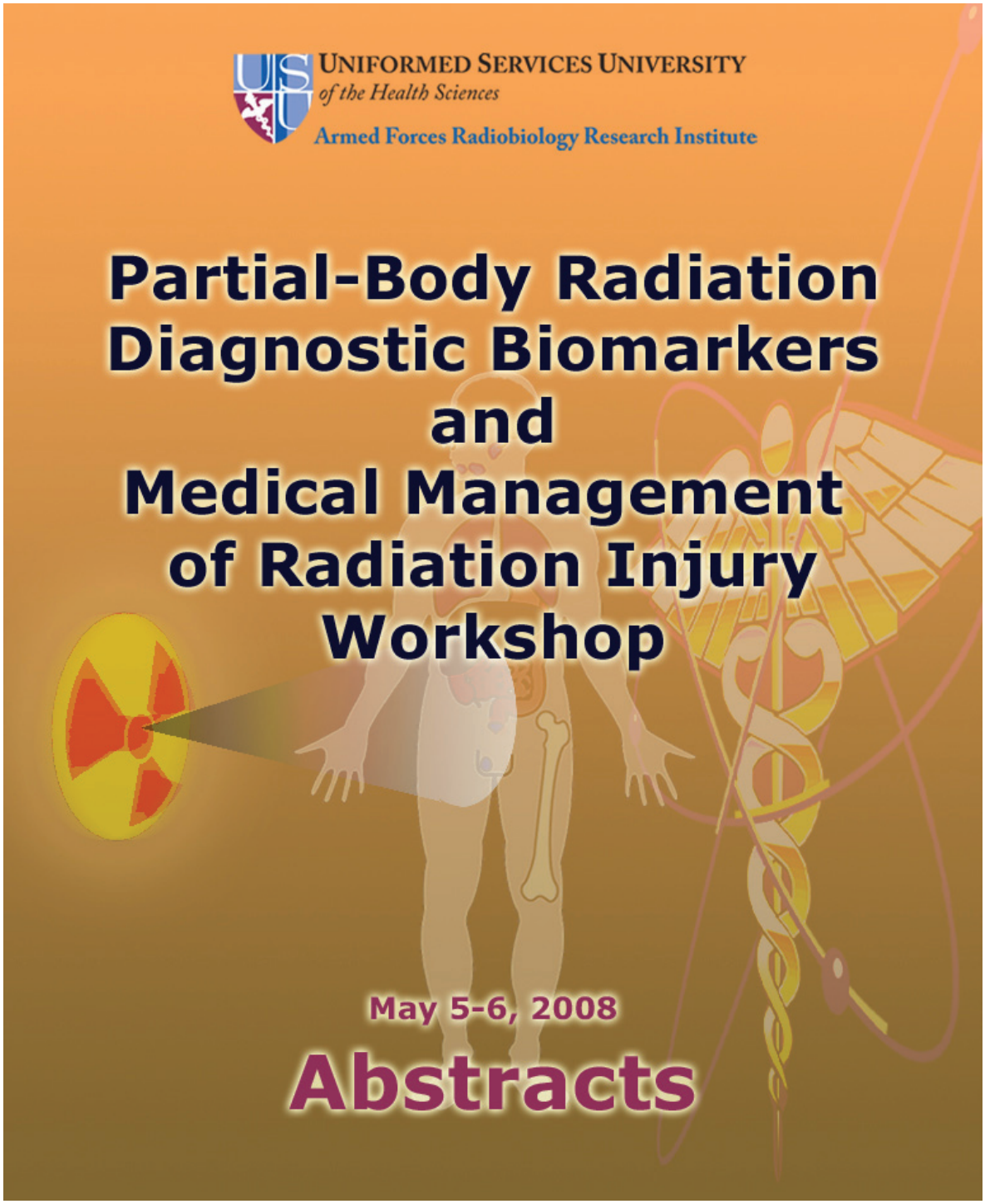

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Partial-Body Radiation Diagnostic Biomarkers and Medical Management of Radiation Injury Workshop

May 5–6, 2008

Sponsored by
Armed Forces Radiobiology Research Institute
Uniformed Services University of the Health Sciences
8901 Wisconsin Avenue
Bethesda, MD 20889-5603

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Contents

* Workshop speaker

SESSION IV
Gut

Poster Abstracts

Foreword

We hope that those who traveled to Bethesda, Maryland, had a safe and pleasant trip and welcome you to the Partial-Body Radiation Diagnostic Biomarkers and Medical Management of Radiation Injury Workshop.

The Workshop Program Committee has organized a relevant scientific agenda for the workshop, addressing many topics: radiation dose assessment models for partial-body exposures, biodosimetry for organ system injury, novel diagnostic approaches, and current approaches for treatment of partial-body exposures. We expect the lectures and poster presentations to be both informative and thought-provoking. We also anticipate that all workshop participants will contribute to the roundtable discussion led by our panel of experts. We aim to develop a plan for a path forward to address the need for partial-body diagnostic biomarkers.

It is our goal to make your brief stay here both productive and enjoyable. The workshop's agenda includes a scientific as well as a social program, where we encourage discussions and interactions. The workshop scientific program will be held in the AFRRI Conference Room.

We look forward to your participation in our workshop. Thank you for your contribution to the workshop theme both in your presentations and discussions.

Patricia K. Lillis-Hearne, COL, MC, USA
Director, AFRRI

Preface

Radiation mass casualties that occur in urban areas are likely to be partial-body exposures. Treatment based on whole-body dose assessments may not be appropriate for partial-body exposures, especially when local doses are high.

Actively proliferating systems such as bone marrow, the gastrointestinal tract, and skin are among organs critically affected by radiation. The current concept for treating radiation injuries is to provide supportive care and available countermeasures to the critically exposed cohort. Treatment depends on knowledge of an individual's injury due to the absorbed dose and dose distribution.

The Armed Forces Radiobiology Research Institute's May 5–6, 2008, workshop provides a forum for discussing diagnostic biomarkers, interlaboratory comparisons, dose assessment approaches, and treatment strategies for partial-body radiation exposures. We aim to develop a consensus on the best way forward to address the requirement for partial-body diagnostic biomarkers.

Workshop lectures include oral and poster presentations. Topics will include potential scenarios of radiation mass casualties, biodosimetry emergency preparedness, emergency medical management, diagnostic markers of partial-body exposures and dose assessment, assessment of dose to critical organs impacting acute survival, development of statistical models for partial-body exposure assessment, and expert panel discussions.

Abstracts have been published on the workshop website. Following the workshop, a consensus paper/meeting report will be developed. We anticipate that participants will reflect government, academia, regulatory, and industry communities.

Patricia K. Lillis-Hearne, COL, MC, USA
AFRRI Director

Terry C. Pellmar, PhD
Scientific Director, AFRRI

Pataje G.S. Prasanna, PhD
Research Biologist

William F. Blakely, PhD
Program Advisor, Biodosimetry

About the Speakers, Moderators

Jean-Marc Bertho, PhD, HDR
Senior Scientist, IRSN, Fontenay aux Roses, France

Jean Marc Bertho has an initial formation in immunology and hematology. He joined IRSN in 1991, and started experimental studies on the radiation-induced hematopoietic syndrome, both in mice, in non human primates and in humans. These studies lead to the development of a new biological indicator of radiation-induced damage to the hematopoietic system, the blood Flt3-ligand concentration. He also developed studies about the use of hematopoietic stem cell expansion and cytokine injection in the treatment of the hematopoietic syndrome. He is a radiopathologist strongly implicated in the follow-up of radiation accident victims that are treated in France. Recently, he is working in radiation toxicology, on the effects of chronic contamination through ingestion of radionuclides.

William F. Blakely, PhD
Senior Scientist, Scientific Advisory Board Member/Biological Dosimetry, Armed Forces Radiobiology Research Institute (AFRRI), USA

Dr. Blakely received his PhD in 1980 at the University of Illinois-Urbana-Champaign in radiation biology; his doctoral advisor was Dr. Howard S. Ducoff. He completed his postdoctorate study on DNA radiation chemistry in Dr. John F. Ward's laboratory at the University of California, San Diego. In 1983 he joined the Armed Forces Radiobiology Research Institute, his present affiliation. Dr. Blakely's research activities have focused on molecular mechanisms of radiation sensitivity, cell cycle effects, DNA damage and repair, and biological dosimetry. He presently is the Biodosimetry Research Group Advisor for his Institute, which is a component of Uniformed Services University of the Health Sciences. He also serves as a U.S. representative on the ISO TC85/SC2 (Radiation Protection) Working Group 18 (Performance Criteria for Service Laboratories Performing Biological Dosimetry by Cytogenetics), Chair of a NATO Research Study Group-Radiation Bioeffects and Countermeasures (RTG-033), and on Council for the National Council on Radiation Protection & Measurements (NCRP). Additional information can be obtained online at his website http://myprofile.cos.com/wfblakely

Doran M. Christensen, DO (Moderator)
Oak Ridge Institute for Science and Education (ORISE), USA

Dr. Doran Christensen hails from the state of Iowa. He was a U.S. Army Medic in the late 1960s and served in France, Belgium and the Republic of Viet Nam. After graduating from the University of Iowa with a baccalaureate degree, he worked as a graduate teaching assistant in physiology and genetics while working on a master's degree at the University of Iowa. He was awarded a U.S. Navy Health Professions Scholarship to attend the College of Osteopathic Medicine and Surgery in Des Moines, from where he graduated in 1975 with his DO degree. His post-graduate training in medicine was at the Philadelphia Naval Regional Medical Center. Subsequently, he served at the Portsmouth NRMC and aboard the USS Guam, LPH9. Dr. Christensen has spent almost 30 years in the practice of Emergency Medicine and Occupational Medicine and most recently spent 12 years as Medical Director at the U.S. DOE Fernald Environmental Management Project outside of Cincinnati, Ohio. He was trained in Aerospace Medicine in the U.S. Air Force and served on active duty with the 906th Tactical Fighter Group during Operation Desert Storm. He became the Associate Director of the Radiation Emergency Assistance Center/Training Site (REAC/TS), Oak Ridge, Tennessee, in 2004.

John P. Chute, MD
Duke University, Durham, NC, USA

Dr. John Chute is an Associate Professor of Medicine at Duke University Medical Center. Dr. Chute received his medical degree at Georgetown University and completed his training in Internal Medicine at the National Naval Medical Center. He subsequently completed training in medical oncology and hematology at the National Naval Medical Center and National Cancer Institute. Dr. Chute directs a laboratory in stem cell biology at Duke University and his research focuses on characterizing the intrinsic and extrinsic pathways which regulate hematopoietic stem cell fate. His current research focuses on the role of the bone marrow vascular niche in controlling hematopoietic response to myelotoxicity and in the development of peripheral blood signatures of radiation response.

