activity. Promising hit combinations are actively under further development. The third and potentially most challenging component of this CRISPR-based MCM development strategy is the nanoparticle oral delivery vehicle. Chitosan has been selected as a base structural unit in combination with polyethyleneimine (PEI) for oral nanoparticle construction. This cationic carrier has been shown to effectively condense nucleic acids and make them orally available in mice (*80*). Early testing of chitosan-PEI as a carrier for the CRISPRa/i demonstrated >50% in vitro functional transfection efficiency in colorectal cells and hepatocytes (H. Wang). This approach has also been demonstrated to functionally activate *Ttn* gene targets (encoding the protein titin) in the small intestine and liver of mice using a *Ttn*-targeted CRISPRa complex as cargo.

A further outcome of these studies was the development of methods to conduct non-invasive gut exfoliome profiling for GI monitoring, via an amplicon-based approach to directly sequence stool RNA from exfoliated intestinal cells (Exfo-seq). Exfo-seq can assess up to 1,000 genes at a time from exfoliated cells in the stool and can differentiate multiple GI compartments (upper and lower GI). In a mouse model, this technique is capable of monitoring radiation injury and recovery in the GI tract. This approach is now being employed to longitudinally assess the murine GI response to various radiation exposures and MCMs. This information can also be linked to changes and effects in the host microbiome. This approach may lead to new biomarkers for radiation-induced GI responses and possibly identify new therapeutic targets.

Patient sorting with dosimeter-embedded ID cards in a mass casualty radiation/nuclear event. Radiation retrospective physical dosimetry was next presented, in the context of a radiation mass casualty incident by using novel non-resonant broadband electron spin resonance (ESR) measurements of alanine dosimeters. Although alanine dosimeter technology was first identified in the 1970s, the application proposed here uses uniquely small and reference-grade alanine pellets embedded in personal items, such as identification badges, that individuals are likely to carry on their person (I. Pazos). The National Institute of Standards and Technology (NIST) developed this technology as the dosimetry standard for transfer of NIST standards in the 1990s for doses ranging between 20–100,000 Gy. Alanine has remained the NIST dosimeter standard since that time. The alanine ESR signal in response to radiation exposures is immediate, permanent, and cumulative, which makes this an invaluable tool for dosimetry. The proposed approach applies this technology to quickly assess doses to an individual (clinically significant range of 2–10 Gy of combined neutron and gamma irradiation), and in a distributed fashion across large groups of people and geographic areas after a no-notice mass casualty nuclear/radiological event.

The conceptual basis for this work is the critical need to make rapid tactical decisions in the immediate aftermath of a radiological or nuclear incident, while also protecting the health and safety of first responders, military personnel, and others. This Emergency Response Dosimetry System (ERDS) will provide identification and assurance for the minimally- or non-exposed populations and ensure a record of exposure for future follow-up. It will further allow for the identification of survivors who may have received significant acute radiation exposures (2 Gy and above) and who need to rapidly seek medical evaluation and potential MCM interventions. Such rapid sorting is significant because of both the limited stocks of such MCMs and the time-sensitive nature of administering them. For example, cytokines for the treatment of H-ARS should be administered "as soon as possible" after exposure.[17] Without a means for efficiently and objectively measuring radiation exposure, the healthcare infrastructure will quickly be overwhelmed, and such decision-making would be uninformed and essentially impossible. The ERDS addresses these requirements for early rapid exposure sorting in a forward-deployed pre-hospital setting, which enables a fast, efficient, and locally-based response to an incident. This complements and enhances existing limited options of biodosimetry, other means of retrospective physical dosimetry, and symptom-based clinical triage. This will be made feasible by pre-deployment of ERDS cards (e.g., embedded in ID badges or as a stand-alone "companion" card) among first responders, military personnel, other government and private industry employees, and high-risk

[16] https://www.uniprot.org/.

