SPECIAL THANKS TO

Winthrop Rockefeller Charitable Trust

AND OUR OTHER MAJOR SPONSORS

[Logos of UAMS College of Pharmacy and DRH]

CONFERENCE PARTNERS

[Logos of UAMS and Winthrop Rockefeller Institute]

# Table of Contents

Welcome .............................................................................................5
Agenda...............................................................................................6
Speaker Biographies ........................................................................10
Chairperson Biographies ....................................................................13
Space Radiation: Plenary Speaker Abstracts ......................................15
  - Deficits in Attention and Social Recognition Memory Following Space Radiation Exposure ..........................................................17
  - Effects of Low-dose Radiation on Neurovascular Remodeling in the Mouse Brain and Eye ..............................................................19
  - Do You Have the Guts to Travel to Mars? Understanding Gastrointestinal Effects of Space-like Radiation ........................................21
Oral Presentation Abstracts .................................................................23
  - Radiation-induced Non-targeted Changes in Arterial Tissue Mechanics .................................................................25
  - The Effects of Obesity on Intestinal Health after Radiation Exposure .........................................................................................27
  - Kruppel-like Factor 2 (KLF2): A Novel Radiation Target is Suppressed in the Mouse Intestine ............................................................29
  - Aberrant TLR4 Signaling Promotes Radiation-induced Intestinal Injury in Cebpd-deficient Mice ..........................................................31
Radiological Emergencies: Plenary Speaker Abstracts .........................33
  - Cell Therapy Treatment for Patients with Severe Radiotherapy Sequelae: Lessons Learned from the Radiotherapy Accident of Epinal, France ...................................................35
  - Delayed Effects of Acute Radiation Exposure on Normal Tissues: Insights from Nonhuman Primate Studies ........................................37
  - Against Fat Man and Little Boy: A Sequel ...........................................39
Oral Presentation Abstracts .................................................................41
  - Neulasta Regimen for the Hematopoietic ARS: Effects Beyond Neutrophil Recovery .................................................................43
  - BBT-059, a Potential Prophylactic Radiation Countermeasure as well as a Mitigator for Acute Radiation Injury, Protects Bone Marrow and Spleen .........................................................45
  - Delayed Effects of Acute Radiation Exposure in BBT-059 Treated Survivors .................................................................47
  - Anti-ceramide Antibody as Treatment for the Acute Radiation Gastrointestinal Syndrome ...........................................................49
Survival Risk Modeling as a Function of Related Histological and Functionality End Points in Radio Induced Gastro-intestinal Syndrome (RIGS): a Monte Carlo-based Cox Model Approach .....................51
  - Circulating Ano1 RNA as a Biodosimeter and Indicator of Radiation-induced Gastrointestinal Toxicity ............................................53
  - Gamma-Tocotrienol Restores Mucosal Barrier Integrity in Mice after Total Body Irradiation ..............................................................55
  - Design and Development of Novel Structure Based Vitamin E Analogues, the Tocoflexols, as Radiation Protectors ..........................57
Post-therapy Side Effects: Plenary Speaker Abstracts .........................59
  - Dissecting the Role of p53 in Mediating Normal Tissue Injury Following Radiation and Radiation Carcinogenesis ..........................61
The Use of Consomic Rat Models to Identify Factors that Modulate Normal Tissue Radiation Sensitivity ................................................................. 63
Metabolomics Pre-empts Radiation Induced Antecedent Tissue Injury in Hippocampal Tissue .......... 65
Oral Presentation Abstracts ................................................................................................................................................. 67
A Personalized, In Vivo Method to Quantify the Number and Repair of DNA Base Changes Induced by Radiation and Other Carcinogens ........................................................................................................ 69
Computing Regional Myocardial Radiation-Dose Response in Left-sided Breast Cancer Patients ........ 71
FGF-P: A Mimetic Biobetter for Mitigation of Gastrointestinal Syndrome ................................................................. 73
Design and Development of I-PARTS, an Integrated Platform for Anti-cancer Radiation Therapeutics Screening ................................................................................................................................. 75
Development of Partial Body Irradiation Model Using Small Animal Radiation Research Platform .... 77
Loss of Sirt3 Exacerbates IR-induced Liver Injury ...................................................................................................................... 79
Dietary Methionine Modulates the Gastrointestinal Response to Radiation ................................................. 81
Greetings and welcome to the Conference on Normal Tissue Radiation Effects and Countermeasures (CONTREC), a Winthrop Rockefeller Conference. We are honored that you have chosen to join us here at the Winthrop Rockefeller Institute atop beautiful Petit Jean Mountain.

Our program this year—which we hope will build upon the success of our 2015 conference—focuses on side effects of radiation during and after cancer therapy; radiological and nuclear emergencies; and exposure to radiation in space. These important areas of inquiry represent some of the outstanding work being done in the state of Arkansas, and we are eager to learn more about them from our distinguished lineup of speakers and presenters.

CONTREC is held in partnership with the University of Arkansas for Medical Sciences’ Division of Radiation Health and the Winthrop Rockefeller Institute. This conference is made possible by generous support from the Winthrop Rockefeller Charitable Trust with additional major sponsorships from the Division of Radiation Health and the UAMS College of Pharmacy.

Throughout the conference, we hope that you take advantage not only of the opportunities to learn about new research and emerging technologies, but also to connect with colleagues and develop relationships that will benefit you both personally and professionally. This conference links the best and brightest from Arkansas and the mid-South with leading researchers from around the country and the world. We hope you find your time here this week to be beneficial and invigorating.

With warm regards,

Martin Hauer-Jensen, M.D., Ph.D.   Marta Loyd, Ed.D.
Director, Division of Radiation Health   Executive Director
University of Arkansas for Medical Sciences  Winthrop Rockefeller Institute
### MONDAY, MAY 14

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>2:00 p.m.</td>
<td>Registration/Check-in – Front Lobby</td>
<td></td>
</tr>
<tr>
<td>6:00 p.m.</td>
<td>Welcome reception – President’s Lodge Great Room</td>
<td></td>
</tr>
<tr>
<td>8:00 p.m.</td>
<td>Movie – Rock Theater</td>
<td></td>
</tr>
</tbody>
</table>

### TUESDAY, MAY 15

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>8:00 a.m.</td>
<td>Breakfast – River Rock Grill</td>
<td></td>
</tr>
<tr>
<td>9:00 a.m.</td>
<td>Welcome by Dr. Marta Loyd and opening remarks by Janet Harris and Dr. Martin Hauer-Jensen – Rock Theater</td>
<td></td>
</tr>
</tbody>
</table>

**Space Radiation: Plenary Presentations**
Chaired by Dr. Marjan Boerma, UAMS Division of Radiation Health
Co-chaired by Dr. Igor Koturbash, UAMS Department of Environmental & Occupational Health

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
<th>Speaker</th>
<th>Institution</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>9:15 a.m.</td>
<td>Deficits in Attention and Social Recognition Memory Following Space Radiation Exposure</td>
<td>Catherine Davis-Takacs, Ph.D.</td>
<td>Johns Hopkins Medicine, Bethesda, MD, U.S.A.</td>
<td>Rock Theater</td>
</tr>
<tr>
<td>9:50 a.m.</td>
<td>Effects of Low-dose Radiation on Neurovascular Remodeling in the Mouse Brain and Eye</td>
<td>Xiao Wen “Vivien” Mao, M.D.</td>
<td>Loma Linda University, Loma Linda, CA U.S.A.</td>
<td>Rock Theater</td>
</tr>
<tr>
<td>10:25 a.m.</td>
<td>Do You Have the Guts to Travel to Mars? Understanding Gastrointestinal Effects of Space-like Radiation</td>
<td>Igor Koturbash, M.D., Ph.D.</td>
<td>University of Arkansas for Medical Sciences, Little Rock, AR, U.S.A.</td>
<td>Rock Theater</td>
</tr>
<tr>
<td>10:55 a.m.</td>
<td>Break – Flagstone Foyer</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Space Radiation: Oral Presentations**

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
<th>Speaker</th>
<th>Institution</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>11:10 a.m.</td>
<td>Radiation-induced Non-targeted Changes in Arterial Tissue Mechanics</td>
<td>Sourav S. Patnaik, Ph.D.</td>
<td>University of Texas at San Antonio, San Antonio, TX, U.S.A.</td>
<td>Flagstone Foyer</td>
</tr>
<tr>
<td>11:30 a.m.</td>
<td>The Effects of Obesity on Intestinal Health After Radiation Exposure</td>
<td>Laura E. Ewing, M.S.</td>
<td>University of Arkansas for Medical Sciences, Little Rock, AR, U.S.A.</td>
<td>Flagstone Foyer</td>
</tr>
<tr>
<td>11:50 a.m.</td>
<td>Kruppel-like Factor 2 (KLF2): A Novel Radiation Target is Suppressed in the Mouse Intestine</td>
<td>Rupak Pathak, Ph.D.</td>
<td>University of Arkansas for Medical Sciences, Little Rock, AR, U.S.A.</td>
<td>Flagstone Foyer</td>
</tr>
<tr>
<td>12:10 p.m.</td>
<td>Aberrant TLR4 Signaling Promotes Radiation-induced Intestinal Injury in Cebpd-deficient Mice</td>
<td>Sudip Banerjee, Ph.D.</td>
<td>University of Arkansas for Medical Sciences, Little Rock, AR, U.S.A.</td>
<td>Flagstone Foyer</td>
</tr>
<tr>
<td>Time</td>
<td>Event</td>
<td>Presenter</td>
<td>Institution/Location</td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>----------------------------------------------------------------------</td>
<td>-----------------------------------------------</td>
<td>---------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>12:30 p.m.</td>
<td>Effects of Space Radiation on Inflammatory Infiltration in the Heart</td>
<td>Vijayalakshmi Sridharan, Ph.D.</td>
<td>University of Arkansas for Medical Sciences, Little Rock, AR, U.S.A.</td>
<td></td>
</tr>
<tr>
<td>12:50 p.m.</td>
<td>Lunch – River Rock Grill</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:50 p.m.</td>
<td>Cell Therapy Treatment for Patient with Severe Radiotherapy Sequelea: Lesson Learned from the Radiotherapy Accident of Epinal, France</td>
<td>Marc Benderitter, Ph.D.</td>
<td>IRSN, Fontenay-aux-Roses, France</td>
<td></td>
</tr>
<tr>
<td>2:25 p.m.</td>
<td>Delayed Effects of Acute Radiation Exposure on Normal Tissues: Insights from Nonhuman Primate Studies</td>
<td>Mark Cline, D.V.M., Ph.D., DACVP</td>
<td>Wake Forest University, Winston-Salem, NC, U.S.A.</td>
<td></td>
</tr>
<tr>
<td>3:00 p.m.</td>
<td>Against Fat Man and Little Boy: A Sequel</td>
<td>Nukhet Aykin-Burns, Ph.D.</td>
<td>University of Arkansas for Medical Sciences, Little Rock, AR, U.S.A.</td>
<td></td>
</tr>
<tr>
<td>3:30 p.m.</td>
<td>Break – Flagstone Foyer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3:40 p.m.</td>
<td>Neulasta Regimen for the Hematopoietic ARS: Effects Beyond Neutrophil Recovery</td>
<td>Maria Moroni, Ph.D.</td>
<td>AFRRI/USUHS, Bethesda, MD, U.S.A.</td>
<td></td>
</tr>
<tr>
<td>4:00 p.m.</td>
<td>BBT-059, a Potential Prophylactic Radiation Countermeasure as Well as a Mitigator for Acute Radiation Injury, Protects Bone Marrow and Spleen</td>
<td>Sanchita Ghosh, Ph.D.</td>
<td>AFRRI/USUHS, Bethesda, MD, U.S.A.</td>
<td></td>
</tr>
<tr>
<td>4:20 p.m.</td>
<td>Delayed Effects of Acute Radiation Exposure in BBT-059 Treated Survivors</td>
<td>Neel Sharma, Ph.D.</td>
<td>AFRRI/USUHS, Bethesda, MD, U.S.A.</td>
<td></td>
</tr>
<tr>
<td>4:40 p.m.</td>
<td>Anti-ceramide Antibody as Treatment for the Acute Radiation Gastrointestinal Syndrome</td>
<td>Vijay Singh, Ph.D.</td>
<td>AFRRI/USUHS, Bethesda, MD, U.S.A.</td>
<td></td>
</tr>
<tr>
<td>5:00 p.m.</td>
<td>Survival Risk Modeling as a Function of Related Histological and Functionality End Points in Radio Induced Gastro-intestinal Syndrome (RIGS): a Monte Carlo-Based Cox Model Approach</td>
<td>Mohamed Benadjaoud, Ph.D.</td>
<td>IRSN, Fontenay-aux-Roses, France</td>
<td></td>
</tr>
</tbody>
</table>
## TUESDAY, MAY 15 (CONT.)