Eric P. Cohen, MD
Medical College of Wisconsin (MCW), Milwaukee, WI, USA

Eric P. Cohen, MD, is a Nephrologist at the Medical College of Wisconsin and Froedtert Hospital, Milwaukee, Wisconsin. He has studied radiation nephropathy, experimental and clinical, for over 15 years, in collaboration with John Moulder, PhD. Their studies have shown that radiation injury may be mitigated by antagonists of the renin-angiotensin system. Current studies have focused on persistent oxidative stress in this model, its measurement and its treatment.

Ronald E. Goans, PhD, MD, MPH
Senior Medical Consultant, MJW Corporation, USA
Clinical Associate Professor, Tulane University School of Public Health and Tropical Medicine
Affiliate Faculty, Radiation Emergency Assistance Center/Training Site (REAC/TS), USA

As Senior Medical Consultant to MJW Corporation, Dr. Goans provides radiation medicine consultation to the NIOSH dose reconstruction project operated under the Energy Employees Occupational Illness Compensation Program Act. He also provides radiation accident consultation to the Nuclear Regulatory Commission, the International Atomic Energy Agency, and the Radiation Emergency Assistance Center/Training Site. A member of the faculty of Tulane University, Dr. Goans teaches courses in health physics and the pathological basis of disease. He developed two of the clinical tests commonly used in early evaluation of radiation injuries. His current research interests include mathematical modeling of radiation accidents, early radiation accident triage techniques, and ultrasound techniques for the evaluation of acute local radiation injury. Dr. Goans is an Associate Editor of the Health Physics Journal. He is on the Council of the NCRP. Most recently, he participated in writing NCRP Commentary 19, "Key Elements of Preparing Emergency Responders for Nuclear and Radiological Terrorism," and the report of Committee SC 4-1, "Management of Persons Contaminated with Radionuclides."

Marcy Beth Grace, PhD
Research Biologist/Biodosimetry Group, Armed Forces Radiobiology Research Institute;
Research Assistant Professor/Uniformed Services University, USA

Dr. Grace is a Research Biologist at the Armed Forces Radiobiology Research Institute and an Assistant Professor of Radiobiology in the School of Medicine at the Uniformed Services University of Health Sciences, Bethesda, Maryland. She earned her PhD in Genetics from George Washington University (1996) where her research was based on identifying the underlying molecular mechanisms associated with functional gene mutations. Starting from January 2000, Dr. Grace's research goals at AFRRI include the development of rapid, noninvasive techniques that use peripheral whole blood to establish radiation-responsive DNA damage and gene expression biomarkers. Utility of these biomarkers for biodosimetry applications are based on elucidation of the molecular mechanisms of radiation sensitivity, regulation of cell-cycle checkpoints, and integration of DNA damage/repair circuitry associated with cellular responses to ionizing radiation. The ultimate intention of her

research is to develop forward deployable molecular biodosimetry tools of practical use to the military.

P. Richard Hill, PhD
Ontario Cancer Institute (OCI)/Princess Margaret Hospital (PMH), Canada

Dr. Hill's research program focuses on laboratory and translational research studies in tumour and normal tissue radiobiology, metastasis and aspects of the tumour microenvironment, notably tumour hypoxia. Dr. Hill trained in Physics at St John's College, Oxford, and in Radiation Biology at St Bartholomew's Hospital Medical College in London. He has been a member of the senior scientific staff of Ontario Cancer Institute/Princess Margaret Hospital (OCI/PMH), which is part of the University Health Network (UHN) in Toronto since 1973. He is currently a Professor in the Departments of Medical Biophysics and Radiation Oncology at the University of Toronto. His research is funded by the National Cancer Institute of Canada with funds raised by the Terry Fox Run, by the Canadian Institutes of Health Research, and by NIH/NIAID (grant numbers U19 AI067734 and U19 AI067733).

Patricia K. Lillis-Hearne, MD, MHA
Director, Armed Forces Radiobiology Research Institute (AFRRI), USA

COL Lillis-Hearne was selected to be the 15th Director of the Armed Forces Radiobiology Research Institute after completing the Air War College at Maxwell Air Force Base in Montgomery, AL. Immediately prior to that, she was Commander of the 67th Combat Support Hospital and Wuerzburg MEDDAC in Wuerzburg, Germany. During that assignment, she commanded Medical Task Force 67 in support of Operation Iraqi Freedom (OIF). COL Lillis-Hearne received her undergraduate degree in Chemistry and Biology from the University of South Carolina, and her medical degree from the Medical University of South Carolina, Charleston. Her residency training in Internal Medicine and subsequent fellowship in Hematology/Oncology were completed at Brooke Army Medical Center in San Antonio. She trained in Radiation Oncology at the University of California, San Francisco (UCSF). COL Lillis-Hearne is board certified in Internal Medicine, Medical Oncology and Radiation Oncology. She also holds a master's degree in Health Care Administration from Seton Hall University. Other key assignments have included serving as Deputy Commander, Europe Regional Medical Command, and Deputy Commander for Clinical Services at WMEDDAC in Germany. She served as Chief of Radiation Oncology at Brooke Army Medical Center (BAMC) and also as Chief of Medical Oncology at Eisenhower Army Medical Center (EAMC). As Staff Internist with the 121st Evacuation Hospital in Korea, she served as Chief of Pulmonary Medicine. COL Lillis-Hearne's awards include, among others, the Legion of Merit, the Bronze Star, the Meritorious Service Medal with three Oak Leaf Clusters, a Joint Meritorious Unit Citation, the Global War on Terrorism Expeditionary Medal and the Global War on Terrorism Service Medal.

David C. Lloyd, PhD
Senior Group Leader, Cytogenetics Health Protection Agency, Centre for Radiation, Chemical and Environmental Hazards (HPA-CRCE), UK

David Lloyd trained as a zoologist in the University of Wales. In 1971 he joined the UK National Radiological Protection Board (NRPB) as leader of the Cytogenetics Group. This institute, located close to Oxford, was incorporated in 2005 into the Radiation Protection Division of the UK Health Protection Agency. For the past 38y David has specialised in biological dosimetry, undertaking an extensive research programme in the field (in excess of 250 publications) and providing a biological dosimetry service for the UK and several other countries. He has undertaken numerous consultancies with IAEA, WHO and ISO promoting the introduction and spread of biological dosimetry capabilities worldwide. He is currently involved with programmes for evaluating and improving the UK preparedness for responding to large scale radiological events.

Ludy C.H.W. Lutgens, MD, PhD
Maastricht Clinic, Netherlands

Ludy C.H.W. Lutgens, MD, PhD, is a radiation oncologist at the Maastricht Radiotherapy and Oncology Clinic, Maastricht, the Netherlands. He is specialized in treating patients with gynaecologic, urologic and gastrointestinal cancers. His research focuses on small bowel radiation damage. He coordinated a collaborative research project at the Maastricht University. Expertise on clinical radiation toxicity, experimental radiation toxicity, inter-organ metabolism and digitised imaging techniques were thus joined. The project group has demonstrated citrulline as a biomarker for measuring and monitoring cytotoxic treatment-induced small bowel functional epithelial cell loss.

Viktor Meineke, MD
Director, Bundeswehr Institute of Radiobiology, affiliated to the University of Ulm

COL Prof. Dr. Viktor Meineke is the Director of the Bundeswehr Institute of Radiobiology, affiliated to the University of Ulm and liaison institute to WHO REMPAN since 2004. He is a dermatologist and radiobiologist and his special expertise is in the field of cutaneous radiation injury as well as radiation-induced multi-organ interactions and failure. COL Meineke was appointed as a senior lecturer at the Technical University of Munich in 2004. He is an Adjunct Associate Professor of Radiation Oncology at Northwestern University, Chicago, Illinois, since 2006 and Adjunct Professor of the University of Ulm since 2007. COL Meineke has been a member of different expert groups of IAEA and WHO and among other commitments he currently is a member of the subgroup radiation protection in medicine within the German Federal Radiation Protection Board as well as the Bavarian Commission for Quality Assurance in Radiotherapy.

Natalia I. Ossetrova, PhD
Research Assistant Professor, Armed Forces Radiobiology Research Institute/HMJF, USA

Dr. Ossetrova is a Research Assistant Professor at the Armed Forces Radiobiology Research Institute at the Uniformed Services University of Health Sciences, Bethesda, Maryland. She has received her PhD in Experimental Particle Nuclear Physics at the Institute for Nuclear Research of the Russian Academy of Sciences (INR), Moscow, Russia, in 1999. In 2002 she joined BioTraces, Inc., Herndon, Virginia. Her research activities have focused on the development and optimization of immunoassays and the development of improved methods in proteomics. Dr. Ossetrova has extensive research experience in algorithm design; mathematical modeling and Monte Carlo simulations of the nuclear physics processes; investigation and application of electron, gamma and neutron detectors; development of Multi Particle Detection (MPD) technology instrumentation for biology and medicine. In 2005 Dr. Ossetrova joined the Biodosimetry research group at the Armed Forces Radiobiology Research Institute. Her research activities have focused on the validation of radiation-responsive protein biomarkers for biodosimetry applications in order to evaluate their utility as diagnostic biomarkers for early dose and injury assessment.

Terry C. Pellmar, PhD
Scientific Director, Armed Forces Radiobiology Research Institute (AFRRI), USA

Dr. Pellmar is Professor and Chair of the Radiation Biology Department at the Uniformed Services University. In addition, she is Scientific Director at the Armed Forces Radiobiology Research Institute at the Uniformed Services University, where she oversees the institute's various research programs. Dr. Pellmar has extensive research experience in radiation biology, depleted uranium toxicity, free radical effects in neural systems, medical countermeasures for radiological/nuclear threats, and behavioral health policy. She has recently established a Doctoral Program in Radiation Biology at the Uniformed Services University. Currently she is serving on the Radiological/Nuclear Threat Countermeasures Working Group (a US government advisory panel); NATO Research Task Group 033 (co-chairing Subpanel 4: Combined Injuries and Treatment); the CANUKUS Radiation Medicine

Subgroup of the Medical Countermeasures Coordinating Team; Editorial Advisory Board for the Journal of Medical Chemical, Biological and Radiological Defense; and external advisory panels for a number of academic research programs.

Pataje G.S. Prasanna, PhD
Research Biologist, Armed Forces Radiobiology Research Institute (AFRRI), USA

Dr. Prasanna is a Research Biologist at the Armed Forces Radiobiology Research Institute and an Assistant Professor of Radiobiology in the School of Medicine at the Uniformed Services University of Health Sciences, Bethesda, MD. He has been studying the effects of ionizing radiation on mammalian systems beginning with his thesis work in India for over 15 years. He has participated in several national and international research efforts in biological dosimetry by cytogenetics and harmonization of cytogenetic biodosimetry methods for radiation dose assessment (e.g., ISO TC85/SC2 Working Group 18, Performance Criteria for Service Laboratories Performing Biological Dosimetry by Cytogenetics). His laboratory's current focus is on the automation of cytogenetic methodologies for radiation dose assessment in radiation mass casualties.