[17] https://remm.hhs.gov/cytokines.htm.

civilians. In addition, pre-deployment/stockpiling of networkable Automated Dosimetry Readers (ADRs) that are easy to use by individual ERDS card holders (no training required, simply insert the card in the ATM-like ADR) will permit rapid high-throughput dosimetry assessments and allow subsequent ongoing re-testing for accumulated dose as needed. Networkability of ADR units will allow data flows to and from emergency operations centers. This will enable rapid evaluation of the demographic and geospatial ranges of exposure, enhance situational awareness, and allow Radiation SMEs to modify sorting cutoffs (e.g., up or down from the standard initial 2 Gy cutoff) based on healthcare resource availability.

The ADR is currently configured as a benchtop prototype, but deployable systems will be rugged for field deployment, portable, low-power-consumption machines that will provide end-users with quick (less than two minutes per person), easy-to-interpret onscreen instructions in response to the reading obtained from their EDRS card. Examples of such output instructions are, "evacuate & recheck in 24 hours," or "seek medical attention at [healthcare facility name and location]." The ADR employs a NIST-developed non-resonant broadband ESR technology that is 20,000 times more sensitive than standard commercial alanine-pellet ESR readers (*81*, *82*). The EDRS is poised to establish a rapid real-time dosimetry response system that can be implemented in the immediate aftermath of a radiation public health emergency. This project and system are an example of combining advanced scientific capabilities from the lab with the needs of emergency responders to create a solution that is responsive to the unique challenges of being in the field after such an incident. It will permit dynamic interpretation and response to such an event at the individual and the geospatial levels. This system will provide an additional level of data granularity and act in concert with already existing physical and medical dosimetry evaluation systems. Further development is ongoing to improve low dose sensitivity and response time of the dosimeter and ADR device to achieve an integrated field deployable EDRS.

Engineered probiotics to deliver therapeutics. By leveraging the natural adaptations of *Limosilactobacillus reuteri*, research is underway on a promising new system, by which therapeutics can be delivered to the GI tract (J. P. van Pijkeren). Select strains of *L. reuteri*, a naturally occurring gut symbiont, have undergone millions of years of evolution to thrive in mammalian GI tracts. Although many wild isolates are recalcitrant to genome editing, multiple genome editing tools have been developed for select *L. reuteri* strains. Armed with these new tools, the interaction of *L. reuteri* with other members of the microbiome can be assessed. Specifically, *L. reuteri* has been developed as a model to study bacteriophages and understand their basic biology in the GI tract. This research found that *L. reuteri* has multiple prophages (genetic material of bacteriophages) embedded in their genome, and these prophages are activated during GI tract transit, leading to the lysis of ~80% of the *L. reuteri* that passes through the GI tract (*83*, *84*).

Leveraging this discovery, utilizing phage-mediated lysis to deliver therapeutics to the GI tract has been explored. While using bacteria to deliver recombinant proteins is not a novel concept, it has previously relied on engineering complex secretory and signaling systems in the bacteria. Harnessing naturally occurring lysis simplifies the delivery system and leads to robust recombinant protein release. Furthermore, a lysis-based system contributes to the containment of the bacteria delivery vehicle, reducing the likelihood of GI tract colonization and reducing the load of live engineered microbes entering the environment. Therefore, the efficacy of this method of probiotic therapeutic delivery was tested in models of radiation survival. A single oral dose of *L. reuteri* engineered to release interleukin-22, a known radiation mitigator, significantly improved the survival of mice exposed to 9.25 Gy total-body irradiation (*85*, *86*). Moreover, interferon-β released through *L. reuteri* delivery also demonstrated a protective effect in mice exposed to total-body irradiation, whole-abdomen irradiation and partial-body irradiation (*87*). Due to the ultimate goal of human use, further safety considerations need to be investigated before the *L. reuteri* delivery system can be tested in larger models of irradiation. Although *L. reuteri* has not been observed to colonize the GI tract of the tested mouse models, the natural symbiont possesses the genes necessary to colonize the human gut if the niche is available. Therefore, a biocontainment strategy targeting adhesin genes is being developed to decrease colonization likelihood.