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
<th>Presenter &amp; Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>5:20 p.m.</td>
<td>Circulating Ano1 RNA as a Biodosimeter and Indicator of radiation-induced Gastrointestinal Toxicity</td>
<td>Sadasivan Vidyasagar, M.B.B.S., Ph.D. University of Florida Health, Gainesville, FL, U.S.A.</td>
</tr>
<tr>
<td>5:40 p.m.</td>
<td>Gamma-Tocotrienol Restores Mucosal Barrier Integrity in Mice after Total Body Irradiation</td>
<td>Sarita Garg, Ph.D. University of Arkansas for Medical Sciences, Little Rock, AR, U.S.A.</td>
</tr>
<tr>
<td>6:00 p.m.</td>
<td>Design and Development of Novel Structure Based Vitamin E Analogues, the Tocoflexols, as Radiation Protectors</td>
<td>Ujwani Nukala University of Arkansas at Little Rock, Little Rock, AR, U.S.A.</td>
</tr>
</tbody>
</table>

**Networking Reception – River Rock Grill Bar**

**Dinner – River Rock Grill**

**Networking Reception – River Rock Grill Bar**

## WEDNESDAY, MAY 16

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
<th>Presenter &amp; Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>8:00 a.m.</td>
<td>Breakfast – River Rock Grill</td>
<td></td>
</tr>
<tr>
<td>9:00 a.m.</td>
<td>Dissecting the Role of p53 in Mediating Normal Tissue Injury Following Radiation and Radiation Carcinogenesis</td>
<td>David Kirsch, M.D., Ph.D. Duke University, Durham, NC, U.S.A.</td>
</tr>
<tr>
<td>9:35 a.m.</td>
<td>The Use of Consomic Rat Models to Identify Factors that Modulate Normal Tissue Radiation Sensitivity</td>
<td>Carmen Bergom, M.D., Ph.D. Medical College of Wisconsin, Milwaukee, WI, U.S.A.</td>
</tr>
<tr>
<td>10:10 a.m.</td>
<td>Metabolomics Pre-empts Radiation Induced Antecedent Tissue Injury in Hippocampal Tissue</td>
<td>Amrita Cheema, Ph.D. Georgetown University, Washington, D.C., U.S.A.</td>
</tr>
<tr>
<td>10:40 a.m.</td>
<td>Break – Flagstone Foyer</td>
<td></td>
</tr>
</tbody>
</table>

**Post-therapy Side Effects: Oral Presentations**

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
<th>Presenter &amp; Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>10:55 a.m.</td>
<td>A Personalized, In Vivo Method to Quantify the Number and Repair of DNA Base Changes Induced by Radiation and Other Carcinogens</td>
<td>Paul Okunieff, M.D. University of Florida Health, Gainesville, FL, U.S.A.</td>
</tr>
</tbody>
</table>
### WEDNESDAY, MAY 16 (CONT.)

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
</tr>
</thead>
</table>
| 11:15 a.m. | **Computing Regional Myocardial Radiation-dose Response in Left-sided Breast Cancer Patients**  
Shruti Kumar, Ph.D.  
AFRRI/USUHS, Bethesda, MD, U.S.A. |
| 11:35 a.m. | **FGF-P: A Mimetic Biobetter for Mitigation of Gastrointestinal Syndrome**  
Steven Swarts, Ph.D.  
University of Florida Health, Gainesville, FL, U.S.A. |
| 11:55 a.m. | **Design and Development of I-PARTS- An Integrated Platform for Anti-cancer Radiation Therapeutics Screening**  
Rao Papineni, Ph.D.  
University of Kansas Medical Center, Kansas City, KS, U.S.A. |
| 12:15 p.m. | **Development of Partial Body Irradiation Model Using Small Animal Radiation Research Platform**  
Vidya Kumar, Ph.D.  
AFRRI/USUHS, Bethesda, MD, U.S.A. |
| 12:35 p.m. | **Loss of Sirt3 Exacerbates IR-induced Liver Injury**  
Kimberly Krager, Ph.D.  
University of Arkansas for Medical Sciences, Little Rock, AR, U.S.A. |
| 12:55 p.m. | **Dietary Methionine Modulates the Gastrointestinal Response to Radiation**  
Isabelle Miousse, Ph.D.  
University of Arkansas for Medical Sciences, Little Rock, AR, U.S.A. |
| 1:15 p.m. | Lunch – River Rock Grill |
| 2:15 p.m. | Networking/Sightseeing  
Art in its Natural State Walking Tour – led by Payton Christenberry  
Walks with the Governor Bus Tour – led by Janet Harris |
| 6:00 p.m. | Dinner – Show Barn Hall |
| 7:30 p.m. | Bluegrass band, Runaway Planet – River Rock Grill Bar |

### THURSDAY, MAY 17

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
</tr>
</thead>
<tbody>
<tr>
<td>8:00 - 9:30 a.m.</td>
<td>Breakfast – River Rock Grill</td>
</tr>
<tr>
<td>9:30 - 11:00 a.m.</td>
<td>Guests Check-out – Front Desk</td>
</tr>
</tbody>
</table>
SPEAKER BIOGRAPHIES

**Nukhet Aykin-Burns, Ph.D.,** is assistant professor of pharmaceutical sciences at University of Arkansas for Medical Sciences and a member of the College of Pharmacy’s Division of Radiation Health. Dr. Aykin-Burns’s research focuses on reactive oxygen species (ROS), radiation induced (IR and UV) normal tissue damage and wound healing as well as polychlorinated biphenyl (PCB) induced oxidative stress. Dr. Aykin-Burns received her Ph.D. degree from the University of Missouri-Rolla (currently known as Missouri University of Science and Technology) focusing on antioxidant-based therapies in lead poisoning. She received postdoctoral training at the Free Radical and Radiation Biology Program (FRRBP), Department of Radiation Oncology at the University of Iowa.

**Marc Benderitter, Ph.D.,** is the head of the Department of Radiobiology and Regenerative Medicine in IRSN, France. His group is interested in identifying the mechanism of severe tissue radiation effects (radiotherapy side effects and accidental exposure) and develops medical countermeasures mainly based on stem cell therapy. He is chairman of the International Association of Radiopahology (IAR), and expert for the IAEA in case of radiation emergency medical response and assistance.

**Carmen Bergom M.D., Ph.D.,** is an assistant professor in radiation oncology at the Medical College of Wisconsin (MCW). She treats breast cancer patients at the MCW Cancer Center, and she also has a translational research laboratory focusing on using innovative genetic models to improve the therapeutic ratio of radiation therapy by identifying targets to enhance tumor radiosensitivity and minimize normal tissue toxicities. Dr. Bergom obtained undergraduate degrees in chemical engineering and biology from the Massachusetts Institute of Technology, a master’s degree in epidemiology at Cambridge University, and M.D. and Ph.D. degrees from MCW. She completed her medical residency in radiation oncology at MCW. During her residency, she was named a Leonard B. Holman Research Pathway Fellow by the American Board of Radiology. Throughout her training and career, Dr. Bergom has been interested in coupling basic research findings with translational and clinical research.

**Amrita Cheema Ph.D.,** is a professor of oncology and biochemistry at Georgetown University Medical Center. She also co-directs the Waters Center of Innovation in Metabolomics at GUMC. Her extramurally funded research program is focused on delineating small molecule biomarkers that are predictive of exposure to ionizing radiation as well for adverse outcomes of radiotherapy. Her laboratory has also developed several tools and workflows for furthering metabolomics based molecular phenotyping for clinical and translational research. Cheema’s work has been documented in more than 40 peer reviewed publications and five biomarker patents.
Mark Cline D.V.M., Ph.D., DACVP, is professor of pathology/comparative medicine, radiation oncology, and translational sciences at Wake Forest School of Medicine, and vice chair for research in the Department of Pathology. Dr. Cline received his D.V.M. and Ph.D. degrees at North Carolina State University, and completed a residency in anatomic pathology at Cornell. His PhD work focused on tumor hypoxia in canine patients, and for many years he studied hormonal and dietary effects on breast and reproductive cancer risk.

Currently, he has an active and well-funded research program in comparative cancer biology and oncology, with a particular focus on spontaneous primate cancer models and delayed normal tissue injury after radiation exposure. He directs a national Primate Radiation Survivor Core as part of a collaborative U19 within the Centers for Medical Countermeasures against Radiation. This core allows study of long-term multisystemic radiation injury, including radiation-induced neoplasms in primates. His complementary Department of Defense Focused Program Award explores mechanisms of radiation co-morbidities including diabetes, radiation-induced heart disease, and immune injury, and includes genomic and RNAseq signatures of radiation injury.

He is a member of the Wake Forest Comprehensive Cancer Center, the Translational Science Institute, the Office of Women in Medicine and Science, the Hypertension Center and the Center for Vaccines at the Extremes of Aging. He has served on many national committees, task forces and boards for scientific societies including the North American Menopause Society, the American College of Veterinary Pathologists, the Society for Toxicologic Pathology and the Radiation Research Society. He has served on NIH study sections (Comparative Medicine, Reproductive Endocrinology, and ad hoc Center reviews), and serves annually as an Integration Panel member for the Congressionally Directed Medical Research Programs Breast Cancer Research Program.

He has served as a mentor for 11 D.V.M. fellows, four M.D. fellows, and many Ph.D. students, undergraduates and pathology residents. He directs a T32 post-doctoral fellowship program for veterinarians, and founded a T35 Summer Fellowship program. His past trainees are productive investigators at the NIH, NIEHS, EPA and universities in the US, Europe and Asia. Recent publications from his group have focused on delayed cardiac injury, diabetes and mechanisms of cerebrovascular injury after brain irradiation.

Catherine Davis-Takacs, Ph.D., is an assistant professor of behavioral biology in the Department of Psychiatry and Behavioral Sciences at Johns Hopkins University School of Medicine. She received her Ph.D. in 2009 from the Behavior, Cognition, and Neuroscience program at American University. Her research focuses on determining the behavioral, neurochemical and physiological differences that impact an individual’s sensitivity to neurobehavioral deficits following exposure to ionizing radiation. Using behavioral, immunohistochemical, pharmacological and chemogenetic techniques, she aims to investigate the mechanisms underlying radiation-induced deficits in sustained attention and memory and to design successful strategies to mitigate these deficits. Davis-Takacs was a 2011 NSBRI First Award Postdoctoral Fellow and a 2015 recipient of the NSBRI Career Advancement Award.
David Kirsch, M.D., Ph.D., is the Barbara Levine University Professor at Duke in the Departments of Radiation Oncology and Pharmacology & Cancer Biology. After graduating from Duke with a B.S. in biology, he completed the M.D./Ph.D. program at Johns Hopkins School of Medicine, where he performed his thesis research with Dr. Michael Kastan. After an internship in internal medicine, Dr. Kirsch trained in radiation oncology at Massachusetts General Hospital. He worked as a post-doc in the laboratory of Dr. Tyler Jacks at M.I.T., where he developed a genetically engineered mouse model of soft tissue sarcoma. He utilized the Jacks’ lab model of non-small cell lung cancer to study radiation response in vivo. In 2007 Dr. Kirsch moved to Duke, where he uses radiation therapy to care for patients with sarcomas at the Duke Cancer Center. Dr. Kirsch is the leader of the Radiation Oncology & Imaging Program in the Duke Cancer Institute and serves as vice chair for basic and translational research in the Department of Radiation Oncology. Dr. Kirsch’s laboratory utilizes sophisticated genetically engineered mouse models to study mechanisms of sarcoma and normal tissue response to radiation.

Igor Koturbash, M.D., Ph.D., is an associate professor at the Department of Environmental and Occupational Health, University of Arkansas for Medical Sciences. He received his M.D. from the State Medical University in Ivano-Frankivsk, Ukraine (2001), and his Ph.D. in biomolecular sciences from the University of Lethbridge, Canada (2008). Dr. Koturbash completed his training as an Oak Ridge Institute for Science and Education (ORISE) Fellow at the National Center for Toxicological Research, US Food and Drug Administration in Jefferson, Arkansas.