Alexander Romanyukha, PhD
Technical Manager, Naval Dosimetry Center, USA

Dr. Romanyukha is a Technical Manager of the Naval Dosimetry Center and an Assistant Professor of Radiology and Preventive Medicine and Biometrics in the School of Medicine at the Uniformed Services University of the Health Sciences, Bethesda, MD. He has been working in the field of electron paramagnetic resonance (EPR) retrospective dosimetry since 1992. He served as a Chief Scientific Investigator of the IAEA research project Electron Paramagnetic Resonance Biodosimetry, a member of the ICRU Report Committee for Retrospective Assessment of Exposures to Ionizing Radiation, co-authored the IAEA-TECDOC-1331, ICRU report 68. Currently his research is focused on the EPR dosimetry in tooth enamel, bone and fingernails, TLD and OSL dosimetry.

Julie D. Saba, MD, PhD
Senior Scientist, Children's Hospital Oakland Research Institute (CHORI), USA
Co-Medical Director, Hematopoietic Stem Cell Cryopreservation Laboratory, Alta Bates Summit Medical Center, USA

Dr. Saba received her MD from the University of Maryland School of Medicine in 1985, completed a residency in pediatrics (1989) and a fellowship in pediatric hematology/oncology (1993), both at Duke University Medical Center (DUMC). She simultaneously initiated graduate studies in the area of sphingolipid metabolism and signaling under the mentorship of Yusuf Hannun, MD. She became Assistant Professor of Pediatrics at Duke in 1994 and completed her PhD there in 1996. In 1996, Dr. Saba initiated an independent research career at the Children's Hospital Oakland Research Institute. Her studies focus on the role of sphingolipid metabolism and signaling in the regulation of cell growth and death pathways, DNA damage and stress responses, immune cell trafficking, and in the biology and treatment of cancer. She was the first to clone and characterize the enzyme sphingosine-1-phosphate lyase (S1P lyase), which is responsible for catabolism of sphingosine-1-phosphate (S1P), an endogenous lipid metabolite that acts as a radioprotectant. Her current studies are focused on targeting S1P lyase for protection of normal tissues from various stresses and insults, including radiation injury. Dr. Saba is the recipient of an NIAID award under the program "Medical Countermeasures to Restore Gastrointestinal Function After Radiation."

Daniel Weisdorf, PhD
Director, Adult Blood and Marrow Transplant Program, University of Minnesota, USA

Dr. Daniel Weisdorf is Professor of Medicine and Director of the Adult Blood and Marrow Transplant Program at the University of Minnesota. He had Internal Medicine training in Chicago and subsequent Hematology/Oncology Fellowship at the University of Minnesota

where he remained on the faculty. He also serves as Scientific Director of the National Marrow Donor Program (NMDP) and Senior Research Advisor of the CIBMTR (Center for International Blood and Marrow Transplant Research). His research interests include complications of hemopoietic stem cell transplantation and immunotherapy for hemalogic malignancies. He serves on the Executive Committee of RITN (Radiation Injury Treatment Network) sponsored by the ASBMT (American Society for Blood and Marrow Transplantation) and NMDP.

Andrzej Wojcik, PhD
Professor, Stockholm University, Dept. of Genetics, Microbiology and Toxicology, Sweden

Andrzej Wojcik has since 1984 worked in the field of radiation biology in Austria (Forschungzentrum Seibersdorf), Germany (University Clinics Essen), Netherlands (EC Joint Research Centre—Institute for Energy, Petten) and Poland (Institute of Nuclear Chemistry and Technology). Starting from April 1, 2008, he moved to the GMT Department of Stockholm University, where he leads a radiobiology research group. Dr. Wojcik has been active for many years in the field of biological dosimetry. While working in Poland he was involved in the follow up and assessment of doses absorbed by radiotherapy patients during the Bialystok accident in 2001. He conducted a number of research projects, among others one on the analysis of individual radiosensitivity of human chromosomes 2, 8 and 14 (assessed in human peripheral blood lymphocytes by chromosome painting) for the purpose of biological dosimetry. In addition, a number of research topics relevant to biological dosimetry were carried out in collaboration with medical clinics. These include the analysis of micronuclei in lymphocytes of patients with thyroid cancer undergoing radiotherapy with I-131 or the analysis of micronuclei in lymphocytes of patients with restenosis undergoing brachytherapy with P-32, and the analysis of markers of individual sensitivity in lymphocytes of radiotherapy patients. He also participates in the normalization of cytogenetic techniques for biological dosimetry (19238 ISO recommendation, coordinated by P. Voisin of IRSN). Recently he coordinated the development of a statistical software dedicated to biological dosimetry.

Eduardo G. Yukihara, PhD
Assistant Professor, Physics Department, Oklahoma State University, USA

Dr. Yukihara has been involved in research on thermoluminescence (TL) and optically stimulated luminescence (OSL) since 1996. He received his PhD in 2001 at the University of São Paulo under the supervision of Dr. Emico Okuno, and in the same year joined Dr. Steve McKeever's group at Oklahoma State University as a postdoctoral fellow. Since 2004 he holds an Assistant Professor position at Oklahoma State University, where he has been developing the OSL technique to address challenges in radiological/nuclear accidents, neutron dosimetry, space dosimetry, and medical dosimetry. The OSU group is currently collaborating with the U. S. National Cancer Institute and Oak Ridge National Laboratory to develop the technology to use the OSL from dental enamel in medical triage in the aftermath of a radiological/nuclear accident.

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A Review of Partial-body Radiation Accidents

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The history of radiology provides an instructive introduction to the effects of radiation exposure to the skin. The discovery of X-rays was first announced by Roentgen in the public press on January 4, 1896. Also during January, 1896, Grubbe, a manufacturer of Crookes X-ray tubes in Chicago, IL, USA noted erythema, edema, hyperemia, blisters, epilation and hyperesthesia on the back of his left hand and sought medical attention on January 27, 1896, 23 days after Roentgen's announcement. Realizing from his own experience the destructive effects of high intensity X-rays and, in spite of his own pain, he treated a patient with carcinoma of the breast two days later. Historically, acute radiation accidents involving local injury to the skin are difficult to diagnose and treat because of the relatively long delay between the accident and the appearance of signs and symptoms. In order to devise an optimal care plan for such victims, it is necessary for the treating physician and nursing staff both to make the correct diagnosis in a timely manner, and to ascertain the relative magnitude of the accident. The medical history is particularly crucial for partial-body injury since signs and symptoms usually take days to weeks to manifest. It is also helpful that approximate deterministic thresholds exist for low LET radiation dose to skin (1) ~ 3 Gy for epilation, beginning 14–21 days post-accident; (2) ~6 Gy for erythema, transient post-accident, and appearing again 14–21 days thereafter; (3) 10–15 Gy for dry desquamation of the skin secondary to damage to the germinal layer; 4) 20–30 Gy for wet desquamation appearing at least 2–3 weeks post-exposure, and dose-dependent. The pathophysiology for erythema includes arteriolar constriction with capillary dilation and local edema. There is usually diminished mitotic activity in cells of the basal and parabasal layers with thinning of the epidermis and desquamation of large macroscopic flakes of skin. In cases of moist desquamation, microscopically, there is intracellular edema, coalescence of vesicles to form macroscopic bullae, and a wet dermal surface, coated by fibrin. For a skin dose >50 Gy, one observes overt radionecrosis and ulceration secondary to endothelial cell damage and fibronoid necrosis of the arterioles and venules in the affected area. A cutaneous syndrome, arising from high-level whole-body irradiation along with local injury, has also been described by Peter and various colleagues. The REAC/TS Radiation Registry sponsored by the US Department of Energy has been used to investigate the incidence since 1945 of partial-body exposure to skin, either alone, or in conjunction with whole body exposure. As of March 27, 2008, the Registry included 222 cases of pure local injury (532 patients, 28 fatalities) and 67 cases of local injury associated with at least some whole-body exposure (103 patients, 25 fatalities). Currently the Registry contains n=2408 radiation accidents and incidents. Analysis of a sample of those cases which were well documented indicates historically that sealed sources caused the majority of local injury (67%), while accelerators and X-ray devices caused the remainder. Of the sealed sources, only four isotopes were responsible for 89% of the accidents involving local injury (Ir-192 46.3% Co-60 31.1%, Cs-137 8.5% and Sr-90 3.0%). In the vast majority of these cases, diagnosis of skin dose was made by observing the clinical evolution of symptoms and often augmented by cytogenetic dosimetry. Analysis of overdispersion of dicentric chromosomes from the expected Poisson distribution using the Qdr technique of Sasaki was the most common mathematical tool.

In Vitro and Animal Models of Partial-Body Dose Exposure: Use of Cytogenetic and Molecular Biomarkers for Assessment of Inhomogeneous Dose Exposures and Radiation Injury

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The world-wide use of ionizing radiation that spans many disciplines for beneficial purposes has also led to hundreds of instances in which one or more persons were accidentally overexposed (Gonzalez 2007). International generic guidelines for early medical diagnosis and biodosimetric assessment of overexposed individuals are well established (Alexander et al. 2007; Blakely et al. 2005). These approaches, however, generally apply for assessment of whole-body exposures when, frequently, individuals involved in radiation accidents and cancer radiation-therapy patients exhibit non-homogeneous dose-exposure profiles. Research efforts have focused on development and validation of biodosimetric approaches applicable for radiation dose and injury assessment for partial-body or non-uniform distribution of dose.

Simulated partial-body exposures are typically modelled using *in vitro* blood or lymphocyte cultures. These studies often involve mixing increasing amounts of irradiated to non-irradiated cells immediately after *in vitro* radiation exposure. Various cytogenetic bioassays for radiation dose assessment, including dicentric, premature chromosome condensation, micronuclei, and fluorescent *in situ* hybridization or FISH assays (Lloyd et al. 1973, 1987; Blakely et al. 1995; Darroudi et al. 1998; Duran et al. 2002; Gotoh et al. 2005), have been applied in these simulated partial-body exposures or "mixing studies." Cytogenetic bioassays useful for partial-body dose assessment generally have dual features. First, radiation exposed cells can be discriminated from non-exposed cells and exhibit high percentage yields at low doses. Second, the degree of damage on a cellular basis can be quantified and exhibits meaningful dose dependency (Lloyd et al. 1973; Blakely et al. 1995; Gotoh et al. 2005).