Microbial-based delivery systems hold the potential for therapeutic delivery due to a combination of unique advantages. First, microbial systems are cheap, partially because producing purified recombinant protein is not necessary. Furthermore, they can be delivered through oral administration. Additionally, they have months of shelf-life in a lyophilized state and can be recovered with high viability. *L. reuteri* is poised to be one of the primary models for GI therapeutic delivery because it has already evolved to thrive in GI systems and therefore does not need any additional genetic editing for survival purposes. *L. reuteri*'s phage-mediated pathway also overcomes the bottlenecks of secretory and signaling pathways and contributes to containment. Finally, *L. reuteri* exhibits a very low natural mutation rate compared to other gram-positive bacteria providing increased genetic stability and possibly safety. Moving forward, there are still many steps before *L. reuteri* can be used for clinical therapeutic delivery, and safety and efficacy studies are needed in more advanced models, such as swine and NHPs. However, the research so far highlights the potential of this novel therapeutic delivery system to treat many diseases, including ARS.

A novel ex vivo human fascio cutaneous flap perfusion model to investigate radiation-induced injuries. Human-derived model systems to study skin injury provide a new

frontier for more representative injury research, especially for radiobiology. To that end, a full-thickness human skin model is being developed, which recapitulates the physiological repercussions of cutaneous radiation exposure and could be applied to other areas of injury study (A. Ejaz). The need for new human-based models to study skin injury are manifold. While in vitro tissue culture and organoid models can represent human biology, they are often still too simplistic to accurately replicate pathophysiological processes and responses. Although in vivo animal models address these concerns, there are drawbacks. Primary among these challenges are species-specific physiological and behavioral differences. For example, mouse and human skin vary anatomically, including differences in dermal thickness, distribution of hair follicles, extracellular matrix composition, and other factors. Swine skin is much more comparable to human skin, but with this increase in animal size comes increased costs and ever-present behavioral differences, which can lead to variability in wound research.

For this reason, a human cutaneous flap perfusion model derived from the skin removed during abdominoplasty has been developed. The skin removed in this procedure is normally considered surgical waste; however, the tissue flap can be successfully perfused through the superficial arterial and venous system. A bioreactor that semi-automatically regulates temperature, pH, oxygen, and humidity can then be employed to continuously perfuse this tissue flap, creating a non-submerged $\sim$16 $\times$ 16-inch human skin model that is viable for $\sim$4 weeks. This model's basic physiological function and structure are preserved for over 12 days, as can be seen by a stable metabolic rate and realistic gene expression recovery after perfusion stress. Furthermore, various physiological challenges demonstrate that the vasculature and metabolic activity of the model remains responsive to stimuli at least 12 days after establishment. Moreover, no major histological differences or cell damage were detected at the 12-day time point. (A. Ejaz).

The previous observations confirm the stability, reliability, and reproducibility of this human skin model, allowing for pathophysiological applications including radiation, chemical, thermal, trauma, and combined radiation injury research. A primary area of investigation among these possible applications is CRI. X-ray doses of 20 and 40 Gy were tested in the model. At day six postirradiation, approximately 80% cell death was found in the dermal layer, as visualized by Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining. By day 12, both radiation doses led to the loss of the epidermal layer. Genes commonly found to be upregulated after radiation injury, including those coding for interleukins and NF-κB, were also significantly elevated in the skin model after irradiation. Continuing validation of this model for radiation injury research will be performed to confirm reproducibility, which would allow for studies investigating mitigators to radiation injury, such as metformin and JP4-039 (a mitochondrial-targeted nitroxide under investigation as a radiation MCM for ARS (*88*) and skin injuries (*89*). Beyond radiation injury, further studies have been performed looking at chemical-induced skin injury with nitrogen mustard, which has shown promising results that recapitulate the expected response to such an injury (A. Ejaz). This model is also being tested in other wound and injury situations to demonstrate the system's flexibility. The promising results highlight the feasibility of complex human-based models for radiation injury that avoid the pitfalls of the common research paradigms.