The focus of Dr. Koturbash’s research is to understand: 1) epigenetic and metabolic mechanisms of the normal and cancer tissue responses to radiation, and how the diet can modulate those responses; and 2) safety, efficacy and mechanisms of action of dietary supplements. He has published over 80 peer-reviewed articles and book chapters, and his research has received uninterrupted extramural funding since the beginning of his independent career. Dr. Koturbash is a recipient of numerous prestigious awards and honors, including the Michael Fry Award from the Radiation Research Society and an Award for Faculty Excellence in Research from UAMS. Igor is a current president of the South-Central Chapter of the Society of Toxicology, and serves as an Associate Editor for Radiation Research and an Editorial Board Member for Chemico-Biological Interactions.

Xiao Wen “Vivien” Mao, M.D., is a research professor within the School of Medicine at Loma Linda University. She has over 15 years of experience in radiobiology studies, and her research focuses on radiation-induced normal vascular changes in the central nervous system. Her present work involves using a controlled ground-based mouse model to identify factors and cellular mechanisms that trigger chronic irradiation and unloading-induced brain microvascular and tissue remodeling. More recently, she has been awarded funding for investigating the spaceflight environment-induced remodeling of vascular network in mouse retina through flight and ground-based studies. Her research approaches combine advanced imaging, neurobiological analysis, complex “omics” analysis and behavioral endpoints.
CHAIRPERSON BIOGRAPHIES

Alexei Basnakian, M.D., Ph.D., D.Sc., received his M.D. from Sechenov Moscow Medical School and Ph.D. and D.Sc. degrees from the Russian Academy of Medical Science, both in the field of biochemistry. He has completed postdoctoral training in molecular biology at the Harvard Medical School and in toxicology/cancer research at the National Center for Toxicological Research, Food and Drug Administration. Dr. Basnakian is a tenured professor in the Department of Pharmacology and Toxicology at the University of Arkansas for Medical Sciences, Director of the DNA Damage and Toxicology Core Center at UAMS, and Research Career Scientist at the Veteran’s Hospital in Little Rock. He is the author of 85 peer-reviewed papers and 14 reviews or book chapters. Dr. Basnakian is an editorial board member of three biomedical journals, and a member of NIH, AHA and VA grant study sections. His research interests are drug discovery in the field of DNA endonucleases and DNA damage associated with radiation, toxicity, tissue injury and cell death.

Marjan Boerma, Ph.D., has 15 years of experience in radiation biology. She received her Ph.D. from the University of Leiden, the Netherlands, where she used pre-clinical models to investigate biological mechanisms of radiation-induced heart disease, a long-term side effect of radiation therapy of intrathoracic and chest wall tumors.

Dr. Boerma is currently associate professor in the Division of Radiation Health at the University of Arkansas for Medical Sciences, continuing her research on cardiovascular effects of ionizing radiation. Her lab aims to identify biological mechanisms of and potential interventions in radiation-induced heart disease. In addition, together with investigators of Loma Linda University, the University of Arizona and Georgetown University, she formed the Center for Space Radiation Research that is funded by the National Space Biomedical Research Institute and investigates acute effects and cardiovascular disease risks from exposure to space radiation.

Martin Hauer-Jensen, M.D., Ph.D., is a professor of pharmaceutical sciences, surgery and Pathology at the University of Arkansas for Medical Sciences (UAMS), associate dean for research in the UAMS College of Pharmacy, as well as director of the UAMS Division of Radiation Health in Little Rock, Arkansas. He also holds an appointment at the affiliated VA facility, Central Arkansas Veterans Healthcare System. He is internationally recognized as an authority on radiation effects in normal tissues. His research focuses on determining mechanisms of injury and on developing strategies to prevent adverse effects after radiation therapy in cancer patients. A major emphasis of his research is also on developing effective, non-toxic medical countermeasures for use in radiological or nuclear emergencies.

Dr. Hauer-Jensen has published more than 200 scientific articles and book chapters. His research currently receives funding from the National Cancer Institute (NCI), National Institute of Allergy and Infectious Diseases (NIAID) and the Biomedical Advanced Research and Development Authority (BARDA) of the Department of Health and Human Services (DHHS). He has been recognized with many honors and awards, including listing by Best Doctors in America nine times and the Legends in Cancer Research Award from the American Cancer Society. He is also a recipient of the prestigious MERIT Award from the National Cancer Institute (NCI).

Dr. Hauer-Jensen serves on numerous national and international advisory boards, review panels and editorial boards. He has been chair of the Radiation Study Section of the National Institutes of Health (NIH), is a consultant on radiological emergencies to the World Health Organization (WHO) and currently serves on the councils of both the National Council on Radiation Protection and Measurements (NCRP) and the Radiation Research Society (RRS).
Deficits in Attention and Social Recognition Memory Following Space Radiation Exposure

Catherine M. Davis-Takacs, Ph.D.

Johns Hopkins University School of Medicine, Baltimore, Md., USA

Future long-duration missions will involve travel outside of the protection of Earth’s magnetosphere and will result in astronauts being exposed to protons and high energy and charge (HZE) particles through galactic cosmic rays and solar particle events. The biological consequences of these exposures are a major factor limiting human space missions beyond low Earth orbit. Ground-based studies simulating space radiation exposure show that protons and HZE particles can damage multiple tissues, including the central nervous system (CNS). Recent data suggests that these exposures result in structural changes to various brain regions and alterations in normal cellular processes underlying cognition, including long-term changes in neurotransmission, the availability of neurotransmitters and associated signaling proteins, and decreases in synaptic complexity in brain regions important for neurobehavioral function, including the medial prefrontal cortex. These neurobiological changes are often associated with long-term deficits in various cognitive domains, such as learning, memory and attention, and more recently, social processing. Importantly, these neurobehavioral deficits persist for months following exposure and at certain proton or HZE exposure levels, appear to be irreversible without dietary, pharmaceutical or biological interventions. Recent data suggests that these neurobehavioral deficits are specifically correlated with neurobiological changes in individual subjects, raising the possibility that certain individuals might be more susceptible to the deleterious effects of proton and HZE exposures. Work from our laboratory and others has shown that the CNS is sensitive to low doses of several different particles and for specific cognitive domains, such as sustained attention and cognitive flexibility, these behavioral deficits are more severe in a subset of irradiated subjects. Recent work from our laboratory supports the role of the medial prefrontal cortex in radiation-induced deficits, including deficits in social odor recognition memory. Taken together, the neurobiological and behavioral data examining simulated space radiation exposures in rodent models demonstrates the sensitivity of the CNS to high LET radiation and that these exposures pose a significant hazard for manned, long-duration space missions beyond low Earth orbit.
Effects of Low-dose Radiation on Neurovascular Remodeling in the Mouse Brain and Eye

X. W. Mao, M.D.

Department of Basic Sciences, Division of Biomedical Engineering Sciences (BMES), Loma Linda University School of Medicine and Medical Center, Loma Linda, Calif., USA

Low doses of high linear energy transfer (LET) radiation are critical elements of the space radiation environment and dominate the health risks for astronauts. Low to moderate radiation doses are also clinically relevant as radiotherapy often involves exposure of relatively large normal tissue volumes. Although the link between high doses of ionizing radiation and vascular damage in the central nervous system (CNS) has been well studied clinically, the association between lower dose exposures and late changes in the microvasculature is less defined. Microvascular networks, which control the delivery of oxygen and nutrients to tissues, as well as the removal of metabolic waste products, are the most radiosensitive components of the vascular system. Indeed, the microvasculature is among the most critical, dose-limiting tissue of the CNS. Endothelial cell damage and dysfunction are two of the earliest tissue-level responses to radiation exposure and likely play an important role in both acute and late tissue damage in the brain. Our studies demonstrated that low-dose radiation induces adverse remodeling in the hippocampal microvasculature. There were substantial changes in this network after only 0.5 Gy $^{56}$Fe or 1 Gy proton exposures, with a complex dose response and regional differences. Endothelial cell losses and vascular topological changes are also associated with transient and permanent changes in cross talk between endothelial cells and astrocytes, and likely contribute to the development of blood-brain barrier (BBB) disruption. Some evidence also showed that low-dose radiation induced extracellular matrix (ECM) remodeling in the brain. Given that radiation is known to increase reactive oxygen species (ROS) levels, it should not be surprising that increased oxidative stress has been implicated as a possible mechanism by which radiation promotes vascular damage. Studies showed that even low doses of radiation can induce significant levels of oxidative stress in microvessel endothelial cells. A variety of effects in these cells, ranging from a dose-dependent apoptosis to activation of redox-regulated genes, were observed. The retina is considered an extension of CNS. The retina and the retinal vasculature play important roles in vision. Changes in microvascular topology are a common cause of vision loss in aging individuals and in patients with diabetes, as well as after exposure to X-rays. Nonetheless, the retina and the retinal vasculature have not been studied extensively in relation to space travel and space radiation. We have shown that space travel and space-like radiation exposures cause oxidative changes and apoptosis in the retina and continuous remodeling of retinal microvessel architecture over the course of a year after irradiation. Significant changes in retinal endothelial cells occur at doses as low as 0.1 Gy. Understanding how radiation and environmental insults impact on neurovasculature will help focus the approach toward more effective countermeasures during human spaceflight and planetary exploration. Mechanistic studies may also lead to new efficacious therapies that can prevent, reverse or stop the progression of neurovascular-related diseases and retinal degeneration.
Do You Have the Guts to Travel to Mars? Understanding Gastrointestinal Effects of Space-like Radiation

Igor Koturbash, M.D., Ph.D.

Department of Environmental and Occupational Health, University of Arkansas for Medical Sciences, Little Rock, Ark., USA

One-carbon metabolism unifies a number of important biochemical reactions and is critical for gene regulation, amino acid synthesis and DNA methylation, affecting nearly all cellular functions. Alterations in one-carbon metabolism are linked to a number of diseases; however, knowledge of the effects of exposure to ionizing radiation on the one-carbon metabolism and associated radiation-induced health effects is limited.

In this study, we sought to investigate the effects of exposure to space environment-relevant low-mean absorbed doses of protons in the mouse gut. We report that exposure to 0.5 and 1 Gy of protons substantially and persistently affected the one-carbon metabolism in both mouse proximal jejunum and colon. Specifically, exposure to protons caused significant decreases in the tissue methionine and glutathione concentrations, and DNA hypomethylation in a dose-dependent manner. These effects, as observed nine months after irradiation, were more pronounced in the proximal jejunum and associated with altered gut microbiome profiles, malnutrition and weight loss. At the same time, histomorphological evaluation did not reveal any evidence of inflammation, mucosal necrosis or mucosal blunting. Analysis of molecular signatures of exposure to ionizing radiation showed significant up-regulation of Casp14 and concomitant down-regulation of Casp4, nearly 20-fold loss in the expression of Nos2, and increases in the expression of the number of tight junction-related proteins — Cldn5, Cldn6 and Cldn10.

Altogether, these findings suggest that despite the absence of manifested histomorphological changes, exposure to space environment-relevant doses of proteins causes long-term metabolic and epigenetic effects and may result in altered gut physiology.

Funding: P20 GM109005, NSBRI RE03701 through NCC 9-58, ASGC NNX15AK32A.
Radiation-induced Non-targeted Changes in Arterial Tissue Mechanics

Sourav S. Patnaik, Ph.D. 1, Catherine M. Davis, Ph.D. 2, Mirunalini Thirugnanasambandam, M.S. 1, Senol Piskin, Ph.D. 1, Anthony G. Lau, Ph.D. 3, and Ender A. Finol, Ph.D. 1

1University of Texas at San Antonio, San Antonio, Texas, USA; 2Johns Hopkins University School of Medicine, Baltimore, Md., USA; 3The College of New Jersey, Ewing Township, N.J., USA

**Introduction:** Exposure to ionizing radiation leads to direct (oxidative damage, inflammation, etc.) and indirect (induced gene expression, innate immune response, etc.) tissue injuries, ultimately yielding vascular tissue damage and cellular death [1]. In this study, we investigated the collateral effect of non-targeted high energy particulate radiation exposure in deep-seated, soft tissues such as the abdominal aorta. We hypothesized that the biomechanical properties of the abdominal aorta will exhibit stiffer characteristics in head-only irradiated animals, as compared to the sham irradiated animals.