These cytogenetic bioassays and other bioindicators (e.g., erythema, hair diameter) for radiation dose and injury assessment have also been used in animal partial-body radiation models. An external photon or high-LET radiation source is typically used with a radiation field that involves partial shielding to permit selective irradiation of discrete partial-body regions of animals (e.g., abdomen, lung, head, testes, isolated skin, etc.). In other cases, selective body regions (e.g., tibia, femur, abdomen, oral cavity, head, etc.) and organs (e.g., exteriorized intestine) are shielded and the remaining body parts are irradiated. For example, shielding one tibia of rats results in exposure of 95% of the marrow. These partial-body exposure models are often focused to address one or more of the acute radiation syndrome or sickness (ARS) sub-syndrome organ systems (e.g., haematopoietic, gastrointestinal, cutaneous) and other organ injuries (e.g., kidney and lung). Additional animal models used for this purpose include: mice, Syrian hamsters, dogs, miniature pigs, swine, and rhesus nonhuman primates. An x-ray dosimetry intercomparison was held among a number of laboratories involved in a partial-body irradiation study using mailed acrylic plastic rat phantoms. They demonstrated the value of improvements in dosimetry and irradiation procedures for partial-body irradiations (Puite et al. 1980). Animal studies have also used radioisotopes that target specific organs (e.g., radioiodine therapy of thyroid) to permit partial-body exposures. Use of intensity-modulated radiation therapy (IMRT) offers promise for radiation dose painting to specific organs for enhancing research to identify and validate bioassays for partial-body exposures.

Blood biochemical markers of radiation exposure have been advocated for use in early triage and injury assessment of radiation casualties (Bertho et al. 2001; Blakely et al., 2003a, 2003b, 2007; Roy et al. 2005; Ossetrova et al. 2007). Biomarkers can fall into two classes: early expressed biomarkers of radiation injury or organ-specific injury biomarkers exhibited at varied intervals after radiation exposure in a dose- and time-dependent fashion and which are based on specific organ and tissue transit times. The blood plasma biomarker approach has several advantages. Early biomarkers of radiation exposure may contribute along with other early biodosimetric indices, clinical signs and symptoms, and evidence of physical dose to initiate use of non-toxic medical countermeasures that demonstrate greater efficacy when initiated 24 h after radiation exposure (Waselenko et al. 2004; MacVittie et al. 2005). Organ- and tissue-specific biomarkers, representing cell and tissue response to radiation injury, will leak tissue- and organ-specific bioindicators into blood. These measurements can provide useful diagnostic information about the temporal onset and severity of specific organ and tissue system injury. For example, blood biomarkers have been shown to be correlated with radiation-induced hematopoietic ARS severity (Mal'stev et al. 2006), cell loss in bone marrow (Roy et al. 2005), and small-bowel epithelial mass (Ludgens et al. 2004).

Biomarkers along with clinical classification systems can assist in the prediction of clinical outcome, add new aspects for further research in understanding of ARS and, therefore, offer the ability to develop new strategies in medical care. Only a combined approach using clinical classification systems, biomarkers, other biodosimetric indices, and physical measurements will ensure the best strategy to formulate early medical-treatment decisions.

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Statistical Models for Partial-Body Dose Assessment: Gaps and Approaches

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Cytogenetic biological dosimetry is based on the analysis of chromosomal aberrations in peripheral blood lymphocytes (PBL). The absorbed dose is estimated by comparing the level of cytogenetic damage in PBL of an exposed person with an *in vitro* dose-response curve (called calibration curve). An advantage of the cytogenetic biodosimeter is that, due to blood circulation, a certain level of cytogenetic damage will be detected after exposure of any part of the body. Due to mixing of the lymphocyte pool, the level of damage following a high dose exposure to a small part of the body may be the same as the level of damage following exposure to a low dose exposure to the whole body. A practical question is how to distinguish these two exposure scenarios, based on the analysis of cytogenetic damage in PBL.

Here it must be recalled that chromosomal aberrations, notably dicentric chromosomes and centric rings, induced in PBL by a whole-body exposure to low-LET ionising radiation are Poisson-distributed. A unique characteristic of the Poisson distribution is that it can be described by a single variable Y which, in the case of chromosomal aberrations, is the mean number of aberrations per cell and is equal to the variance of Y. A consequence of this is that the distribution of aberrations, if Poissonian, can be computed based on the knowledge of Y.

Two methods have been developed that, following an acute partial-body exposure, allow to calculate the dose absorbed by the irradiated fraction of blood: the Dolphin method and the Qdr method. Both methods are based on the assumption that cells containing aberrations are those that have been exposed. Based on the distribution of aberrations, the number of exposed cells with no aberrations and the value of Y are computed. The dose to the irradiated blood is then estimated based on the calibration curve. In addition, it is possible to estimate the size of the fraction of body exposed.

Both methods are based on a number of assumptions: 1. that the dose to the irradiated fraction of blood was homogeneous, 2. that the exposure time was so short that only a fraction of blood was exposed, 3. that blood is distributed evenly in the body, 4. that the level of interphase death of PBL *in vivo* and *in vitro* is the same and individually not variable. The available data indicate that these assumptions are in fact simplifications.

The Dolphin and Qdr methods will be presented and their limitations explained. Also the problems of estimating the dose absorbed during exposure to fractionated doses (as in teleradiotherapy) will be discussed. Finally, an attempt will be made to highlight the research directions that should be undertaken in order to improve the applicability of biodosimetry to detect and estimate the dose following partial-body exposure.

A New Therapeutic Approach of Radiation Burns by Mesenchymal Stem Cell Transplantation

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The therapeutic management of severe radiation burns remains a challenging issue. Conventional surgical treatment (excision and skin autograft or rotation flap) often fails to prevent unpredictable and uncontrolled extension of the radiation-induced necrotic process. This is mainly due to two major causes on the first hand the difficulties to delineate extend and severity of radiation damages because of the unpredictable dynamic evolution of the lesions and on the other hand the very frequent delay in the recognition of the radiological nature of the lesions. Here we present two cases of radiation burns that occurred recently. The first accident occurred on December 15, 2005, in Chile, where a 27-year-old picked-up a gammagraphy source (^{192}Ir, 3.3 TBq) with his left hand and put it in the back left pocket of his trousers, where he kept it for approximately ten minutes before the alert was given. The patient rapidly exhibited multifocal lesions to the left hand and the buttock, and at the request of Chilean authorities, the patient was hospitalized at the burn treatment center of Percy military hospital on December 27, 2005. During this time, a physical reconstitution of the accident indicated more that 2000 Gy at the center of the buttock lesion. On the basis of the 20 Gy isodose determined by the physical dosimetry, an excision of the buttock radiation burn was made on day 21 post irradiation (PI), followed by a wound closure by a skin allograft, and in a second step by a skin autograft. However, due to a rapid lysis of the skin allograft together with an infected ulceration, a new therapeutic strategy was applied, using mesenchymal stem cell (MSC) autograft. For that purpose, a bone marrow harvest was made on day 75 PI, and MSC were expanded in vitro. A second excision was then performed on day 90 PI, followed by a second skin autograft together with local injection of 168×10^6 MSC. A second local transplantation of 226×10^6 MSC was made on 99 days PI and the lesion was further dressed with artificial derma. Following MSC injections, pain disappeared and the active clinical evolution was stopped. A complete healing was observed by 75 days post treatment (5.5 months PI) without any functional impairment.

The second case of radiation burns occurred in Dakar (Senegal) during June and July 2006. Following a technical failure, an iridium source was retained in the source ejection system. The material containing the source was stored near a work place during a 2-month period. The reconstitution of the accident allowed the identification of 63 potentially irradiated victims, of which 4 patients exhibited skin lesions of various severities. One of the most severely irradiated victims was hospitalized in Percy military hospital, 27 days after the discovery of the accident. At that time, a diagnosis of an acute irradiation syndrome together with a severe radiation burn to the left arm was evidenced. Biological dosimetry gave a mean global radiation of 2.6 Gy, but with strong evidence of heterogeneous exposure. The physical reconstruction of the radiation dose was not possible, due to the difficulty of defining a clear-cut scenario. The hematopoietic syndrome was evidenced by blood Flt3-ligand concentration of 2700 pg/ml, and was treated by G-CSF and EPO injections as soon as day 31. The hematopoietic syndrome resolved by day 35. By contrast, the evolution of the radiation burn to the left arm was worse. After a period of dry desquamation followed by moist desquamation, ulceration appeared. A first excision was made on day 100, followed by a succession of two rotation flaps, 5 MSC transplantations and 2 skin allografts. The detailed evolution of the lesion will be presented. During one of the excision, a fragment of the humerus was harvested for ESR dosimetry. Results indicated that the humerus

received a mean radiation dose of 40 Gy. However, more than 300 days post hospitalization the clinical evolution of the lesion was stopped and healing was observed, with some functional impairment due to the severity of the lesion.

Overall, these two cases of accidental irradiation showed opposite characteristics. In the Chilean case, the radiation was localized, and the radiological nature of the accident was recognized immediately. In the Senegal case, there was a combination of a global irradiation together with a localized burn, and the radiological nature of the lesions was recognized with a one month delay. However, in these two cases, the general therapeutic strategy was the same. Necrotic lesions were excised, the wound was covered with skin allogaft, and autologous MSC were locally injected around the lesion. Results were similar in the two cases, with a first immediate effect which is the disappearance of pain. A second effect was observed, with a progressive healing of the lesions. Although there are only two cases of radiation burn treatment by local injection of autologous MSC, the comparison with historical cases strongly suggest that this therapeutic strategy may be highly efficient.

Role of Damage to the Cutaneous System in Radiation-Induced Multi-Organ Failure

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Radiation damage to the skin is a key diagnostic and prognostic parameter for patients who accidentally have been exposed to radiation. The skin, moreover, is one of the key organs in radiation-induced multi-organ involvement and failure.

For systematic as well as for practical reasons, different radiation-exposure situations have to be distinguished. When discussing clinical aspects of the cutaneous radiation syndrome (CRS), these different scenarios have always to be taken into consideration. First of all, there is a need to differentiate between an acute and chronic radiation syndrome of the skin. The determinants in this case are time after radiation exposure as well as radiation quality and dose. From a biological and clinical point of view, damage to the skin organ and potentially other organs involved in the radiation field, including distant effects, is the important endpoint. Moreover, it must be differentiated between localized radiation injuries (e.g., one to several radiation ulcers) and the situation of whole or at least significant partial body exposures. These totally different situations may not only show up with different clinical courses (including varying patterns of biological indicators) but, furthermore, significant different requirements for therapeutic strategies. The existence of radiation-induced multi-organ interactions is a fact that must be faced nearly in all radiation exposure situations. The progression of these radiation-induced organ interactions, a so to say cascade-like process into a radiation-induced multi-organ failure, depends not only on the amount of damage to single organs but rather on interactions between more- and less-affected organs (and even not irradiated organs) and, thus, has a potentiating or self-augmenting effect. The crucial point of radiation response in organs and tissues is the individual capacity of these organs and organ systems to cope with radiation damage in a way other than the development of multi-organ failure.