Applications of machine learning in transcriptomics-based radiation biodosimetry. A large-scale radiation exposure event will necessitate rapid and accurate radiation dose assessment to triage and treat exposed individuals. Current biodosimetry methods, such as the dicentric chromosome assay and measurement of micronuclei in human peripheral blood (HPBL), are time-consuming and require sophisticated equipment and highly trained personnel. Therefore, scalable biodosimetry approaches, including gene expression profiles in peripheral blood cells, are being developed (*90*). The ML algorithms have long been central to analyzing transcriptomic data and classifying samples based on gene expression profiles. Studies to evaluate changes in radiation-induced gene expression profiles as a rapid biodosimeter to determine acute- and long-term risks to exposed individuals have been carried out. Using whole genome microarray expression profiling as a discovery platform to identify genes in irradiated ex vivo HPBL, a 74-gene signature has been established that can discriminate four radiation doses (0.5, 2, 5 and 8 Gy) from controls at six and 24 hours after radiation exposure (*91*). Thus, the ability of a single gene set using a nearest centroid classifier model (i.e., an ML classification model that assigns to observations that label the class of training samples whose mean is closest to the observation) to reliably predict radiation dose throughout a window of time without the need for individual pre-exposure controls represented an essential advance in the development of gene expression algorithms for rapid biodosimetry.

Such ML algorithms could be used in various accidental radiation exposure scenarios, including internal contamination. For example, in nuclear reactor accident fallouts, exposure from ingested or inhaled Cs-137 for an extended duration can have serious health consequences. Global gene expression in the blood of male C57BL/6 mice injected with Cs-137 has been profiled, and the data were analyzed at various post-exposure time points. Depending on the time of exposure, 466–6,213 genes were differentially expressed after Cs-137 administration. At early time points, most responsive genes were expressed above the control levels, while at later times (20–30 days) most responding genes were expressed below control levels. This pattern was consistent with significantly enriched gene ontology categories, including those related to nucleotide binding, protein localization and modification, actin and the cytoskeleton, and the integrin signaling canonical pathways (*92*). More recent studies identified both dose-rate-independent and -dependent gene expression responses in

mice after Cs-137 exposure. Clustering genes by pattern provided additional insights into possible drivers of the dynamic transcriptome response in vivo. The biological response of blood cells to internal radiation exposures is an essential step toward understanding the effects of internal contamination after a nuclear event (*93*).

Overcoming mouse-to-man translational barriers is a crucial need in biodosimetry. To fill this gap, a robust NHP biodosimetry model was also built using interspecies-correlated genes, in which the absorbed dose in human samples could be accurately predicted (*94*). In higher-order mammalian models, NHP rhesus macaques and ex vivo HPBL models that compared gene expression profiles 24 hours after exposure to different radiation doses showed a highly correlated expression of 52 genes between the species. Predominantly, these genes consisted of p53/DNA damage response, apoptosis, and cell cycle-related genes. Using NHP data, this algorithm showed a mean prediction accuracy of about 90% within 1 Gy of the delivered dose in "leave-one-out cross-validation," a standard approach to validate prediction algorithms (*94*). However, tests on human samples indicated that human gene expression values may need to be adjusted before using the NHP model for practical biodosimetry applications. A multi-gene approach utilizing all gene values for cross-species conversion and applying the converted values on the NHP models gave a leave-one-out cross-validation prediction accuracy for human samples highly comparable (up to 94%) to that for NHPs (*94*).

This generalized statistical method can be applied to existing transcriptomic datasets to develop a signature for dose reconstruction for TBI with photons. A proof-in-principle study using statistical methods to select radiation-responsive genes to generate quantitative rather than categorical radiation dose reconstructions based on a blood sample was recently described in which a normalization method is used to reduce the effects of variability of signal intensity in unirradiated samples across studies (*95*). This dose reconstruction biomarker was trained using two datasets and tested on two independent datasets. It was able to reconstruct a dose of up to 4.5 Gy on a test dataset using the same platform and up to 6.0 Gy on a test set using a different platform. This gene set classifier shows excellent promise for reconstructing individual radiation doses and may be developed further to be informative for the more complex exposures likely to be encountered in a realistic radiological or nuclear event (*95*). The performance of these dose prediction algorithms can be further amplified by a stacking approach, in which several different ML algorithms are used to build the model. This approach shows promise in dose reconstruction; however, performance still needs to be improved at higher doses.