**Materials and methods:** N = 9 male, Long Evans rats approximately 4 months old and weighing 350-400 gr underwent either acute head-only exposure to 100 cGy dose of proton radiation (150 MeV/n; Group I, n = 7) or sham radiation exposure (Group II, n = 2) at Brookhaven National Laboratory. Post-exposure, after 11-12 months, these animals were sacrificed, their abdominal aortas dissected, and these cut into 5 mm rings for suture strength testing (see Fig. 1(A)). Mechanical testing was performed using an Electroforce T3200® and the specimens pulled to failure at a rate of 1 mm/min. Force and extension data were recorded while mechanical parameters such as stiffness and maximum load were calculated. Differences in these parameters obtained from the two specimen groups (I vs. II) were assessed by ANOVA using SPSS; results were considered significant when p < 0.05.

**Results:** In contrast to our hypothesis, the sham group (II) exhibited a higher stiffness than the radiation group (I). The stiffness of Group I was nearly 45% greater than that of Group II (p < 0.001). No statistical difference was found in the maximum load of both groups (p > 0.05).

**Discussion:** Unexpectedly, the sham tissue specimens were more resilient than the radiated ones. Tissue mechanics is a direct function of its constituents (i.e., collagen, elastin, etc.) and a change to these proteins (due to radiation) could possibly explain the observed biomechanical differences between the groups. Future work should focus on the mechanical changes occurring at the microscopic level due to radiation exposure and the potential translation of this work towards countermeasure target development.

The Effects of Obesity on Intestinal Health after Radiation Exposure

Laura E. Ewing, M.S., Isabelle R. Miousse, Ph.D., Rupak Pathak, Ph.D., Sarita Garg, Ph.D., Charles M. Skinner, B.S., Stepan Melnyk, Ph.D., Howard Hendrickson, Ph.D., Reid Landes, Ph.D., Alan J. Tackett, Ph.D., Annie Lumen, Ph.D., Martin Hauer-Jensen, M.D., Ph.D., and Igor Koturbash, M.D., Ph.D.

Introduction: The interplay of diet and lifestyle factors can influence the susceptibility to radiation-induced gastrointestinal toxicity during radiotherapy and accidental radiation exposure. In a methionine-supplemented diet plus irradiation (MSD + IR) model, we show increased mortality, associated with changes in tight junction-related gene profiles, altered methionine metabolism and shifts in the gut ecology. To further study the effects of lifestyle/body condition, we implemented a total body irradiation scheme in an obese mouse model.

Methods: We subjected male NZO/HILtJ mice, a strain prone to spontaneous obesity and diabetes, to 0, 5.5, 6.37, 7.4, or 8.5 Gy ($^{137}$Cs) of total body irradiation (TBI) and monitored them for 30 days. As the mice succumbed to radiation toxicity, we collected intestinal tissue for gene expression, protein, and histological analyses.

Results: Mice at higher IR doses lost more weight compared to non-IR shams, while mice at 5.5 Gy were characterized by weight gain. There was a sharp delineation in survival between 6.37 (LD$_{0/30}$) and 7.4 Gy (LD$_{90/30}$). At the 5.5 and 6.37 Gy doses, blood sugar was decreased compared to controls and to higher, lethal doses of IR. There was little difference in mitotic figures in jejenum crypts and little evidence of necrotic changes across IR doses. There were marked differences in tight junction gene expression profiles, with Cldn2, Cldn5, Jam2 and Jam3 being highly up-regulated and Cldn4 and OcIn being highly down-regulated. There were also major changes in some cell-cycle regulatory genes, such as Igf1r, as well as genes for inflammatory mediators, such as C3, Ccl11, Ccl4, Il18 and Tlr4. These changes are more pronounced when comparing survivors vs. non-survivors. Western blots were performed to confirm the changes in gene expression.

Conclusions: Pre-diabetic condition and elevated blood glucose levels did not exacerbate the radiation-induced normal tissue toxicity, when compared to survival patterns of other mouse strains. There is a dose-dependent response in expression for tight junction, cell cycle and inflammatory genes in the jejunum between the mice exposed to 5.5 and 6.37 vs 7.4 and 8.5 Gy of TBI. Those differences potentially pre-determined the differences in survival; however, further studies will be needed to delineate the underlying mechanisms.
Kruppel-like Factor 2 (KLF2): A Novel Radiation Target is Suppressed in the Mouse Intestine

Rupak Pathak, Ph.D., Ratan Sadhukhan, Ph.D.1, Sarita Garg, Ph.D.1, Snehalata Pawar, Ph.D.1, Marjan Boerma, Ph.D.1, Jerry Ware, Ph.D.2, and Martin Hauer-Jensen, M.D., Ph.D.1

1Division of Radiation Health, Department of Pharmaceutical Sciences, College of Pharmacy and  
2Department of Physiology and Biophysics, College of Medicine, University of Arkansas for Medical  
Sciences, Little Rock, Ark., USA

The pathophysiology of acute and/or delayed intestinal toxicity after radiation exposure is highly complex and controversial. Within the intestine, post-irradiation dysfunction of vascular endothelial cells (ECs) and activation of immune cells play a critical role in the progression and development of intestinal damage. As the barrier between blood and the extravascular space, EC dysfunction can promote edema, tissue ischemia, hypoxia and leukocyte extravasation to the various intestinal compartments. As a result, additional pathological cascades are triggered, including but not limited to, inflammation, coagulation, oxidative stress and fibrotic changes. Kruppel-like factor 2 (KLF2), a shear-responsive zinc finger transcription factor, critically regulates endothelial functions and quiescence of T cells, B cells and monocytes. KLF2 is a central regulator of endothelial anti-inflammatory, anti-coagulant, anti-oxidant, anti-adhesive and anti-permeability properties. However, it is completely unknown whether radiation alters the level of KLF2, specifically in the intestine. We hypothesize that EC dysfunction and immune cell activation in the irradiated intestine could be due to suppression of KLF2.

To address the issue, we exposed CD2F1 male mice to 8 Gy total body irradiation (TBI) and collected intestinal samples at 4 h, 24 h, 4 d, 7 d, and 21 d after irradiation. Since KLF2 is highly expressed in the lung, we also collected lung samples from CD2F1 mice at 4 h, 4 d and 21 d. To rule out the possibility of a mouse strain effect, a similar experiment was performed using C57BL/6 mice exposed to 8.5 Gy TBI. KLF2 expression was measured both at mRNA and protein levels by qRT-PCR and immuno-histochemistry analysis.

We observed a significant time-dependent suppression of KLF2 both at mRNA and protein levels in the irradiated intestinal tissue of CD2F1 mice. Like CD2F1, C57BL/6 also exhibited post-irradiation suppression of intestinal KLF2 level at the mRNA level. As in the intestine, KLF2 expression was likewise suppressed in the irradiated lung.

These data clearly demonstrated that radiation reduces KLF2 levels in the intestine. As a key transcription factor for normal endothelial cell function, this places KLF2 in a critical position impacting a wide-range of vascular-dependent pathologies. Moreover, pharmacological manipulation of KLF2 could be a novel strategy to protect not only the intestine, but also the other organs from harmful effects of radiation.
Aberrant TLR4 Signaling Promotes Radiation-induced Intestinal Injury in Cebpd-deficient Mice

Sudip Banerjee, Ph.D.¹, Qiang Fu MD, Ph.D.¹, Sumit Shah, M.B.B.S., M.P.H.¹, Stepan B. Melnyk, Ph.D.², Martin Hauer-Jensen, M.D., Ph.D.¹, and Snehalata A. Pawar, Ph.D.¹

¹Division of Radiation Health, Department of Pharmaceutical Sciences, College of Pharmacy, University of Arkansas for Medical Sciences, Little Rock, Ark., USA; ²Arkansas Children’s Hospital Research Institute, Little Rock, Ark., USA

Background: Exposure to ionizing radiation (IR) induces a plethora of responses in the cells/organism to counteract oxidative stress, DNA damage response and inflammation by inducing the expression of inflammatory and anti-inflammatory cytokines. CCAAT enhancer binding protein delta (Cebpd; C/EBPδ) is a transcription factor implicated in the regulation of oxidative stress, DNA damage response and inflammation. We have previously reported that the lethality of Cebpd⁻/⁻ mice to IR occurs due to injury to the bone marrow and intestine. In this study, we investigated the hypothesis that C/EBPδ downregulates TLR4 expression, attenuating the inflammatory and oxidative stress responses to protect from IR-induced intestinal injury.

Methods: Intestine, liver and blood samples were harvested from Cebpd+/+ and Cebpd⁻/⁻ mice after exposure to 8.5 Gy total body irradiation (TBI). The expression of Tlr4, inflammatory cytokines and chemokines and tight junction proteins were measured in intestine tissues by real-time PCR. Plasma levels of lipopolysaccharide-binding protein (LBP) were measured by ELISA. Glutathione (GSH), GSH/GSSG, S-nitrosoglutathione (GSNO) and 3-nitrotyrosine (3-NT) in intestine tissues were analyzed by high performance liquid chromatography. Intestinal in vivo permeability and amplification of 16S rRNA in the liver were measured. The effect of the TLR4-inhibitor (C34) pretreatment for 30 mins prior to IR (3 Gy) exposure was tested on the growth of intestinal organoids derived from Cebpd⁺/+ and Cebpd⁻/⁻ mice.

Results: Cebpd⁻/⁻ mice showed significantly elevated expression of TLR4 at the protein and mRNA levels at 1h and 4 h post-irradiation compared to compared to Cebpd⁺/+ mice. Cebpd⁻/⁻ mice show increased intestinal expression of Il-6, Tnf-α, Tgf-, Mcp-1, Cxcl1, and Mif-1α and Nos2 transcripts compared to Cebpd⁺/+ mice after exposure to IR. Cebpd⁻/⁻ mice expressed decreased GSH levels and elevated 3-nitrotyrosine and S-nitrosoglutathione levels in the intestine which was further exacerbated by IR, indicative of oxidative and nitrosative stress. Claudin-2, a protein associated with in vivo intestinal barrier disruption, was significantly upregulated in irradiated Cebpd⁻/⁻ mice, and correlated with increased intestinal permeability, plasma LBP and increased bacterial translocation at day 3.5 post-TBI. Pre-treatment with a TLR4 inhibitor prior to irradiation showed robust protection of intestinal organoids of both genotypes.

These results identify a novel role for impaired TLR4 signaling in promoting radiation-induced intestinal injury in Cebpd⁻/⁻ mice. These results may have implications for utilizing TLR4 as a therapeutic target to protect the intestine from IR-induced normal tissue injury. Future studies will investigate the role of C/EBPδ in the regulation of target proteins that may inhibit the TLR4 pathway in response to IR.
TUESDAY, MAY 15, 2018

RADIOLOGICAL EMERGENCIES
PLENARY SPEAKER ABSTRACTS
Cell Therapy Treatment for Patient with Severe Radiotherapy Sequelae: Lessons Learned from the Radiotherapy Accident of Epinal, France

M Benderitter, JJ Lataillade, R Tamarat, M Mohty, A Chapel, JM Simon and NC Gorin

1Institut de Radioprotection et de Sûreté Nucléaire, Laboratoire de Radiopathologie et de Thérapie Cellulaire, Fontenay-aux-Roses, France; 2Centre de Transfusion Sanguine des Armées, Département de Recherche et de Thérapie Cellulaire, Hôpital d’Instruction des Armées, Clamart, France; 3CHU Saint Antoine, Département d’Hématologie, Paris, France; 4CHU La Pitié Salpêtrière, Paris, France

Radiation overexposure accidents are rare but can have severe long-term health consequences. The greatest share of reported overexposures occur in the medical fields using radiation therapy and fluoroscopy. The accident of radiation oncology at the Public General Hospital in Epinal was the highest in France. It was classified level 6 on the 10-degree scale of the ASN/SFRO (Autorité de Sûreté Nucléaire/Société Française de Radiothérapie Oncologique). It was linked to errors in the process of treatment and a mistake in the use of dynamic wedges, with an overdosage of 20%. The clinical consequences were severe for 24 patients treated for prostate cancer and exposed to this overdosage. Sequelae were classified grade 2 to 5 on the CTCAE 3.0 scale. A second cohort of 397 was identified, which received an overdose of 10% linked to the daily use of portal imaging, which explained a higher risk of grade 2-3 rectitis.