To illustrate a model of radiation-induced multi-organ failure, the skin is an ideal candidate. A study based on the databank system SEARCH (System for Evaluation and Archiving of Radiation Accidents based on Case Histories) focused on the investigation of the timely course of radiation-induced skin reactions, the percentage of affected skin surface and the severity of affection. The percentage of affected skin surface turned out to be very important criteria for prognostic estimation of the clinical course of the acute radiation syndrome beneath the well-known categories regarding the haematopoietic system. This study underlined the importance of the CRS as a diagnostic parameter and triage pattern. It also revealed the skin as a crucial organ system for the prognosis of radiation victims, both independent of radiation-damage to other organ systems but also in a potentiating way.

The special so-called combined injury situation proved to worsen prognosis of radiation victims a long time ago. In the case of combined injuries, the important role of the skin was shown in several studies on animal models. Radiation exposure was combined with burns and open skin wounds, which became entrance ports for bacteria and could cause septicaemia leading to increased lethality. This fact, therefore, also must be taken into consideration when discussing contingency planning for radiological emergencies.

To a great extent, the pathophysiolgical background of radiation-induced multi-organ failure remains unclear. The recent pathophysiological understanding considers the endothelium as one connecting factor. Exposing the organism to a significant radiation dose causes an immediate response of the capillary bed both by direct and indirect action, resulting in an increased permeability (oedema) and fragility (petechial bleeding) and resembling inflammatory reactions.

Changes and interactions on the level of cytokines and other proteins also are intensively discussed. Therefore, future research on biomarkers of radiation exposure might also help to understand the pathophysiology of cellular and organ damage caused by ionizing radiation.
Due to the complexity of radiation exposure situations and the subsequent clinical pictures, one fact already is clear. Estimating radiation damage in a serious way can only be done by integrating biological and clinical data. There will be no single parameter allowing a reliable diagnosis of radiation injury or, moreover, clinical therapeutic decision-making.

This fact must be taken into account for future clinical research and, in particular, medical management of radiation-exposed patients.

Cytogenetic Assays for Partial-Body Radiation Accidents

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Biological dosimetry by chromosomal aberration analysis is a long-established technique that has value in the clinical management of accidental radiation overexposures. The simplest output from the technique is an estimate of the averaged whole body dose. It was appreciated early on that virtually all accidents involve partial exposure of the body and that where large doses are involved this can be discerned in the distributions of the aberrations among the scored metaphases. Two methods, known as the contaminated Poisson (CP) and Qdr, were developed independently to manipulate the aberration data mathematically to provide a more realistic estimate of the part-body dose and also by CP, an estimate of the percentage of the body volume involved. The techniques are now available in easy-to-use Windows format software. Both methods involve a number of simplifying assumptions but when tested with *in vitro* simulated accidents both produce similar dose estimates which are acceptably close to the known doses. Of course being based on blood lymphocytes, neither method can indicate the area of the body involved but, for acute external irradiation sufficient to be of clinical concern, this would be apparent from skin responses. The immediate value of the data to the clinician is the confirmation that some fraction of the body volume has been spared, or only lightly irradiated, and thus there are likely to be surviving foci of haematopoietic stem cells. Such data can inform the strategy for managing haematopoietic crisis by stem cell replacement or cytokine therapy. As well as *in vitro* cytogenetic experiments the methods have been tested *in vivo* in animals and radiotherapy patients. With Syrian hamsters the CP approach worked well with 1/3 and 2/3 body irradiations provided that the spleen was not in the field. In cancer patients receiving a single hemi-body irradiation the methods also produced dose estimates close to the given dose. However for fractionated radiotherapy the aberration distributions reverted to near Poisson which is to be expected given the inter-fraction circulation and redistribution of lymphocytes. Both the CP and Qdr methods are especially good at detecting situations where small volumes of the body have been spared. For the reverse situation where small volumes were irradiated, these are more difficult to detect. This is particularly so by the CP method because allowance needs to be made for the selective losses by apoptosis and cycle delay of the irradiated fraction of cells in reaching metaphase. For these situations the mitotic fusion/premature chromosomal condensation method that avoids cell culture is much more effective as then the percentage of cells seen with aberrations simply reflects the percentage of the body exposed. This was demonstrated *in vitro* with human, and *in vivo* with rhesus monkey, lymphocytes. However for a severe but just localised burn, there is unlikely to be a generalised haematological crisis and the clinical priorities are quite different. The cytogenetics community is currently much preoccupied with strategies for responding to mass casualty events and one approach is to move into a triage mode. This involves scoring a limited number of metaphases per subject to give approximate dose estimates more rapidly. With *in vitro* simulations it has been shown that even with only 50 cells scored it is possible to detect 50–95% partial-body volume irradiations of 2 Gy and above.

Automated Sample Preparation and Interlaboratory Cooperative Network for Conducting the Dicentric Assay

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The dicentric chromosome assay (DCA) is the "gold standard" biodosimetry method for radiation dose assessment. The DCA can be used for quickly assessing dose to individuals in the early period aftermath of a radiological or nuclear incident for optimum medical aid. DCA's application in radiation mass casualties necessitates greater sample processing and chromosome aberration analysis capacity. Therefore, automated sample processing, chromosome aberration analysis, and establishment of a co-operative network of cytogenetic laboratories are essential.

Recent efforts at the Armed Forces Radiobiology Research Institute (AFRRI) focused on increasing sample processing via automation, technology integration, and implementation of a laboratory information management system (LIMS) for resources and data. We developed a high throughput, flexible, modular, and scalable robotic blood handling system, which represents a "beta" version for automated blood handling aiding increased throughput. Other components of the automated cytogenetic biodosimetry laboratory include sample and reagent bar-code tracking, metaphase harvesters and a spreader, slide stainer, a high-throughput metaphase finder, and multiple satellite chromosome-aberration analysis systems all integrated with LIMS.

Because use of a cooperative network for chromosome aberration analysis and dose assessment by DCA requires routine quality control exercises among partner laboratories, the National Institute for Allergies and Infectious Diseases (NIAID) and AFRRI sponsored an interlaboratory comparison study to determine DCA's validity and accuracy among five laboratories following the International Organization for Standardization guidelines. Blood samples irradiated at the AFRRI were shipped to all laboratories, which constructed individual calibration curves in the 0.0 to 5.0Gy range for ^{60}Co gamma-rays and assessed the dose to dose-blinded samples. For all laboratories, the estimated coefficients of the fitted curves were within the 99.7% confidence intervals (CIs); but the observed dicentric yields differed. When each laboratory assessed radiation doses to four dose-blinded blood samples by comparing the observed dicentric yield with the laboratory's own calibration curve, the actual doses were within 99.75% CI for the assessed dose. Across the dose range, the error in the estimated doses, compared to the physical doses, was from 15% underestimation to 15% overestimation.

Our efforts improve diagnostic biodosimetry response by the DCA aiding optimum medical treatment for radiation-exposed individuals in mass casualties.

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Molecular Biomarkers of Bone Marrow Injury

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Previous work has demonstrated the potential for peripheral blood (PB) gene expression profiling for the detection of disease or environmental exposures. Our group recently has demonstrated that patterns of gene expression can be identified in the PB which predict radiation status and level of radiation exposure in mice and humans (PLos Medicine 2007;4:690–701). We subsequently have aimed to determine the impact of several variables on the PB gene expression profile of ionizing radiation, and to determine the specificity of the PB signature of radiation versus other genotoxic stresses. Neither genotype differences nor the time of PB sampling caused any lessening of the accuracy of PB signatures to predict radiation exposure, but sex difference did influence the accuracy of the prediction of radiation exposure at the lowest level (50 cGy). A PB signature of sepsis also was generated and both the PB signature of radiation and the PB signature of sepsis were found to be 100% specific at distinguishing irradiated from septic animals. We also identified human PB signatures of radiation exposure and chemotherapy treatment which distinguished irradiated patients and chemotherapy-treated individuals within a heterogeneous population with accuracies of 90% and 81%, respectively. We conclude that PB gene expression profiles can be identified in mice and humans that are accurate in predicting radiation status within a heterogeneous population, are specific to ionizing radiation exposure and remain highly accurate over time.

Citrulline: A Serological Parameter for Monitoring Epithelial Small Bowel Cancer Treatment-Induced Injury

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Glutamine is an important substrate for small bowel epithelial cells. Citrulline is an end product of glutamine metabolism in these cells. The typical enzymatic profile of small intestinal enterocytes makes that citrulline is not further metabolized by the enterocyte but instead is released into the circulation, i.e., the portal vein. Citrulline passes the liver without being metabolized. No other citrulline-releasing organ has been identified so far. As a result, the plasma citrulline concentration is directly dependent on the functional epithelial cell mass. This relationship has been confirmed in experimental animals following small bowel resection as well as in patients with celiac and non-celiac disease-associated mucosal atrophy and following small bowel resections. Small bowel epithelial atrophy is a well-known and validated endpoint for small intestinal radiation damage. Radiation damage to the intestinal crypt cell compartment and consequential epithelial denudation is strictly dose dependent. Based on this validated radiation effect on small bowel epithelium, the organ-specific metabolic characteristics of citrulline and the correlation between the citrulline level and the functional epithelial cell mass, we hypothesized that the plasma citrulline level can be used as a surrogate endpoint for epithelial small bowel radiation damage. A dose- and time-dependent effect for the plasma citrulline level following a single whole abdominal radiation was demonstrated in a series of experiments in mice. In addition, the citrulline level was correlated with the small bowel epithelial cell mass as determined by means of surface line measurements. Plasma citrulline level proved to be a reliable and reproducible parameter for measuring epithelial small bowel injury. This biomarker for epithelial small bowel radiation damage was further validated in patients with haematological malignancies following high dose chemotherapy and fractionated whole body radiation. Citrulline was thus correlated with clinical parameters for mucositis and with results obtained with sugar permeability tests as an endpoint for gut mucosal barrier injury. The time course of the citrulline level was more in agreement with known kinetics of epithelial cell loss following radiotherapy and chemotherapy. As compared to the sugar permeability tests, the citrulline assay was more sensitive and more specific for the detection of mucosal injury. In cancer patients with solid tumors treated to limited abdominopelvic volumes with fractionated irradiation, a dose- and volume-dependent decrease of the plasma citrulline level was demonstrated. The time pattern of the citrulline decrease correlated with the occurrence of and freedom from clinical symptoms.