Development of ligand-targeted drugs for radio-, chemo- and immunotherapies of cancer, fibrotic and infectious diseases. Methods are being explored to target drugs specifically to diseased cells, thereby avoiding collateral toxicity to healthy cells. To achieve this specificity, ligands that bind selectively to pathologic cells are linked to drugs that can treat or image the associated diseases (Fig. 4). The drugs are designed to be endocytosed after binding to a receptor on a diseased cell and consist of small molecules (*96*) and antibodies (*97*). This class of drugs could target radioactivity, suppress fibrosis, and reprogram the immune system. For example, agents targeting prostate-specific membrane antigen (PSMA) with high affinity and specificity were developed for imaging and therapy of metastatic castration-resistant prostate cancers, a significant cause of mortality and morbidity in Western society. PSMA is highly expressed in castration-resistant prostate cancer. Lutetium-177 (Lu-177)-PSMA-617, a radioligand that delivers beta radiation specifically to PSMA-expressing cells, was found to prolong progression-free survival when added to the standard-of-care in patients in an open-label, Phase 3 clinical trial (*98*). The U.S. FDA awarded the drug, Lu-177 Vipivotide tetraxetan (Pluvicto) breakthrough status in June 2021 and subsequently was approved in March 2022.[18]

Ligand targeting approaches can also be used to suppress fibrosis. Fibroblast-activation-proteins (FAP) are overexpressed by cancer-associated fibroblasts of several tumor types. Quinoline-based PET tracers that act as FAP inhibitors demonstrated promising results in several cancer types (*99*). One of the consequences of radiation exposure, therapeutic (*1*, *100*) or accidental (*101*), is lung fibrosis. As such, three different approaches to treat fibrosis in the lung are being considered. Using idiopathic pulmonary fibrosis (IPF) as a model, a method has been developed to shut down collagen production in myofibroblasts by the targeted delivery of PI3Ki with FAP, to increase efficacy and reduce systemic toxicities. Immunotherapies of tumors have also demonstrated promise; however, immunosuppressive cells, such as tumor-associated macrophages (TAMs) in the tumor microenvironment prevent the infiltration of immune cells' anticancer functions. Selective reprogramming of the TAMs, with targeted delivery of a drug payload, can improve immunotherapies of several cancers and enable a precision medicine approach at a cellular level. Thus, reprogramming immune cells makes it possible to modulate immune responses and improve outcomes in many diseases, and drugs can be targeted with small molecules to most pathologic cell types to increase potency and reduce toxicities. Targeted delivery of agents can also enhance imaging, and targeted reprogramming of immune cells can help treat most diseases. There remains the need to carefully consider the PK and PD of targeted drugs, since some products may have to be released slowly over weeks vs. others requiring rapid release. Improving therapeutic index with ligand-targeted drugs has been around for a long time, and the pitfalls and significant hurdles from the discovery to U.S. FDA approval and implementation

[18] https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-pluvicto-metastatic-castration-resistant-prostate-cancer.