Four patients presenting serious intestinal radiation-induced lesions after overdosage compassionately received MSC treatment. For all three patients, the systemic administration of MSC was well tolerated; efficient analgesic and anti-inflammatory effects as well as hemorrhage reduction were observed. The results demonstrated the feasibility of cell therapy treatment for patients with severe radiotherapy sequelae. Stem cell therapy must now be improved to the point that hospitals can put safe, efficient and reliable clinical protocols into practice. Preclinical data were accumulated to initiate a phase I/II clinical trial.
Delayed Effects of Acute Radiation Exposure on Normal Tissues: Insights from Nonhuman Primate Studies

J. Mark Cline, D.V.M., Ph.D., DACVP1, Greg Dugan, D.V.M.1, Rachel Andrews, D.V.M.1, Kylie Kavanagh, B.Sc., V.M.S., M.V.S., M.P.H.1, Daniel Bourland, Ph.D.1, David Hanbury, Ph.D.1, Ann M. Peiffer, Ph.D.1, Thomas Register, Ph.D.1, Ryne DeBo, Ph.D.1, Kris Michalson, D.V.M., David Caudell, D.V.M., Ph.D.1, John Olson M.S., Benny Chen, M.D., Gregory Sempowski, Ph.D., and Nelson Chao M.D.2

1Wake Forest School of Medicine, Winston-Salem, N.C., USA; 2Duke University Medical Center, Durham, N.C., USA

Introduction: Acute responses to radiation injury are the focus of most emergency medical response and mitigation efforts, but the major burden of radiation injury lies in delayed effects. These late and usually long-term effects of exposure on normal healthy tissues include cellular, molecular and metabolic changes leading to organ dysfunction and failure, fibrosis and neoplasia.

Methods: We present here the long-term adverse effects of single-dose whole-body exposures at 3.5-8.5 Gy in over 100 rhesus monkeys exposed at a median age of 4 years and observed for up to 13 years, in comparison to 38 non-irradiated controls. Some animals were exposed prior to puberty (<3.5 years of age), and the remainder as young adults (up to 10 years of age). Observations included an annual cycle of clinical examinations, imaging (whole body CT and brain MRI), cognitive testing, hematology, clinical chemistry, and for those animals dying under observation, necropsy and histopathology.

Results: Major disease processes identified to date include (1) type II diabetes mellitus, sometimes with islet hyperplasia, amyloidosis and increased peripheral insulin resistance; (2) myocardial fibrosis and reduced left ventricular diameter consistent with loss of myocardial elasticity; (3) immune compromise manifested as impaired vaccine responses, skin and wound infections, bronchopneumonia, pericarditis and sepsis; (4) chronic pulmonary disease including pneumonitis, fibrosis and epithelial dysplasia; (5) neoplasms including sarcomas, hematopoietic, epithelial and neuroendocrine types; (6) early and later incipient focal MRI-detected brain lesions; (7) renal impairment, and (8) elevated circulating markers of inflammation and microbial translocation. Other stereotypical radiation effects (gonadal atrophy, cataracts) were predictably seen. Multiple disorders in the same animal were common, with diabetes being the most common co-morbid condition.

Conclusions: Our growing body of data indicates that a substantial burden of disease is present in long-term survivor non-human primates, including complex patterns of co-morbidity; metabolic and cardiac disease; injury of “high-dose” tissues such as brain at lower doses than anticipated; immunosuppression and inflammation; and pathologies including both loss and fibrous replacement of functional tissue, and cytoproliferative disorders including neoplasms. Comorbidities are often present in a given animal, necessitating an integrative approach.

Against Fat Man and Little Boy: A Sequel

Nukhet Aykin-Burns, Ph.D.

Division of Radiation Health, Department of Pharmaceutical Sciences, College of Pharmacy, University of Arkansas for Medical Sciences, Little Rock, Ark., USA

Understanding and preventing the detrimental effects of radiation on humans have been challenging tasks ever since the Manhattan Project was initiated. Although several promising radiation countermeasures were developed over the decades, the complex nature of short- and long-term radiation injuries prevented the discovery of a single agent that was safe, effective and readily available for eliminating these adverse consequences. The majority of research efforts have focused on radiation-induced hematopoietic and gastrointestinal injuries; however, strategies to target the cutaneous and cerebrovascular syndromes are also being investigated since multiorgan failure plays a significant role in radiation lethality.

Developing drugs to overcome this multifaceted problem during a radiological emergency is a daunting mission because an ideal radiation countermeasure should be readily available in large quantities, able to withstand extreme environmental conditions, easily handled and administered, cost effective, and have a good bioavailability with no toxic side effects. While numerous novel agents are in various stages of development and assessment for their pharmacokinetics, toxicity, safety, efficacy and stability, only a few agents targeting hematopoietic acute radiation syndrome were able to receive approval by the United States Food and Drug Administration.

As our efforts to develop and improve drugs for radiation protection, radiation mitigation, and radiation therapeutics still continue, finding the correct in vitro and in vivo models to study the pathophysiology of radiation injury also becomes critical. Limitations of animal models and the unethical nature of human experiments recently led to the development of “3D organoid cultures” as well as “organs on chips.” The validity of using such systems to develop countermeasures for radiological emergencies and radiation effects in clinical settings remains open to debate.
TUESDAY, MAY 15, 2018

ORAL PRESENTATION ABSTRACTS
Neulasta Regimen for the Hematopoietic ARS: Effects Beyond Neutrophil Recovery

Betre Legesse, M.S., Amandeep Kaur, D.V.M., Ph.D., Doreswamy Kenchegowda, Ph.D., Bernadette Hritzo, B.S., and Maria Moroni, Ph.D.

The potential for nuclear accidents is expected to grow in the coming years, and the need to develop countermeasures for radiation victims is pressing. Only granulocyte-colony stimulating factor G-CSF (Neupogen, and its pegylated form, Neulasta) has been approved by the FDA for treatment of the hematopoietic acute radiation syndrome (H-ARS) based on its ability to alleviate myelosuppression. Besides its role in hematopoiesis, G-CSF displays cardiovascular and neuro-protective features as well as proinflammatory activity. Here, we administered Neulasta to minipigs exposed to H-ARS doses, and further characterized the protective effects of G-CSF against radiation injury beyond neutrophil recovery. The purpose is to offer insights into novel paradigms for the development of additional medical countermeasures.

Methods: Twenty male Göttingen minipigs were exposed to 2.2 Gy (radiation dose equivalent to LD70/45, total body irradiation, Cobalt-60, 0.6 Gy/min). Animals were assigned to two groups and received either Neulasta (300 mcg/kg; n=10) or dextrose (equivalent volume; n=10) at day 1 and 8 after irradiation. Efficacy of Neulasta on survival and recovery of myelosuppression was monitored over a 45-day period. Blood samples were obtained longitudinally from peripheral veins for blood cell counts and ELISAs; tissue samples were obtained at necropsy for molecular signatures.

Results: Neulasta significantly decreased mortality over the control animals by 50% (p=0.03). Among survivors (minipigs that completed 45-day observation period), Neulasta reduced the nadir and duration of neutropenia and improved the recovery of Absolute Neutrophil Counts (ANCs). Among animals undergoing unscheduled euthanasia, ANC dropped more rapidly in Neulasta treated animals than in controls. Overall, organ hemorrhage and the incidence of frank bleeding episodes were lower in the Neulasta group as compared to controls. Neulasta increased the plasma concentration of the vaso-protective hormone IGF-1, activated the cardioprotective IGF-1/PI3K/Akt/eNOS/NO pathway and increased the membrane expression of VE-cadherin in heart, improving vascular tone and endothelial barrier function. Expression of NADPH oxidase was downregulated, whereas the activity of catalase and SOD were marginally affected. In plasma, the acute phase protein CRP was induced by Neulasta, but the magnitude of induction was less than that of IGF-1.

Conclusion: Amelioration of vascular tone, barrier function and inflammation contribute to the beneficial effect of Neulasta for the treatment of the H-ARS.
BBT-059, a Potential Prophylactic Radiation Countermeasure as well as a Mitigator for Acute Radiation Injury, Protects Bone Marrow and Spleen

Sanchita P. Ghosh, Ph.D. 1, Shukla Biswas, M.S. 1, Neel K. Sharma, Ph.D. 1, Sasha Stone, B.S. 1, Gregory Holmes-Hampton, Ph.D. 1, Christine Fam, Ph.D. 2, George Cox, Ph.D. 2, and Vidya P. Kumar, Ph.D. 1

1Armed Forces Radiobiology Research Institute, Uniformed Services University of the Health Sciences, Bethesda, Md., USA; 2Bolder Biotechnology, Boulder, Colo., USA

BBT-059, developed by Bolder Biotechnology (BBT), is a long acting PEGylated IL-11 analog created using site-specific PEGylation technology. A single branched 40 kDa-PEG was added to the C-terminus of the protein at a cysteine residue (PEG-*179C). BBT-059 is being developed as a potential treatment for thrombocytopenia, myelodysplastic syndromes, bleeding disorders and acute kidney injury. In this study, we demonstrate, for the first time, that BBT-059 is effective as a radiation countermeasure in CD2F1 male mice when a single dose was administered from 24 h pre- to 24 h post-total body irradiation (TBI). In addition, we have shown that BBT-059, administered 24 h pre-TBI, accelerated recovery from radiation-induced peripheral blood cytopenia, restored sternal bone marrow, protected bone marrow progenitor cells, as well as attenuated protein biomarkers of H-ARS (hematopoietic acute radiation syndrome). Dose reduction factor (DRF) was determined by probit analysis using mortality as the end point at six radiation doses and surviving animals were monitored for 6 months post-TBI.

A single dose (0.3 mg/kg) of BBT-059 was found to be efficacious in 12 week old CD2F1 male mice, injected 24 h pre-, 12 h pre-, 2 h pre-, 2 h post-, 4 h post-, 12 h post- and 24 h post-TBI. A DRF of 1.32 was calculated for BBT-059 compared to saline. There was significantly accelerated recovery from radiation-induced peripheral blood neutropenia and thrombocytopenia in animals treated with BBT-059 prior to irradiation. The drug also increased bone marrow cellularity and megakaryocytes and accelerated multi-lineage hematopoietic recovery. In addition, BBT-059 inhibited the induction of radiation-induced hematopoietic biomarkers, thrombopoietin (TPO), erythropoietin (EPO) and Flt-3 ligand (Flt3L). Real time PCR of spleen lysates from irradiated animals indicated that certain cell cycle regulator genes (ccnb1, ccnb2, ccna2) were significantly down-regulated on day 1 post-TBI, and were rescued in the drug treated group. Mapk12 and Smad4 were significantly down-regulated in the spleen 15 days post-TBI; however, recovery was observed in drug treated animals.

We have shown that BBT-059 is effective starting from 24 h pre-radiation to 24 h post-radiation exposure, which is a fairly long window of administration for protection of military personnel (before sending them to fields with probable radiation exposure for clean-up operations) as well as civilian population in a radiation fall-out field. Significant survival benefit with BBT-059 suggests that the drug could be developed as a novel radiation countermeasure for soldiers, which can be used either before or after radiation in the aftermath of a radiation event.

Disclaimer: The views expressed here are those of the authors and do not reflect the official policy of AFRRI, USUHS, DoD or the US government.

Funding: NIAID IAA and AFRRI Intramural Research
Delayed Effects of Acute Radiation Exposure in BBT-059 Treated Survivors

Neel K. Sharma Ph.D. 1, Shukla Biswas, M.S. 1, Sasha Stone, M.S. 1, Christine Fam, Ph.D. 2, George Cox, Ph.D 2, Vidya P. Kumar, Ph.D. 1, and Sanchita P. Ghosh, Ph.D. 1

1Armed Forces Radiobiology Research Institute, Uniformed Services University of the Health Sciences, Bethesda, Md., USA; 2Bolder Biotechnology, Boulder, Colo., USA

BBT-059, developed by Bolder Biotechnology (BBT), is a long acting PEGylated IL-11 analog. Previously, we demonstrated that BBT-059 is effective as a radiation countermeasure in CD2F1 male mice when a single dose was administered either at 24 h pre- or 24 h post-total body irradiation (TBI). In this study, we show that surviving animals remain healthy up to 6 months post-TBI.

Methods: Twelve to 14-week-old CD2F1 male mice used in these studies. BBT-059 was prepared in formulation buffer (10mM sodium phosphate, 4% mannitol, 1% sucrose, pH 6.2) at the specific doses used in studies. Formulation buffer and saline (9%) were used as controls. Drug and controls were injected as a single dose (0.1 mL) subcutaneously (SC) at the nape of the neck. The experimental animals received a single exposure of 60Co gamma TBI at an estimated dose rate of 0.6 Gy/min in the AFRRI radiation facility. Survived animals post-30 day after radiation were monitored up to 6 months. Blood and bone marrow analyzed for CBC counts, serum chemistry and colony forming units (CFU) to understand the longterm effects of the survivors at 1 and 6 months post-TBI. Histopathological and immunohistochemical analysis of major tissues and organs were performed.