Taken together, our results and those obtained in patients with functional epithelial cell loss from varying causes suggest that plasma citrulline level is a reliable parameter for the loss of functional small bowel epithelial cells, independent of the underlying cause. The sensitivity, the specificity, the methodological simplicity and relatively low costs make this assay the first choice for measuring and monitoring cytotoxic treatment-induced epithelial small bowel injury. Its use as a predictive assay for acute and late epithelial small bowel damage is another possible application to be determined.

Novel Sphingolipid Biomarkers of Gut Injury Induced by Radiation

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Sphingolipid metabolites are ubiquitous regulators of the cellular response to stress[1]. Ceramide is a sphingolipid metabolite that accumulates in cells treated with radiation and in the serum of irradiated patients [2–5]. This is due in large part to the radiation-induced hydrolysis of sphingomyelin by sphingomyelinases (SMase) in the plasma membrane[5–7]. Ceramide clusters membrane rafts, induces apoptotic signaling cascades and contributes to epithelial and endothelial cell death in radiation-induced enteritis[8,9]. Reducing ceramide by blocking SMase attenuates radiation injury and mucositis in rodent models[10]. However, ceramide can be generated by more than one route. Further, inhibitors of SMase and enzymes of ceramide biosynthesis are not specific and have associated toxicities that may preclude their use in children, pregnant women, the elderly and in large populations where risk of radiation exposure may be uncertain. Thus, alternative or complementary approaches are warranted.

Ceramide can be further catabolized to sphingosine-1-phosphate (S1P), a bioactive lipid that acts through well-defined signal transduction pathways to promote proliferation and survival of many cell types, including endothelial cells, stem cells and enterocytes[11–16]. S1P signaling is also essential for angiogenesis and vascular maturation[17–19]. S1P antagonizes growth-inhibitory and apoptotic pathways including those induced by ceramide and radiation[20–28]. Importantly, S1P promotes cell survival in response to radiation and prevents radiation-induced oocyte apoptosis and sterility in mice[29–34]. Thus, while ceramide contributes to radiation enteritis, its metabolite S1P provides an internal fine-tuning signal limiting the intensity of radiation responses by acting as an angiogenic factor and radioprotectant.

S1P is irreversibly degraded by the enzyme S1P lyase (SPL)[35]. SPL is a ubiquitously expressed, intracellular enzyme, and its highest levels of expression and activity are found in intestinal villi, where it metabolizes dietary sphingolipids. It has been proposed that high SPL expression and corresponding low tissue S1P levels in intestinal epithelium facilitate the rapid cell turnover characteristic of this tissue. SPL expression is induced by DNA damage, and its increased expression and activity promote apoptosis in an S1P-dependent manner (i.e., S1P addition can reverse the effects of SPL)[27]. In contrast, SPL is downregulated in intestinal adenomas, leading to S1P elevation, which drives proliferation[14,15]. SPL can be considered to function as an anti-oncogene whose downregulation may contribute to tumorigenesis. Long-term S1P accumulation could be tumor-promoting and, thus, is undesirable. However, in the scenario of acute radiation injury, a short period of SPL inhibition and S1P accumulation could enhance the survival of endothelial cells and enterocytes, thereby promoting crypt restoration, angiogenesis and recovery.

Our current study aims to use small molecule inhibitors to block (murine) SPL and raise circulating and tissue S1P levels for radioprotection. However, we have made the serendipitous observation that gut SPL activity rises on day 4 after 15 Gy TBI and, at the corresponding time point, S1P levels in blood plasma fall in a dose-dependent manner, with a reduction of 10% at 8 Gy, 24% at 10 Gy and 50% at 15 Gy. It is likely that intestinal SPL activation may be the explanation for reduced circulating S1P after radiation exposure. However, multiple tissues and cell types including erythrocytes, platelets and endothelium are now recognized as contributors to circulating S1P levels; in the case of endothelium, changes in endothelial SPL expression in response to shear stress produce alterations in circulating S1P[36–39]. This finding suggests the

possibility that plasma S1P levels could be an indicator of radiation-induced changes throughout the body and in different vascular beds. Based on these observations and the knowledge that circulating S1P levels can be reliably determined in blood plasma using tandem MS, HPLC and TLC methods, we hypothesize that S1P and possibly other sphingolipid metabolites may serve as useful biomarkers for radiation exposure. To adequately assess the utility of plasma S1P as a radiation biomarker, future studies will need to address remaining gaps in our current understanding of S1P regulation, such as potential effects of fasting, stress and other factors on circulating S1P levels, degree of correlation between TBI dose and S1P levels, and effects of partial body exposures and/or partial body shielding on circulating S1P levels. Additionally, development of rapid and simple methods for quantification of S1P in blood, such as by an ELISA system using the S1P monoclonal antibody, would be advantageous[40].

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Biomarkers for Renal Radiation Injury

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Biomarkers are molecular signs of an injury that are not necessarily critical to its mechanism or expression. Thus, serum levels of troponin are very reliable biomarkers of myocardial injury, but do not in themselves cause morbidity or mortality. Biomarkers may precede expression of tissue injury, and thereby provide a time window for use of agents that can mitigate injury. Truly useful biomarkers must be linked to later outcomes such as organ failure, and their modification should be correlated with improved later outcomes.

In clinical and experimental acute kidney injury (AKI, also known as acute renal failure) biomarkers under study include KIM-1 (kidney injury molecule 1), NAG (N-acetyl glucosaminidase), and IL-18, and others. In AKI, their increase in the serum precedes the elevation of serum urea or creatinine (i.e., it precedes the expression of reduced kidney function). KIM-1 and NAG derive from damaged renal tubular epithelium, while IL-18 is a cytokine that is induced after AKI. While promising, biomarker testing is not used clinically in the setting of AKI, in part because of variable sensitivity and specificity of the biomarkers. No single marker is able to discriminate common causes of AKI, such as sepsis with hypotension, gentamicin toxicity, or cis-platinum toxicity. Markers for chronic kidney disease are less well-studied.

Although urinary protein is elevated within weeks of sufficient kidney or total body irradiation, it is a manifestation of injury rather than a true biomarker. It is also non-specific because proteinuria occurs in most kidney injury, acute or chronic.

We have shown that n-acetyl-glucosamine is increased in the urine of rats exposed to renal irradiation; it coincided with proteinuria, and preceded azotemia. In these studies, there were no consistent changes in gamma-glutamyl transpeptidase, an enzyme of the brush border of proximal renal tubular epithelium. More recently, we have shown early glomerular structural and functional changes within hours of 10 Gy single-fraction total body irradiation (TBI). Analysis of the rat urinary proteome at 24 hours after 10 Gy TBI shows over 700 proteins of interest, compared to unirradiated controls. Notable proteins found after TBI include kallikrein-like peptides and cystatin-C. Gene ontology analysis suggests the presence of proteins from all cellular compartments, nucleus to membrane, and of diverse function, e.g. anti-apoptotic, proteolytic, inflammatory, and others. KIM-1 and IL-18 were unchanged, which is different than is the case of AKI.

It is possible, but unlikely, that a single protein or other chemical biomarker will be found for renal radiation injury. Non-renal effects of TBI, with urinary excretion of resulting substances, require consideration; and studies urine of proteome changes after local renal irradiation are in progress. In a combined injury, such as body fluid depletion and hypotension plus irradiation, interpretation of urinary biomarkers would be very difficult.

Biodosimetry in Skin and Mitigation in Lung following Radiation Exposure

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Following accidental exposure of humans to irradiation, the dose to different parts of the body is likely to be heterogeneous. We have been developing a biological approach that can be used to assess dose to the skin with a particular emphasis on DNA damage in cells of the dermis and epidermis. Our work has demonstrated that it is possible to detect micronuclei (MN) in skin fibroblasts from both mice, rats and humans and that it should be possible to estimate skin dose (in the range 0–10 Gy) using a small punch (3 mm) biopsy that could be taken within a week after radiation exposure. A potential practical problem with this assay is that it requires the early processing of the fresh tissue sample. Consequently we are also investigating the potential for predicting the dose received by the skin using sections obtained from fixed skin biopsies by measurements of radiation-induced foci (RIF) of proteins in the cell nuclei. Preliminary data indicate that this may also be possible in the same dose range, using specimens obtained up to 1 week after irradiation. This result indicates the possibility of using DNA damage in skin as a biodosimeter that could potentially be used in many parts of the body.

Analysis of micronuclei is also possible in fibroblasts obtained from the lung following irradiation. However, we have found that such DNA damage can be observed in both irradiated and shielded areas of rat lung, whereas such damage is not observed in shielded regions of rat skin. We have been using this endpoint (among others) to examine approaches to mitigating lung damage following irradiation. The lung is a relatively radiation sensitive organ with a response to irradiation that is complex, involving killing of lung cells, death of endothelial cells, influx of inflammatory cells and waves of inflammatory cytokines and ROS production. These latter two processes are believed to be major factors driving the development of the two major functional outcomes that are observed, radiation pneumonitis (at 2–3 months) and radiation fibrosis (at 4+ months). Protection against functional and histopathological damage has been demonstrated for a number of different agents when given before irradiation but the extent to which radiation-induced lung damage can be mitigated by agents given only after irradiation is uncertain. Our studies have demonstrated that treating Sprague-Dawley rats with a genistein diet (0.75g genistein per Kg of food) post irradiation can mitigate the formation of micronuclei completely and can partially prevent an increased breathing rate at 2–3 months after the irradiation suggesting a reduced level of pneumonitis. However, the genistein diet was unable to prevent an increase in breathing rate and death of the animals at later times when fibrosis had developed. This was despite the fact that the genistein treatment reduced the number of activated macrophages and the amount of collagen (as assessed by Masson Trichrome staining) in the lung at the 28 week endpoint of the study. Radiation caused increased levels of inflammatory cytokines (TNF-α, IL-1α, IL-1β, IL-6, TGF-β) in the tissue at this late time but genistein prevented most of this increase only for three of the cytokines (TNF-α, IL-1β, TGF-β). Further studies demonstrated that fluctuating levels of cytokines occurred at earlier times in the lung tissue despite the genistein diet. These studies indicate that complete mitigation of micronucleus formation (thought to be due to ROS production in the lung post irradiation) by genistein does not translate into complete mitigation of radiation-induced functional damage in the lung. This may suggest that there are different sources of ROS within the irradiated lung and some may be more effective in causing DNA damage and others more effective in inducing changes which lead to functional deficits. Since genistein is well tolerated and has low toxicity, it may be appropriate to examine the efficacy of larger doses of this agent in mitigating radiation-induced functional damage in the lung.