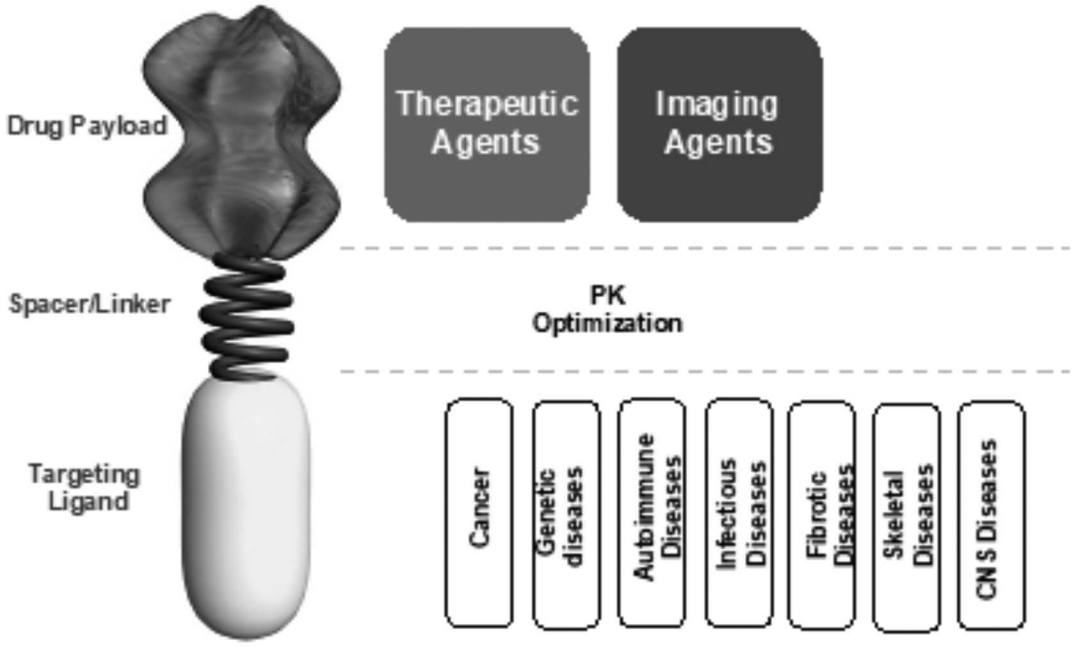

FIG. 4. Model of ligand-targeting for drug pharmacodynamic optimization. Model shows a potential drug payload (e.g., therapeutic or imaging agent) adjoined via a linker to a ligand target. The process of PK optimization allows ligand-targeted drugs for radio-, chemo- and immune-therapies for various diseases to be developed. Used with permission P. Low.

appear to be a loss of specificity, which can be addressed with this novel technology.

SESSION IV: DISCUSSION

Session IV discussions surrounded the notion that mastering advanced technologies has the potential to transform current modeling systems into robust tools. The discussions generally addressed: 1. Are current physical and digital model systems sufficiently versatile to support advanced technology development? 2. What technical challenges need to be overcome to advance the development of new technologies? 3. Will these model systems be appropriate/adequate for U.S. FDA submissions involving the development of MCMs? 4. Are there any unique regulatory challenges for the successful development of technologies into MCMs or commercial products? and 5. Besides funding, are there constraints in developing these systems, such as finding technically knowledgeable partners in the extramural research community?

The conversation started with whether any speakers had introduced their technology to the U.S. FDA, specifically as an alternative to animal models for device/drug approval. The human ex vivo cutaneous flap perfusion model is the closest model system to in vivo human research that is not in in vivo animal models. While the ex vivo model may lack the involvement of complete systems, it is human-derived, and its structure, composition, and functions essentially mimic human skin. It was noted that non-in vivo models will most likely not be feasible or recapitulate the totality of interactions occurring in an animal model, for example, modeling the effects of gut microbiota. In vivo animal models are the standard, even with their downsides, because they allow addressing the impact of variables under controlled conditions. However, research is iterative, and U.S. FDA regulations evolve along with advances in research and technology; therefore, scientists should continue developing model systems for MCM development.

The discussion then shifted to the importance of the correlation of the composition of the microbiota to health and disease. One problem with the current understanding of the microbiome is the need for mechanistic insights into whether the changes in the microbiota cause or are the result of disease. Studies must use correct models and systems to address this issue. Data from mice often do not correlate at all with humans. Further, the impact of the microbiome on data needed for the drug approval processes is also underdeveloped (i.e., the microbiome by itself could contribute to the disease, response to a treatment, and/or serve as a biomarker); all are pertinent to the development of MCMs. Therefore, the microbiome of the organ under study should be considered during MCM evaluation, as it is an additional variable. With the evolution of sequencing, there is an opportunity to incorporate microbiome research further and leverage it for therapeutic benefit.