Results: Mortality was monitored up to 6 months post-TBI. There was an increase in the CBC counts and CFU in the 6 months post-TBI survivors compared to the 1 month group. Increased Glomerular with mesangium and lung fibrosis was observed at 6 months post-TBI. In heart, after 6 months some vessels had evidence of smooth muscle hypertrophy of the arterial/arteriolar wall as compared to naïve. Immunohistochemistry revealed an increase in the B-Catenin expression after 1 and 6 months in kidney. However, after six months serum biochemistry for blood urea nitrogen, creatinine, aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase did not show any difference between naïve, untreated and treated groups. The results indicate that significant delayed effects of acute radiation exposure occur in lung, heart and kidney in survivor animals.

Conclusion: We have shown that BBT-059 treated animals survived up to 6 months post-TBI. Significant survival benefit with BBT-059 and its long term effect suggest that the drug could be developed as a novel radiation countermeasure for civilians exposed in a fallout field for use in the aftermath of a radiation event.

Disclaimer: The views expressed here are those of the authors and do not reflect the official policy of AFRRI, USUHS, DoD, or the US government.

Funding: NIAID IAA
Anti-ceramide Antibody as Treatment for the Acute Radiation Gastrointestinal Syndrome

Vijay K. Singh, Ph.D.,1,2 John Fuller, B.S., 3 Jimmy A. Rotolo, Ph.D.,4 Zvi Fuks, M.D.,5 and Richard Kolesnick, M.D.3

1Department of Pharmacology and Molecular Therapeutics, F. Edward Hébert School of Medicine, Uniformed Services University of the Health Sciences, Bethesda, Md., USA; 2Armed Forces Radiobiology Research Institute, Uniformed Services University of the Health Sciences, Bethesda, Md., USA; 3Department of Molecular Pharmacology, Memorial Sloan-Kettering Cancer Center, New York, N.Y., USA; 4Ceramide Therapeutics, LLC, Brooklyn, N.Y., USA; 5Ceramedix Holding, LLC, New York, N.Y., USA

Background: Accumulating evidence indicates ceramide-mediated apoptosis within the gastrointestinal (GI) microvascular endothelial compartment regulates radiation sensitivity of GI epithelial stem cells. Recent studies describe a novel murine anti-ceramide IgM monoclonal antibody (Mab) that inhibits ceramide-mediated endothelial apoptosis, providing radioprotection from the lethal radiation GI syndrome (RGS) in mice. Further, prophylactic administration of humanized anti-ceramide IgG1 inhibits high-dose radiation-induced microvascular endothelial apoptosis within the GI tract, and dose-dependently protects crypt stem cells. Administering humanized Mab at 24 h post-irradiation similarly protects crypt stem cells, indicating that anti-ceramide may represent a bone fide mitigator of the lethal RGS. The current study investigates a novel anti-ceramide single chain variable fragment (scFv) as mitigator of the RGS.

Methods: Various doses of full length antibody or scFv were administered to C57BL/6 mice at various time points in relation to radiation exposure with supralethal dose, and jejunum was harvested for crypt analysis 3.5 days after irradiation. For 30-day survival studies, antibody or scFv was administered either 15 min before or 24 h after irradiation and bone marrow cells from healthy mice were administered 16 h after irradiation.

Results: When tested against supralethal doses of gamma-radiation in mice, anti-ceramide antibody or scFv demonstrates efficacy in protecting crypt survival against radiation doses as high as 15 Gy when administered through various parenteral routes. This antibody or scFv demonstrated dose response in protecting crypts and optimal dose for antibody and scFv was 1 and 0.1 mg/mouse, respectively. Anti-ceramide antibody or scFv was effective in combination with bone marrow cell administration in significantly improving mice survival when administered 15 min prior to or as late as 24 h post-irradiation.

Conclusion: In brief, anti-ceramide antibody appears to be a promising radiation countermeasure for acute RGS among all candidate radiomitigators currently under development. Our data validate anti-ceramide antibody as a potent countermeasure for mitigation of the RGS in the event of high-dose radiation exposure, such as a terrorist attack or radiological disaster. We are initiating its investigation in large animal model, nonhuman primates, toward advanced development, providing the pre-clinical rationale for initiation of investigational new drug (IND)-enabling studies and furthering this agent along the path towards human therapeutics following the “Animal Rule.”
Survival Risk Modeling as a Function of Related Histological and Functionality End Points in Radioinduced Gastro-intestinal Syndrome (RIGS): a Monte Carlo-based Cox Model Approach

Mohamed Amine Benadjoud, Ph.D. 1, Marc Benderitter, Ph.D. 1, and Radia Tamarat, Ph.D. 1

1 Department of Research in Radiobiology and Regenerative Medicine, Institute for Radiological Protection and Nuclear Safety, Fontenay-aux-Roses, France

Animal studies of countermeasures to protect against or mitigate the radiation-induced gastrointestinal syndrome generate a variety of data, which include survival data as well as histological and functionality end points (HFEP).

A simultaneous observation within the same animal of HFEP and survival results is impossible in practice since the former data require the animal sacrifice, which precludes a direct observation of the future events. Thus, connection between HFEP and survival is often treated qualitatively, arguing analogy trends across the experiment groups since this design makes inefficient the traditional statistical approach, which imposes that predictive and response variables should be observed on the same subject.

To address this issue, we propose a Monte Carlo-based survival Cox model. This approach is based on the concept of multiple imputation by sampling from the observed probability distribution of the (missing) HFEP data within experiment groups. The integrated Cox partial likelihood is then approximated by averaging over the simulations and maximized to provide the risk estimates with their confidence intervals, thereby quantifying the expected contribution of each HFEP on the observed survival.

The proposed approach is illustrated on a study demonstrating the beneficial effect of exosomes derived human MSC on the survival of mice with RIGS. We expressed, for the first time in this experimental model, the death hazard ratio as a function of villus height, apoptosis and proliferating cells in the small intestinal crypts and intestinal permeability.

In conclusion, the proposed work could help researchers in testing radioprotectors or mitigators in a small animal by explicitly and quantitatively bridging from the HFEP modifications to their survival effectiveness.
Circulating Ano1 RNA as a Biodosimeter and Indicator of Radiation-induced Gastrointestinal Toxicity

Reshu Gupta, Ph.D.¹, Xiaodong Xu, M.D. ², Natalie Lockney, M.D. ², Jing Guo, M.S. ², Astrid Grosche, D.V.M., Ph.D. ¹, Steven Swarts, Ph.D. ², Paul Okunieff, M.D. ², and Sadasivan Vidyasagar, M.B.B.S., Ph.D.²

¹Entrinsic Health Solutions, Norwood, Mass., USA; ²Department of Radiation Oncology, University of Florida Health Cancer Center, Gainesville, Fla., USA

**Background:** Anoctamin 1 (Ano1), also known as TMEM16, is a calcium-activated chloride channel that is expressed at higher levels in intestinal epithelial cells following radiation exposure and mediates intestinal chloride secretion, thereby contributing to radiation-induced diarrhea.

**Materials and methods:** All studies were performed on 8-week-old NIH Swiss mice. Mice were irradiated at 0 Gy, 0.5 Gy, 1 Gy, 3 Gy, 5 Gy, or 7 Gy. RNA was isolated from irradiated plasma samples using the TRIzol method. Real-time quantitative reverse transcription PCR (qRT-PCR) was performed using Ano1 gene-specific primers (5’-CCCGAGAAGTACTCGACGCT-3’ and 5’-TCCTCGATGGCGCAGATGTT-3’). The quality of cDNA as a template of real-time qRT-PCR was confirmed by detection of reference gene (TBP) expression. Ion flux studies were done on ileal sheets mounted in Ussing chambers to measure transepithelial current (Isc), conductance (G) and net chloride flux (Jnet).

**Results:** Anion secretion increased with increasing radiation dose (3.3 μeq.cm⁻².h⁻¹, -1.8 μeq.cm⁻².h⁻¹, -1.6 μeq.cm⁻².h⁻¹, -0.8 μeq.cm⁻².h⁻¹, and -0.8 μeq.cm⁻².h⁻¹ at 0, 0.5, 1, 3, 5, and 7 Gy, respectively). The plasma RNA specific for Ano1 levels corresponded to an increase in anion secretion measured in Ussing chamber studies (1.3-fold, 1.6-fold and 2.7-fold at 3 Gy, 5 Gy and 7 Gy, respectively, when compared with 0 Gy). Ano1 protein levels measured using western analysis showed a radiation dose-dependent increase. Similarly, Ano1 expression in intestinal epithelial cells increased with radiation. A clinical study to measure plasma levels of circulating Ano1 RNA in prostate cancer patients (n=53) receiving proton therapy or intensity-modulated radiotherapy showed that plasma RNA levels increased with radiation exposure and correlated with acute CTCAE grade 1-2 gastrointestinal toxicity.

**Conclusion:** This work defines the relationship between Ano1 levels and radiation dose and identifies the time range over which this biodosimeter is effective. These studies illustrate that circulating RNA specific for Ano1 has great promise as a point-of-care biodosimeter following intentional or accidental radiation exposure, given that it appears in plasma as early as 24 hours post-irradiation and is stable for at least 15 days as it is likely packaged in extracellular vesicles.
Gamma-Tocotrienol Restores Mucosal Barrier Integrity in Mice after Total Body Irradiation

Sarita Garg Ph.D., Rupak Pathak Ph.D., Ratan Sadhukhan Ph.D., Junru Wang M.D., Ph.D., and Martin HauerJensen M.D., Ph.D.

Division of Radiation Health, Department of Pharmaceutical Sciences, College of Pharmacy, University of Arkansas for Medical Sciences, Little Rock, Ark., USA

Background: The intestine is highly sensitive to radiation and is a major site of injury during radiotherapy and environmental overexposure. The intestinal mucosa not only sustains the most severe injuries in response to radiation, but also undergoes profound changes in loss of immune cells. Gamma-tocotrienol (GT3), a potent radioprotector, in addition to being an antioxidant, protects vascular endothelium from radiation injury in vivo, possibly by its ability to inhibit 3-hydroxy-3-methylglutaryl-coenzyme-A (HMG-CoA) reductase and accumulate in endothelial cells at much higher levels. The present study was therefore undertaken to determine if GT3 (a) could restitute the loss of mucosal immune cells and (b) contribute to restoration of the mucosal barrier dysfunction following total body radiation in a mouse model.

Methods: Male CD2F1 mice were exposed to various doses of TBI using a $^{137}$Cs irradiator. GT3 (200mg/kg body weight) was administered 24 hours prior to TBI as a single subcutaneous dose. Groups of mice exposed to sublethal dose of TBI (8.0 Gy) were euthanized at 0 h, 4 h, 1 d, 4 d, 7 d and 21 d, and segments of proximal jejunum, lung and peripheral blood samples were procured. For gut permeability assay at day 4, 10.0 and 12.0 Gy TBI was used. Changes in intestinal immune cell populations, intestinal tight junction-related proteins and gut permeability were assessed.

Results: Radiation (8.0 Gy) induced a sharp decrease in the numbers of mucosal macrophages and neutrophils. Recovery of mucosal macrophages occurred within 1 week, whereas neutrophils remained low until 3 weeks post-TBI. Interestingly, GT3 significantly enhanced the recovery of both macrophages and neutrophils by day 4 post-TBI. Most importantly, GT3 inhibited the post-radiation increase in intestinal permeability in mice ($p = 0.001$) at day 4. Furthermore, GT3 restored post-TBI reduction in intestinal occludin levels by day 4, as assessed by Western blot analyses.