Genetic Molecular Markers for Radiation Exposure: Applications of the Gene Expression Bioassay

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Gene expression changes represent an early bioindicator of radiation exposure. Our work aims to decipher mechanisms by which cells detect damage to DNA from ionizing radiation (IR) and oxidative stress and then signal DNA repair and cell-cycle delay. These studies permit us to identify sentinel radiation-responsive gene targets that we then validate for biodosimetry applications. It is clear from work in many laboratories that genes involved in cell-cycle checkpoints, together with DNA repair and apoptosis, are integrated into a circuitry that determines the ultimate cellular response to oxidative damage caused by IR. Changes in radiation-responsive gene expression reflect the overall health status of the organism, comprising differences in genetic determinants, prior exposures to genotoxic agents, therapeutic treatments (pharmaceuticals, nutraceuticals, anti-oxidants, immune modulators, etc.), and other epigenetic determinants (i.e., imprinting, gene-silencing, X-chromosome inactivation, maternal effects, and the progress of carcinogenesis). MicroRNAs (miRNAs) are a recently discovered family of highly conserved, small non-protein-coding RNAs known to negatively regulate expression of protein-coding genes. At least one third of the human genes may be regulated by miRNAs (Lewis *et al.*, 2004, Lim et al., Lewis *et al.,* 2005). MiRNAs appear to show tissue and organ specificity and represent another mechanism of epigenetic control. Radiation was reported to cause no change in expression of miRNAs in human lymphoblastoid cells (Marsit *et al.* 2006), but an increase in expression of miRNAs using murine embryonic stem cells (Ishii and Saito, 2006) and lung cancer cells (Weidhass *et al.* 2007).

In order to elucidate the quantitative and qualitative changes in a gene expression-DNA damage response to IR, we developed a four-prong approach to (1) identify radiation-responsive miRNAs by microarray (Amundson *et al.* 2004), (2) measure sentinel gene expression biomarkers by QRT-PCR assay (Grace *et al.* 2002, 2003), (3) screen DNA damage/repair capacity of individual cells by immuno-fluorescent detection of damage-induced foci of phosphorylated histone H2AX (γH2AX) at specific sites of DNA double-strand breaks (DSBs) and eroded telomeres using fluorescence-activated cell sorting (FACS), and (4) assess an individual's inherent radiation sensitivity with a genotoxic challenge assay where whole blood samples are irradiated *ex vivo* or treated with a radiomimetic agent followed by QRT-PCR and γH2AX assays.

Our findings using the human *ex vivo* blood radiation model demonstrate meaningful IR dose responses for multiple targets (i.e., *gadd45a*, *cdkn1a*, *ddb-2*, *bax*, *bcl-2*, and the ratio of *bax*:*bcl2*) and are derived from i) three healthy donors over a broad dose range (0-3 Gy) and ii) 20 healthy donors at two doses (0.25 and 2.5 Gy) (Grace and Blakely, 2007). Results using nonhuman primate and mouse *in vivo* radiation models show that several gene expression changes in apoptotic, DNA repair, and cell-cycle pathways are dose-dependent, although these are limited data sets that vary in the shapes of dose– and time–response curves. Countermeasure agents such as genistein (Grace *et al.* 2007) and 5-AED (submitted for publication) can influence the levels of selected radiation responsive gene targets. Expression profiles of 1500 miRNA molecules were screened by quadruplicate microarray hybridizations using pooled blood samples from BALB/c mice at 0, 3.5, and 6 Gy. Differential miRNA expression patterns were observed temporally, coinciding with our radiation-responsive gene targets. The type of information generated by these studies provides a promising foundation for developing mechanism-based gene expression signatures of radiation dose and injury that correlate with

the time of absorbed dose and level of radiation injury.

While our multiparametric approach is currently at an early stage of development for applications to triage for mass casualties, it is a potentially useful approach that might include bioassays applicable to partial-body exposure scenarios. Gene expression signatures in isolated cells or tissues can provide organ specific diagnostic information. Further studies are clearly needed to achieve the in-depth understanding of the complete dynamics of miRNAome patterns in regulating cellular differentiation, proliferation, and apoptosis, as well as bystander effects and tissue specificities, particularly useful for partial-body exposure scenarios. The genotoxic challenge assay appears potentially very informative for monitoring populations for previous or chronic genotoxic exposures. Concomitant analysis of gene expression changes in DNA damage effector target pathways (i.e., cell-cycle, DNA repair, apoptosis, etc.) by QRT-PCR assays, with rapid screening of DNA damage/repair capacity by γH2AX foci and/or micronuclei as reference targets, can be used as complementary methods to gauge potential exposure levels to IR.

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Improvement of Radiation Dose Assessment Using Multiple-Protein Expression and Hematological Profiles

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There is a present need to rapidly identify severely irradiated individuals that require prompt medical treatment in mass-casualty incidents, as well as to distinguish exposed vs. non-exposed individuals (Blakely et al. 2005). Early treatment of populations exposed to ionizing radiation (MacVittie et al. 2005; Waselenko et al. 2004) requires accurate and rapid biodosimetry with a precision as high as possible to determine an individual's exposure level and risk for morbidity and mortality. The early medical-management situation in the Chernobyl nuclear power accident was made difficult because for several days after the incident the doses to individuals were not known with precision (Guskova et al. 2001). The development of accurate methods for rapid individual dose assessment possesses some challenges. A major source of uncertainty is individual variability in radiation response.

Hematological biomarkers of exposure to ionizing radiation are well characterized and used in medical management of radiological casualties (Dainiak et al. 2003). Measurements of lymphocyte depletion kinetics (Baranov et al. 1995; Goanz et al. 1997) and time- and dose-dependent changes in neutrophil cell numbers observed after irradiation (Fliedner et al. 2001) provide clinical information soon after exposure. However, because of large variation in lymphocyte and neutrophil counts among normal individuals, it necessitates repeated measurements over a prolonged period. Normalization of the inter-individual variations in the ratio of neutrophils to lymphocytes has been evaluated and used along with lymphocyte depletion kinetics to get an enhanced discrimination index of radiation exposure (Zlang et al. 2004; Blakely et al. 2007).

Proteomics is an area offering hope for potential new biological indicators of radiation exposure. Radiation responsive proteins have considerable potential as biodosimeters. Evaluation of specific changes in radiation-induced protein profiles will likely identify sentinel responsive targets and hence provide a practical means to measure tissue- and organ-systems radiation injury. A proteomic approach may evaluate an individual's responses to radiation exposure, since the individual's characteristic and dynamic protein expression profile will reflect their unique biological system. Tissue specific protein biomarkers detected in peripheral blood can provide diagnostic information of organ specific radiation injury. Proteomic analyses may also be applicable for triage purposes, providing rapid estimation of individual exposure doses (Marchetti et al. 2006). The advancement in this type of research might also provide a powerful tool for the accurate assessment of an individual's radiation risk response, hence, determine appropriate pre- as well as post-exposure interventions.

We recently reported results from a study in a nonhuman primate (*Macaca mulatta*) total-body irradiation model and showed that a multiple protein expression profile (i.e., p53, p21 WAF1, IL-6, salivary α-amylase, and CRP) measured in blood of 10 animals irradiated to 6 Gy 250-kVp x-rays (0.13 Gy/min) and 8 animals to 6.5 Gy ^{60}Co γ-rays (0.4 Gy/min) analyzed with use of multivariate discriminant analysis established very successful separation of samples from exposed animals vs samples from the same animals before irradiation. An enhanced separation

was observed as the number of biomarkers increased (Ossetrova et al. 2007a). We also recently presented results from on-going murine (Balb/c) *in vivo* irradiation studies and demonstrated for the first time that a protein expression profile can be developed not only to predict radiation exposure in mice but also to distinguish the level of radiation exposure, ranging from 1 to 7 Gy (0.1 Gy/min). The SAS-based multivariate statistical procedures algorithm was established for dose assessment and dose-dependent discrimination of study animal groups. We showed that for individual biomarkers there is considerable individual variability in response to radiation which makes their diagnostic utility limited, but still feasible when analyzed according to a multiple biomarkers pathway (Ossetrova et al. 2007a, 2007b).

Here we present results from on-going murine (Balb/c) and nonhuman primate (*Macaca mulatta*) *in vivo* studies demonstrating that a panel of protein biomarkers, selected from distinctly different pathways, each with different radiation responses, coupled with peripheral blood cell counts, may provide more accurate radiation dose assessment as well as an enhanced discrimination index of radiation exposure. These results also demonstrate proof-in-concept that proteomics shows promise as a complimentary approach to conventional biodosimetry for early assessment of radiation exposures and coupled with peripheral blood cell counts provides early diagnostic information to effectively manage radiation casualty incidents. This approach, with additional refinement, could provide a method for practical application of a rapid screening test for the diagnosis of radiation exposure. [AFRRI supported this research under projects BD-10, GIB250-01, and RAB3AG.]

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Use of Optically Stimulated Luminescence (OSL) in Radiation Dosimetry

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The Optically Stimulated Luminescence (OSL) technique has now been used for almost a decade in personal dosimetry, but its utility covers many other applications, such as quality assurance and dose verification in radiotherapy and radiology, dosimetry of heavy charged particles in radiotherapy and space, neutron dosimetry and imaging, retrospective dosimetry, and dating of sediments. In OSL dosimetry, light is used to stimulate the radiation-induced luminescence in natural and artificial dosimetric materials, which can then be detected using an appropriate apparatus and related to a calibration dose to determine the relevant dosimetric quantities. Some of the advantages of the technique are the precision, degree of control, and flexibility provided by the all-optical nature of the process, which even allows the use of optical fibers for remote readout of the dosimeter. These advantages, combined with the fact that OSL phenomenon is frequently observed in many natural materials, makes OSL a promising emerging technique for applications in accident dosimetry and triage. OSL has now been observed in electronic components from telephone and ID cards with a linear dose response and sensitivity suitable for triage. OSL has also been observed in dental enamel, although in this case the low sensitivity remains one of challenges that need to be addressed. This presentation reviews the fundamentals of the OSL technique, from the basic factors influencing the OSL signal to the advanced OSL systems available today, and presents the state-of-the-art in accident dosimetry using both fortuitous dosimeters and dental enamel. Results from the literature and from the collaboration between Oklahoma State University, Oak Ridge National Laboratory, and the National Cancer Institute to characterize dental enamel and further develop the OSL technique for accident and retrospective dosimetry are presented. Finally, current issues and future research are discussed.