While models such as a cutaneous perfusion model provide an opportunity to advance MCM research, the lack of innervation and the need to address the influence of microbiota present additional challenges. Although some antibiotics are used in the perfusion media, some resident microbiota will still be present, and the composition will vary spatially along the model's thickness and temporally

during the experiment. In addition, the absorption of the drugs will also vary across the depth. A steady change in the nutritional requirements and degradation of the model under ex vivo conditions will also present additional challenges, which could be overcome by performing longitudinal analyses of biopsies for time-dependent degradation.

Lung fibrosis is a DEARE complication after significant radiation exposure. The specificity and efficacy of the ligand-based targeting approach to mitigate fibrosis is critical. In this context, there is still a need to conduct longitudinal interventional studies where the specificity and efficacy of the ligand-targeted antifibrotics are tested. Discovery, development, and validation of suitable biomarkers to determine this transition from pro- to anti-inflammatory responses will be crucial. However, to effectively mitigate fibrosis, antifibrotic agents may have to be administered as early as possible, before the onset of pathogenesis, because once fibrosis is onset, it is generally irreversible.

It is essential to understand the effect of the number of genes on the performance of the gene expression analysis biodosimetry model for its ability to predict dose in the presence of other confounders, especially metal toxicities or internal radioisotope exposures compared to external beam irradiations. However, the gene expression models were initially developed with less than 20–30 genes. Separating radiation toxicities from metal toxicities or internal exposures from radioisotopes such as plutonium or cesium is difficult due to experimental limitations. Further, since gene expression analysis in a peripheral blood model for biodosimetry involves RNA extraction (which is inherently destructive), it is difficult to accurately determine the assay's performance characteristics, such as specificity, sensitivity, and dynamic operating range. Furthermore, clustered gene expression network heatmap analysis may be essential to distinguish between reversible and unidirectional and irreversible changes. While complex, single-cell RNAseq dose-response, time-course studies in a peripheral blood model may reveal differential responses in different blood cell subpopulations that may be useful in understanding radiation-induced immune modulation.

The application of CRISPR technology for gene editing to restore tissue function homeostasis after radiation exposure is an open area of research, as tissue function involves a multiprotein system with built-in redundancies to keep the system balanced. One key challenge in using CRISPR-based gene editing is understanding the effect of combinations, confounders, and the time course to execute such studies. For many targets, sustaining a protein or enzyme activation with CRISPR technologies for too long may also be challenging.

The discussants also shared a few personal stories highlighting how the research and clinical studies performed touched the lives of many in different ways and stressed the importance of the work in MCM development with cross utilities in cancer research and clinic.

WORKSHOP OPEN DISCUSSION

As a wrap-up to the two days of presentations, workshop speakers and attendees were invited to engage in an open discussion of the technologies and ideas explored throughout the workshop. To open the session, the group was asked to consider what elements are critical to successfully developing new therapies for radiation injury. An initial response addressed the need to identify robust signatures/biomarkers for radiation damage, one of which is miRNA, which was discussed in some detail in various contexts throughout the meeting. Understanding signatures of effect can aid in identification of appropriate targets for therapeutic development. This effort would require support for large "omics" studies and could also leverage data mining from sources such as ImmPort, GeneLab, and other data repositories. Suggestions on how to best develop these endeavors centered around identifying and supporting networks of data scientists to pursue goal-driven data mining for radiation signature-specific therapeutics that can then be experimentally validated. It was also suggested that pursuit of small molecule-based therapeutics alone may not address or reverse the multiorgan nature of radiation injuries, and efforts involving cellular therapies should not be overlooked. Finally, it is unlikely that a single or universal signature/biomarker of radiation injury can be obtained through "omics" data mining, due to variability of individual exposures and the dynamic nature of injury progression. Therefore, predictive markers of specific outcomes (e.g., lung fibrosis, heart injury, etc.) would be of high value to identify therapeutics for effective interventions.