Conclusion: Taken together, these data suggest that GT3, in addition to enhancing immune system recovery, indirectly helps restore the integrity of the intestinal mucosal barrier. GT3 should be explored further as a therapeutic to alleviate or treat intestinal radiation toxicity.
Design and Development of Novel Structure Based Vitamin E Analogues, the Tocoflexols, as Radiation Protectors

Ujwani Nukala1,3, Awantika Singh1,3, Shraddha Thakkar4, Nukhet Aykin-Burns1, Rajeshkumar Manian4, Mahmoud Kiaei2, Philip J. Breen1 and Cesar M. Compadre1

1Department of Pharmaceutical Sciences and 2Department of Pharmacology and Toxicology, University of Arkansas for Medical Sciences, Little Rock, Ark, USA; 3Joint Bioinformatics Graduate Program, University of Arkansas at Little Rock and University of Arkansas for Medical Sciences, Little Rock, Ark., USA; 4National Center for Toxicological Research/US Food and Drug Administration, Jefferson, Ark., USA; 5Tocol Pharmaceuticals, LLC, Little Rock, Ark., USA

Purpose: There is a need for safe and effective agents that can be used in accidental or terrorist radiological emergencies. In this context, some of the vitamin E components had shown strong radiation protection effects. Vitamin E family includes eight isoforms, four tocopherols (α, β, γ and δ) and four tocotrienols (α, β, γ and δ) with the tocotrienols being more active, but the tocopherols having better bioavailability. Tocotrienols’ shorter circulation half-life is due to their low affinity for α-tocopherol transfer protein (ATTP), the liver protein that maintains the tocol’s plasma levels by recycling them into the systemic circulation. Intrigued by the fact that the tocols show dramatic differences in their pharmacokinetic and pharmacological profile, despite relatively minor structural differences, we have conducted computational and experimental analysis of their structure and properties. The results of our analysis suggest a paradigm in which the observed differences can be explained by a multifactorial function:

\[ \text{Tocol therapeutic efficacy} = F_n \left( \text{intrinsic activity}, \text{elimination rate}, \text{cell-uptake} \right) \]

We have used this paradigm to develop novel vitamin E analogues that show rapid cell-uptake and enhanced bioavailability while maintaining the strong radioprotective activity of the tocotrienols.

Methods: Based on the structural analysis of the complex of the alpha-tocopherol transfer protein (ATTP) with α-tocopherol, we have hypothesized that the flexibility in the tail of tocols is crucial for effective binding to ATTP. Thus, by using in silico molecular dynamics (MD) screening procedure we have identified the novel tocotrienol analogs, named tocoflexols, that have a long isoprenoid dienyl tail with a chromanol head, that have the best potential to bind to ATTP while retaining their bioactivity. MD simulations of the open conformation of ATTP (PDB ID: 1OIZ) in complex with δ-tocoflexol were performed using GROMACS 5.1.4, to test the binding pattern of the tocoflexols to ATTP. The cell uptake of tocols was studied, using mouse NSC-34 cells. The anti-oxidant potential of the tocoflexols was evaluated by measuring their ability to inhibit lipid peroxidation in microsomes using a TBARS assay.

Results: MD simulations showed that ATTP in presence of tocoflexol can readily attain the closed conformation, suggesting that tocoflexol can bind to ATTP and be recycled in to circulation with better bioavailability. Cell uptake measurements showed that the tocoflexols have cell uptake levels comparable to that of tocotrienols and significantly greater than those of tocopherols. Similarly, the TBARS assay showed that tocoflexols inhibit lipid peroxidation at levels comparable to those of the tocotrienols.

Conclusions: Tocoflexols have demonstrated comparable levels of bioactivity and cell uptake levels to tocotrienols, the most potent compounds currently available, and also have the potential for much greater bioavailability than the tocotrienols. Thus, tocoflexols have the potential to be developed into radioprotectors for clinical use.
WEDNESDAY, MAY 16, 2018

POST-THERAPY SIDE EFFECTS
PLENARY SPEAKER ABSTRACTS
Ionizing radiation activates p53 to cause cell-type dependent effects in normal tissues. Using genetically engineered mice with conditional deletion or activation of p53 mutant alleles in different cell types, we are investigating the role of p53 in acute radiation injury and late effects of radiation. These experiments show that p53 acts in gastrointestinal epithelial cells to prevent the radiation-induced gastrointestinal syndrome and late intestinal toxicity. p53 also acts in endothelial cells to promote late effects following radiation to the heart and spinal cord. In contrast, p53 functions in the bone marrow to promote radiation-induced hematopoietic syndrome. Surprisingly, the tumor suppressor p53 acts during total-body irradiation to promote radiation-induced lymphoma development through a non-cell autonomous mechanism. Because different p53 transcriptional targets can regulate these diverse responses to radiation, drugs that manipulate the p53 pathway may protect against radiation injury.
Purpose and objectives: Over 50% of cancer patients receive radiation therapy, but radiation doses are often constrained so the risk of severe damage to normal tissues is < 5-10%, meaning that > 90% of patients could have radiation dose escalation without unacceptable side effects if predictive biomarkers for tissue toxicity were available. The use of animal models with differing genetic backgrounds to assess radiation toxicity, followed by genetic mapping of radiosensitivity phenotypes, has the potential to identify new targets that can predict normal tissue toxicity from radiation therapy.

Materials and methods: We utilized a consomic rat panel, specifically rats where chromosome 3 from the inbred BN rat strain was introgressed into the SS strain background to derive the SS.BN3 consomic strain. To examine cardiac toxicity, adult male and female SS and SS.BN3 rats received image-guided whole heart radiation to a dose of 24 Gy x 1 or 9 Gy x 5 to isocenter (AP and 2 laterals, 1:1:1). Echocardiograms were performed. IHC was used to assess immune cell infiltrates and trichrome staining for fibrosis. RNA-seq was performed on left ventricle tissue. The Student's t-test was used to compare values. Survival curves were analyzed with Kaplan-Meier and log-rank test.

Results: The SS rats exhibited enhanced cardiac toxicity compared to SS.BN3 rats. By 150 days post-radiation, 5 of 11 (5/11) female SS rats died from heart failure, while 0/7 SS.BN3 rats died (p < 0.05). End diastolic and systolic volumes were significantly elevated after radiation in SS rats (p < 0.01). Radial and circumferential strain were significantly worse in SS rats at 3 and 5 months (p < 0.01). In females, moderate/large pericardial effusions were present in 6/6 SS rats compared to 1/7 SS.BN3 rats at 5 months. Similar results were seen using 5 x 9 Gy cardiac radiation. Interstitial fibrosis at 12 weeks was not different between the SS and SS.BN3 hearts, but perivascular fibrosis was significantly increased in SS rats (p < 0.02). IHC staining revealed significantly more CD68+ cells in the SS vs. SS.BN3 hearts at 12 weeks. RNA-seq analysis identified a number of genes on chromosome 3 differentially expressed in SS vs SS.BN3 hearts. Pathway analysis revealed mitochondria dysfunction was one of the most differentially expressed pathways between SS and SS.BN3 hearts.

Conclusion: These results demonstrate that genetic variants on rat chromosome 3 alter the sensitivity to cardiac radiation. Congenic mapping and targeted studies are ongoing to identify the causative host factor(s) on rat chromosome 3. This project has the potential to enhance the effectiveness and toxicity profile of radiation. These methods can be adapted to identify genetic factors influencing radiation sensitivity of a number of normal tissue and tumor types.
Metabolomics Pre-empts Radiation Induced Antecedent Tissue Injury in Hippocampal Tissue

Charles P. Hinzman, Janet Baulch, Khyati Y. Mehta, Kirandeep Gill, Charles Limoli, and Amrita K. Cheema

Although ionizing radiation induced normal tissue toxicity is known to have functional consequences in the brain, the underlying molecular alterations have not been elucidated. We have previously reported cognitive impairments with concomitant changes in dendritic complexity and spine density in mice 12-24 weeks after irradiation. The objective of this study was to delineate metabolic changes in the mouse hippocampus following whole body (4 Gy) or cranial (9 Gy) X-irradiation. Metabolomic and lipidomic profiling of hippocampal tissue revealed that radiation exposure induced dyslipidemia in mice at 2 days and 2 weeks post exposure. Strikingly, significant changes were also observed in metabolites of the hexosamine biosynthesis pathway, a finding that was further confirmed using orthogonal methodologies. We hypothesize that these changes in hexosamine metabolism could induce endoplasmic reticulum stress and contribute to radiation induced cognitive impairments. Taken together, our results show that molecular phenotyping is a valuable approach to identify potentially detrimental pathway perturbations that manifest significantly earlier than gross structural and functional changes in the irradiated brain.
A Personalized, In Vivo Method to Quantify the Number and Repair of DNA Base Changes Induced by Radiation and Other Carcinogens

Paul Okunieff, M.D., Steven B. Zhang, Ph.D., Zhenhuan Zhang, Ph.D., Sadasivan Vidyasagar, M.D., Ph.D., Reshu Gupta, Ph.D., Katherine Casey-Sawicki, M.A., Natalie Lockney, M.D., and Steven G. Swarts, Ph.D.

Department of Radiation Oncology, University of Florida Health Cancer Center, Gainesville, Fla., USA

Background and Significance: Over 90% of cancers are initiated by DNA base changes. The base changes are typically termed single-base substitutions (SBS) or base insertions or deletions (indel), and many are epigenetic. While these base errors are continuously generated due to polymerase errors, carcinogens and error-prone repair pathways, cancer only occurs if the base change hits a specific driver at a specific position on its DNA. Looking for the driver mutations is a late and potentially tragic event. Knowing an individual’s SBS induction frequency, the force leading to their driver mutations, provides an early opportunity to predict personal risk. This is especially valuable for individuals with hazardous occupations (e.g., first responders, radiation workers and astronauts) who would benefit from increased cancer screening.

Methods: Using QClamp technology (DiaCarta, Inc., Hayward, Calif.), we have developed a highly specific method for selective PCR amplification of repeated elements in genomic DNA. The method uses short and long interspersed elements (SINE/LINE) in mouse and human DNA. Using radiation doses from 1 Gy to 9 Gy, times from 2 hr after radiation to days and weeks after radiation, and strains of mice with different natural radiation sensitivities, we measured the accumulation of new base changes in the liver, spleen, bowel and brain. We are currently performing similar studies on human cells in vitro and in patients undergoing therapeutic radiation.

Results: Two hours after irradiation, the spleen demonstrated very high levels of base changes (e.g., 13 to 39 per 104 bases), which at 6 days returned to near normal. These results suggest that mature lymphocytes are sensitive to base changes, whereas progenitor cells are proficient at repair. The liver and bowel had lower levels of mutations at 2 hr (e.g., 5 to 9 per 10^4 bases) and little recovery at 6 days. Brain tissues were resistant to mutations and proficient at fidelity of repair. The relatively resistant C57BL/6 mice had no base changes in the brain; the more sensitive BALB/c mice had 0 to 5 changes per 10^4 bases. No consistent difference was seen between male and female mice.

Conclusion: As cancer drivers are increasingly well known, and the detection of random silent mutations is difficult, most methods for assessing cancer risk have focused on the former despite the importance of the latter. Our novel, yet inexpensive, method allows quantitative, organ-specific evaluation of base changes in non-coding DNA regions using samples as small as 400 pg of DNA and easily interpreted PCR reagents. We are further improving quantification using digital PCR techniques. Future studies will indicate if the accumulation of these base changes is associated with cancer risk.
Computing Regional Myocardial Radiation-Dose Response in Left-sided Breast Cancer Patients

Shruti Siva Kumar, M.S., Julie Bradley, M.D., Christopher Klassen, M.D., and Walter O'Dell, Ph.D.

Introduction: Breast cancer (BC) treatment often involves radiation therapy (RT) to the chest wall, axilla and mediastinal lymph nodes to reduce risk of recurrence and improve survival. However inadvertent radiation exposure to the heart often occurs, especially for patients with left-sided breast cancer, resulting in increased risk for major cardiac events at 5-15 years after RT. Our goal in this project is to correlate regional radiation dose with sub-clinical, regional changes in heart function at early time points to assess cardiac tissue dose-response.

Methodology: Breast cancer patients receiving either IMRT or proton therapy to the chest wall and axilla were enrolled as part of an IRB-approved study.

Regional heart function:

1. Cardiac magnetic resonance (CMR) images were obtained pre-RT and 6-12 months post-RT in BC patients, with both standard T1-weighted, and MR ‘tagging’ sequences.
2. The left ventricle (LV) was semi-automatically segmented using in-house software at 6-8 short-axis and 4-8 long axis slices, and 6-10 time-points from end-diastole and end-systole.
3. 3D surface models in a prolate spheroidal coordinate system (PSCS) were then fit to both the epicardial and endocardial surfaces to define the extent of the LV myocardium.
4. A uniformly-distributed mesh of LV material points was generated from the surface models.
5. A novel deformable image registration approach was used to compute 3D heart motion and mechanical strains from the tagged MR images.

Regional dose:

1. The treatment planning CT images were registered to axial MRI scans acquired pre-RT.
2. The dose field from the planning CT was then transformed onto the 3D scanner coordinates of the MRI series, and mapped to the location of each material mesh point at end-diastole.

Results: Our initial results show an overall decrease in the magnitude of circumferential strain throughout the LV mid-wall post-RT, consistent with an increase in global LV volume measured in this patient. Large regional differences in the magnitude of strain decrease is also evident with the smallest changes in the LV free-wall. The radiation dose to the heart is highly heterogeneous, with doses of 43 Gy measured near the LV apex but negligible dose to the majority of the heart.

Ongoing and future work: We are currently working towards correlating regional dose with regional changes in strain with the intent to extend current dose-response models for cardiac tissue. Future work is oriented towards examining a larger patient cohort to solidify these observations, and to compare the severity of early-stage cardiac toxicity between conventional X-ray vs. proton treated patients.
FGF-P: A Mimetic Biobetter for Mitigation of Gastrointestinal Syndrome

Steven G. Swarts, Ph.D., Amy Zhang, RN, Steven B. Zhang, Ph.D., Zhenhuan Zhang, Ph.D., Lori Rice, Ph.D., Sadasivan Vidyasagar, M.D., Ph.D., Reshu Gupta, Ph.D., Natalie Lockney, M.D., Katherine Casey-Sawicki, M.A., Daohong Zhou, Ph.D., Mohammad Akbar, Ph.D., Hartmut Derendorf, Ph.D., Hardik Chandasana, Ph.D., and Paul Okunieff, M.D.

1Department of Radiation Oncology, University of Florida Health Cancer Center, Gainesville, Fla., USA; 2Department of Pharmaceutical Sciences, University of Arkansas for Medical Sciences, Little Rock, Ark., USA; 3Department of Pharmaceutics, University of Florida, Gainesville, Fla., USA

Introduction: An effective mitigation agent for acute gastrointestinal (GI) syndrome is currently missing from our clinical armamentarium. Members of the fibroblast growth factor (FGF) family have been found to help prevent and mitigate radiation-induced bone marrow syndrome and GI syndrome as well as to improve skin and nerve healing. In particular, several studies have shown that basic FGF (FGF2) benefits both GI and hematopoietic syndromes in mouse models. However, natural FGF2 is severely depleted by total-body irradiation in humans, and replacement with hrFGF2 has logistical challenges and production costs that make clinical use and strategic stockpiling unrealistic. Thus, to fill this gap, our team developed FGF-P, a small peptide mimetic of FGF2. We hypothesize that FGF-P mitigates acute radiation-induced GI syndrome through a variety of cooperating mechanisms, including decreased mucosal cell loss, improved proliferation of small bowel mucosa and improved gut barrier function with reduced bacterial translocation. It also helps maintain progenitor cells through signaling pathways that mimic natural FGFs.

Methods: Different doses and schedules of FGF-P (FGF2 used as positive control) were administered SC beginning 24 hr after irradiation in 8-week old male and female NIH Swiss mice. Animals received sub-total body (a hind leg out of field) gamma irradiation with doses ranging from 17 to 21.5 Gy. Hematopoietic factors, GI function, cellular proliferation and cellular maturation parameters were tested by various methodologies. Pharmacokinetic (PK) modeling of FGF-P was conducted in sham-irradiated and irradiated Wistar rats.

Results: A survival benefit accompanied by reduced GI bleeding and improved stool formation was seen in mice receiving FGF-P at 5-20 mg/kg SC for 1, 3, or 10 days. These results were most pronounced in animals receiving 3 daily doses. The PK of FGF-P was similar and linear in sham-irradiated and irradiated rats, with rapid absorption into systemic circulation, a short half-life and rapid elimination. Single doses of FGF-P or FGF2, given 24 hr after irradiation, induced mitochondrial functional benefits that persisted for at least 30 days.

Conclusion: FGF-P administered SC for 1 to 10 days, beginning 24 hr after irradiation, produced a survival benefit in a GI syndrome mouse model despite rapid clearance kinetics. FGF2 and FGF-P induced signaling and mitochondrial changes that appear consistent with a common mechanism of action. These promising results indicate that FGF-P may be a potential mitigation agent for radiation-induced GI syndrome.
Design and Development of I-PARTS, an Integrated Platform for Anti-cancer Radiation Therapeutics Screening

Rao Papineni, M.Sc., Ph.D.

University of Kansas Medical Center, Kansas City, Kan., USA

Tumor irradiation during cancer treatment induces cell death by apoptosis, mitotic death and clonogenic death. With expanded global access to cancer radiotherapy, there is a need to discover agents with anti-cancer and radiation sensitizing properties. In vitro high-throughput screening (HTS) systems routinely used to screen agents for cytotoxicity assays unfortunately do not directly measure clonogenic potential and fail to accurately predict the efficacy of an agent either in subsequent preclinical animal model testing or in clinical trials. Clonogenic assays unlike colorimetric and metabolic assays for determining cell viability and apoptosis, are less susceptible to artifacts. We are addressing these requirements through an integrated I-PARTS platform development and optimization. Here, the design and the prototype of a fully enclosed imaging/X-ray irradiation system will be presented. The proof of principle experimentation to obtain high content information on the cellular response to specific primary human cells (normal or tumors) to a variety of radiation doses and fraction modality will be described. The initial approaches taken towards the establishment of an integrated platform to screen anti-cancer drugs will be discussed.
Development of Partial Body Irradiation Model Using Small Animal Radiation Research Platform

Vidya P. Kumar, Ph.D., Sasha Stone, B.S., Shukla Biswas, M.S., and Sanchita P. Ghosh, Ph.D.

Armed Forces Radiobiology Research Institute, Uniformed Services University of the Health Sciences, Bethesda, Md., USA

Background: Radiation injury will result in multiorgan dysfunction leading to multiorgan failure. In addition to many factors such as radiation dose and dose rate, the severity of the injury will also depend on organ systems which are exposed. Radiation exposure could be due to many reasons such as nuclear accidents, terrorism or exposure to normal tissue during radiotherapeutic treatments. The strategy for screening for radiation countermeasures can either be carried out on total body irradiated (TBI) mice or partial body irradiation (PBI) targeted to specific system organ. Both options have their pros and cons. Here, we describe one such effort of PBI by targeting either gastrointestinal (gut-PBI) or thoracic region (lung-PBI) using the Small Animal Radiation Research Platform (SARRP). SARRP is an X-ray irradiator with capabilities of image-guided irradiation with a variable collimator. The precise beam geometry of SARRP aids in accurate target exposure with minimized exposure to non-targeted tissues and organs.

Design: Determinations of coordinates and isocenter to establish the field of exposure were done using CT scans of the mice. Mice were anesthetized during irradiation using isoflurane. A fluoroscopic X-ray image using portal imaging camera was used to confirm the desired isocenter and field of exposure. For lung-PBI, C3H/HeN male mice were exposed to a 14 or 16 Gy dose of radiation to the lung area (below the neck to diaphragm). For gut-PBI, the gut area (diaphragm to pelvis, avoiding pelvic bone) was exposed to 12 Gy dose. All the animals were transferred to their respective cages on recovery from anesthesia. Blood and tissues were collected from these animals at various time points post-PBI for analysis to characterize the model.

Results: Precise and accurate irradiation of lung and gut region using SARRP was carried out successfully. Though a low throughput model, it is amenable to “on the spot” changes in field size. As expected, damage to the targeted tissue was observed in the histopathological evaluation. EPO and SAA levels were found to be elevated in response to radiation injury. PCNA and PARP-1 levels were found to be decreased in the irradiated group. Citrulline levels dropped significantly on day 4 post-gut-PBI indicating intestinal tissue injury due to radiation. No damage to other non-targeted organs was observed in histopathological evaluation. There was no change in CBC of irradiated mice in comparison to naïve mice. Bone marrow cells had no damage in irradiated mice.

Disclaimer: The views expressed here are based on my opinion and do not represent the Armed Forces Radiobiology Research Institute, the USUHS or the DoD. There is no conflict of interest, financial or otherwise, to declare.

Funding: CDMRP and NIAID IAA
Loss of Sirt3 Exacerbates IR-induced Liver Injury

Kimberly Krager, Ph.D., Francesca LoBianco, M.S., Gwendolyn Carter, Ph.D., Youzhong Yuan, M.D., Ph.D., Nukhet Aykin-Burns, Ph.D.

Division of Radiation Health, Department of Pharmaceutical Sciences, College of Pharmacy, University of Arkansas for Medical Sciences, Little Rock, Ark., USA

Chronic normal tissue injury due to ionizing radiation (IR) exposure is a significant concern for patients receiving radiation therapy. The late radiation effects in liver tissue as a result of abdominal exposures include radiation hepatitis, fibrosis and in some cases hepatocellular carcinomas in months or years following treatment. Pathophysiology of radiation late effects in liver tissue is not completely understood. However, recent studies suggest mitochondrial dysfunction and oxidative stress as key players for liver disease progression following stress/injury. Sirtuin 3 (SIRT3), the primary mitochondrial NAD-dependent protein deacetylase, plays a major role in maintenance of liver mitochondrial integrity, regulation of intracellular reactive oxygen species (ROS) and/or antioxidant production, and tumor suppression.

In this study 10 week (young) or 10 month (old) male mice (wild type or SIRT3⁻/⁻) were given 24 Gy radiation specifically targeted to the liver using the Small Animal Radiation Research Platform (SARRP). The liver treatments were performed using a 5 x 5 mm collimator. Liver tissue was collected 6 months after IR treatment. H&E scoring suggested a moderate initiation of persistent liver injury and also indicated micro and macrovesicular steatosis in IR young SIRT3⁻/⁻ mice. Irradiated older mice presented an increase in lymphoplasmacytic inflammation in the perivenular space. Inflammatory cytokines TGF-B, IL6 and IL1B as well as TUNEL staining in old SIRT3⁻/⁻ mice were also significantly increased suggesting persistent inflammation and apoptosis 6 months following IR exposure. Our IHC data also indicated increased protein oxidation determined by 3-nitrotyrosine levels in both young and old SIRT3⁻/⁻ irradiated livers.

Although no long term studies following the loss of Sirt3 function and IR-induced liver injury have been completed to date, it has been demonstrated that superoxide mediates acute IR induced liver injury in SIRT3⁻/⁻ mice. There were no changes in MnSOD in any of the groups, however activity of antioxidant enzymes involved in hydrogen peroxide and organic hydroperoxides were significantly altered in irradiated SIRT3⁻/⁻ livers. These results suggest that as a mitochondrial protein, Sirt 3 could cause a peroxide mediated injury in a murine liver long after IR exposure.
Dietary Methionine Modulates the Gastrointestinal Response to Radiation

Isabelle R. Miousse, Ph.D., Laura Ewing, M.Sc., Rupak Pathak, Ph.D., Sarita Garg, Ph.D., Charles M. Skinner, B.S., Stepan Melnyk, Ph.D., Oleksandra Pavliv, Howard Hendrickson, Ph.D., Reid D. Landes, Ph.D., Annie Lumen, Ph.D., Alan J. Tackett, Ph.D., Nicolaas E.P. Deutz, M.D., Ph.D., Martin Hauer-Jensen, M.D., Ph.D., and Igor Koturbash M.D., Ph.D.

Methionine is an essential amino acid required for normal growth and development. Despite its importance to biological systems, methionine is toxic when administered at supra-physiological levels. The aim of this study was to investigate the effects of short- and long-term methionine dietary modulation in conjunction with total body irradiation. Dietary L-methionine supplementation to levels comparable to the ones found in a Western dietary pattern exacerbated radiation-induced gastrointestinal injury 6 days after exposure. Supplementation led to a greater loss of crypt depth and mucosal surface in the proximal jejunum than radiation alone. This was mirrored in plasma citrulline concentrations. Both radiation and methionine supplementation decreased the methionine concentration in the jejunum tissue. The supplemented diet also further decreased the gene expression of many tight junction-related intestinal transmembrane proteins, including Cldn8, Cldn9 and Cldn10. Methionine supplementation exacerbated mortality in mice exposed to ionizing radiation, from 0% to 40% mortality after 3 Gy of radiation and from 50% to 100% after 5.5 Gy. Results from partial body irradiation were consistent with gastrointestinal injury. In contrast, removing L-methionine completely from the diet improved survival when the diet was initiated after irradiation. However, the same diet increased mortality when initiated 2 days prior to irradiation. Restricting dietary methionine to 25% of the recommended daily intake, a level comparable to a vegan dietary pattern, was found to improve survival after 7.4 Gy. In conclusion, dietary modulation of methionine intake within levels consistent with different dietary patterns dramatically alters the response to ionizing radiation.