The need to consider the limitations of the different models, especially animal models, was raised as an important consideration in therapeutic development. A multispecies approach has demonstrated some success in biomarker identification and development. Using new highly multiplexed technologies for proteomics and genomics studies (e.g., $\geq$7,000-plex) that have multispecies capabilities and can be leveraged for radiation injury may reduce the selection of biomarker signatures that might not translate well from one model species to another, and especially to humans. Such highly multiplexed biomarker signatures, including those derived from database mining, could be integrated with various organ injury-specific models to develop sets of biomarker signatures that can be informative for different organ injuries and how they develop over time postirradiation. If achievable, such information may be useful for field-deployable triage applications, and for identifying useful targets for therapeutic development.

Discussion of how to interrogate various model systems for signatures of radiation exposure raised the important question of how to establish that experimental conditions for delivery of radiation dose are consistent and reproducible, either between individual experiments or among different institutions. This is also a question that bears investigation for differing radiation doses, and possibly dose rates, within

individual models, and how this may affect potential biomarker signatures. While efforts are ongoing by the RNCP to assess and harmonize dosimetry within the radiation biology community, meeting participants acknowledged the challenges of variable responses to radiation injury within model organisms and the need to take this into account when developing biomarker signatures. This conversation also suggested that cross-model validation and standardization be used, including for MPS models, and some validation be done using candidate products. With respect to characterization of one MPS technology, the human bone marrow chip, testing and validation were done in the context of 5-fluorouracil administration, and the chip recapitulated the clinical bone marrow response, especially the cytopenia seen in patients. However, to fully validate the model it would be necessary to test responses to multiple drugs with different mechanisms of action and confirm clinically relevant responses. Regarding the radiation response of the bone marrow chip model, when tested with G-CSF, it was shown to recapitulate G-CSF-driven neutrophil recovery. Currently, it is unclear whether developing and validating animal model MPS with direct comparisons to in vivo responses in the animal would be informative for the performance of human model MPS and whether they can fully recapitulate the human condition. However, some of the work presented in this workshop has begun to address this question and promising results have been obtained using this approach. The group's overall opinion is that even if the MPS approaches do not recapitulate all aspects of every in vivo situation, data obtained using these approaches will be highly valuable, and expansion of the technologies available to investigate radiation injuries will provide new insights into biological responses.

Some takeaways from the open discussion were centered on the need to more closely integrate members of the computational community working with AI, ML, and "omics" data, with the researchers in the radiation biology community who are engaged in wet lab experimental work. Such efforts are underway at NASA, with working groups analyzing GeneLab data and biological samples obtained from astronauts and animals that have flown on the International Space Station. This approach was suggested as a model to further expand such efforts into the radiation biology community. The idea of normalizing the use of AI and ML as a commonplace part of the radiation biology scientific toolbox and workflow process, by incorporating of computational biologists and physicists as part of resource cores for large multi-project programs was also recommended. This approach may help address what the discussants perceived as a gap in the current applicability of experimental data for more effective incorporation into AI and ML datasets, and for AI and ML to become readily available tools for experimentalists to employ in their investigations.

CONCLUSION

The overarching workshop goal of engaging with multiple research institutions in a common mission to explore cutting-edge technologies to increase the development of products for use in the radiation space was achieved, in part through strong collaboration among the participating U.S. Government funding agencies and non-government research partners. With a recent surge in advanced methodologies and platforms, such as CRISPR-based gene editing, tissue chips, ML/AI, and imaging techniques that span across multiple scientific areas of focus, the radiation biology field has benefitted from these advances and many researchers in the field have been leaders in developing some of the new and relevant approaches highlighted during the meeting. The valuable meeting presentations and discussions helped identify challenges and gaps for researchers to address and have broadened awareness of opportunities to leverage these exciting new technologies in ongoing and future investigations. Ultimately, government partners and the research community will continue to move forward with this added knowledge, to further support and advance these approaches as potential future platforms to aid in the clearance/approval/licensure of MCMs for radiation injury.

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