Electron Paramagnetic Resonance Biodosimetry in Teeth and Fingernails

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Electron paramagnetic resonance (EPR) biodosimetry is based on the measurement of radiation-induced radicals in human tissues. This physically based type of measurements in tissues provides some useful features, especially for estimating dose with asymmetric exposures. The intensity of the radiation-induced signal is unaffected by biological processes such as stress and reflects only the radiation that impinges directly at the site of measurement (i.e. hands, feet or teeth). Such data can serve as complementary to the dose assessments made based on biological changes that are likely to be affected by physiological stress, injuries and whole body exposure. Tooth enamel has the best EPR dosimetric properties because of the high stability of the radiation induced radicals, which in teeth reside in the hydroxyapatite matrix. During the last decade EPR dosimetry in teeth has made considerable progress towards becoming a routine dosimetric method. It has been applied for dose reconstruction for epidemiological studies of different cohorts, including Hiroshima atomic bomb survivors, Chernobyl clean-up workers and others. In 2002 the International Atomic Energy Agency issued IAEA-TECDOC-1331 that contains a detailed description of the EPR dose reconstruction procedure. International Commission on Radiation Units and Measurements recognized in its Report 68 that EPR dosimetry is the most accurate method of retrospective dosimetry for external gamma exposure. Another important EPR biodosimetry milestone was the completion of four international dose intercomparisions. More than 20 research groups from 14 countries have participated. Most EPR biodosimetry is performed in X-band, which is from 9 to 10 GHz. This band provides a good compromise between sensitivity, sample size, and water content in tooth enamel but requires to have extracted teeth for the dose measurements, making its application for immediate, after-the-fact dosimetry problematic. EPR spectroscopy in other mw bands (both lower and higher than X-band) offers two significant opportunities to overcome this obstacle. The lower frequency of L-band EPR systems (1.2 GHz) makes EPR measurements less perturbed by high water content in a sample and allows in vivo measurements of whole teeth. The higher frequency Q-band (34 GHz) spectrometers require much smaller samples (~2 mg) for the dose measurements which can be obtained by biopsy techniques. Practical utilization of these two opportunities has received a significant development in last years and will be discussed in detail.

Recent studies have also indicated that EPR-based dosimetry in fingernails or toenails can be an effective method for estimating acute exposures in a large number of subjects. Fingernails and toenails contain large amounts of α-keratin and the observed EPR signals appear to be from radiation-induced radicals formed in this component. The use of fingernails and toenails provides an opportunity to measure radiation exposure at four different anatomical sites, complementing the measurements made in teeth.

Medical Treatment of Radiological Casualties

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The US experience in partial-body exposure has usually been high-level low LET exposure to relatively small areas of skin, either from sealed sources or X-ray or accelerator accidents. For a clinically significant lesion to occur, generally greater than 10 cm^2 of the basal layer of the skin must be irradiated. In order to devise a care plan for patients with partial-body irradiation, the treating physician and nursing staff need to ascertain the relative magnitude of the event. The medical history is particularly helpful for partial-body injury since signs and symptoms generally take days to weeks to manifest. In addition, serial color photographs are crucial, possibly along with drawings of the lesion, to document its evolution. In the US, diagnosis of high-level skin dose has generally been estimated by physicians in radiation medicine observing the serial evolution of symptoms and often augmented by cytogenetic dosimetry and diagnostic tools such as PET scans, MRI, ultrasound, and Doppler or laser flow profiles in the affected area. Most physicians in the US have also found it important to engage the consultative services of a plastic and reconstructive surgeon early in the process should skin grafting and/or amputations need to be performed. The key clinical management issues with cutaneous radiation injury are infection control, state-of-the art wound care, and pain management. The US Centers for Disease Control and Prevention have published physician guidelines for grading cutaneous radiation injury: Grade I: >2 Gy; Grade II > 15 Gy; Grade III > 40 Gy; Grade IV: >55 Gy. Generally Grade I lesions will recover early, possibly with minimal erythema or after dry desquamation has healed. Delayed effects may include minimal skin atrophy. Grade II lesions include edema, moist desquamation, late epithelization, but with possible tissue necrosis and blood vessel compromise in the higher dose ranges. The pathophysiology for erythema includes arteriolar constriction with capillary dilation and local edema. There is also generally diminished mitotic activity in cells of the basal and parabasal layers with thinning of the epidermis and desquamation of large macroscopic flakes of skin. In cases of moist desquamation, microscopically, there is intracellular edema, coalescence of vesicles to form macroscopic bullae, and a wet dermal surface, coated by fibrin. Late effects of Grade II lesions will include skin atrophy or recurring ulcer formation and possible telangiectasis many years after the event. In the upper range of Grade II lesions (30-40 Gy), plastic reconstruction with grafting may also be necessary. Grade III lesions (>40 Gy) pose significant clinical challenges with small vessel damage and occlusion, large areas of tissue necrosis and, historically, have involved serial amputation, usually of extremities. Late effects are multiple, including skin atrophy, depigmentation, small vessel occlusion, tissue fibrosis and sclerosis of connective tissue. Grade IV lesions are extremely severe, with rapid tissue ischemia, and these patients generally require multiple amputations and continued reconstructive surgery over several years if they survive the cutaneous syndrome at all. The cutaneous syndrome has been defined and expanded by Peter and colleagues over the past 15 years and this syndrome poses many significant medical challenges in patient management. As with the acute radiation syndrome (ARS), local injuries should be treated symptomatically and any surgical trauma should be dealt with in the first 48 hours. Additionally a baseline CBC with differential should be taken since the ARS can be an additional complication to be dealt with in high-level, partial-body dose. Speaking directly to the management of local partial-body injury, the patient should be treated in a burn unit with reverse isolation if available to prevent infections, use of medications to reduce inflammation, inhibit proteolysis, relieve pain, and stimulate regeneration of the skin and improve circulation. In the early phase of the radiation injury, use of corticosteroids and sedatives should be considered. Later, proteinase inhibitors (such as Gordox®) and antibiotics will be necessary.

Lioxasol® has also been suggested as a possibility to regenerate DNA in the wound and when skin regeneration has started, biogenic drugs such as Actovegin® and Solcoseril® have been recommended by our Russian colleagues. During the third and fourth week when small vessel occlusion and fibrosis will be occurring, Pentoxifylline® has been used successfully in the US to increase blood flow. After immediate stabilization of the clinical phase, there is generally a long and painful healing process for the patient. Here, pain management will be the most crucial issue and physicians and nurses trained in the various aspects of pain management should be consulted. In the later phase of healing, additional medical products could be used to stimulate vascularization of the wound area as well as inhibit fibrosis and infection. These medications would include Pentoxifylline®, vitamin E and interferon gamma. In spite of these medications, proper care of the wound may still require reconstructive surgery. Full thickness grafts and microsurgical techniques often provide the best results. There appears to be no general consensus in the literature in the use of what medical agent for proper treatment of partial-body injury and in what order of application. In addition, hyperbaric oxygen therapy has proven useful in certain specific radio-induced lesions of bone, particularly involving the mandible and other bones. The experiences of the US, Canada, Japan, and Europe, particularly including Russia, will be compared in the use of drug formulations for acute partial-body injury. In conclusion, it is important to remember that psychological support for the patient and his/her family is also crucial. Patients with profound partial-body radiation exposure may express feelings of anger, disbelief, sadness, irritability, arousal, sleep disturbance, dissociation, or increased use of alcohol, or stimulants such as caffeine and tobacco, or drugs. Patients exposed to high-level radiation events that actually threaten their lives are at the highest risk of psychiatric morbidity, which may meet the criteria for psychiatric diagnoses such as Acute Stress Disorder or Post Traumatic Stress Disorder (PTS).

Contingency Planning for Triage, Supportive Care and Transplantation

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The multiorgan injury and myelosuppression accompanying partial body irradiation or marrow toxic exposure can lead to rapid or more slowly evolving life-threatening manifestations of the acute radiation syndrome (ARS). Experience in assessing and managing patients with severe myelosuppression as well as those suffering the cutaneous, mucosal and epithelial injury from chemotherapy and radiation is reminiscent of the care required for patients with acute leukemia or other malignancies undergoing hematopoietic cell transplantation (HCT). Comprehension of the biology of radiation injury, application of estimates of clinical dosimetry and rapid and reproducible determination of the multiorgan consequences of radiation exposure require preplanned education, assessment tools and a therapeutic algorithm. Patients with estimated partial body radiation exposure > 4 Gy may have profound myelosuppression and might benefit from consideration of allogeneic HCT. Many centers on multiple continents have contemplated these concerns and in the U.S., the Radiation Injury Treatment Network (RITN) sponsored by the National Marrow Donor Program (NMDP) and American Society for Blood and Marrow Transplantation (ASBMT) has established coordinated multicenter protocols for evaluation, triage, treatment and as needed, transplantation for marrow injured radiation exposed victims. Victims of total or partial body radiation may have intense myelosuppression but variable and unpredictable immunocompetence. Plans for their support with allogeneic HCT must include additional immunosuppressive therapy to ensure satisfactory engraftment as well as prevent the consequences of graft vs. host disease. This scenario resembles transplant approaches using reduced intensity or nonmyeloablative conditioning, now widely used for older or otherwise compromised cancer patients. Insights gained from this experience can directly inform plans for developing and implementing allogeneic transplantation for radiation victims. In conjunction with detailed protocol outlines the Network has conducted communication drills to assess transplant center capacity, educational seminars to enhance knowledge of these contingency plans in both transplant and evaluation centers and has worked to ensure preparedness amongst those best suited to manage neutropenic myelosuppressed irradiated patients. In addition, coordinated prospective data collection mechanisms have been established to learn from each experience, to formally evaluate Network and individual center performance and to revise protocols as contingencies arise. Radiation and marrow toxic emergencies may seem unlikely but the acknowledged best approach is preparedness, education and contingency planning to improve the evaluation and care of patients experiencing marrow toxic radiation. Education and preparedness are our best defense.

The Biomedical Advanced Research and Development Authority's Biodosimetry Program

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The Biomedical Advanced Research and Development Authority (BARDA) is the focal point within the Department of Health and Human Services (HHS) for the advanced development and acquisition of medical countermeasures to protect the American civilian population against chemical, biological, radiological, and nuclear (CBRN) and naturally occurring threats to public health. The BARDA office manages the advanced development of medical countermeasures for CBRN agents and Project BioShield for their acquisition. Diagnostic and dosimetric products and capabilities are important among medical countermeasures, given their pivotal role in determining appropriate prophylactic, mitigating, or therapeutic treatments.

The HHS Public Health Emergency Medical Countermeasures Enterprise (PHEMCE) is an interagency organization responsible for research, development, acquisition, storage, maintenance, deployment, and provision and guidance for use of emergency medical countermeasures. The PHEMCE Governance Board includes the leadership of the HHS offices of the Assistant Secretary for Preparedness and Response (ASPR), National Institutes of Health (NIH), Food and Drug Administration (FDA), and the Centers for Disease Control and Prevention (CDC), as well as ex officio non-voting participation of (a) other agencies within HHS; (b) the Departments of Homeland Security, of Defense, and of Veterans' Affairs; and (c) the Executive Office of the President. BARDA coordinates the PHEMCE operations as part of its mission to facilitate the research, development, and acquisition of medical countermeasures for CBRN agents and emerging infectious diseases, including pandemic influenza, that threaten the U.S. population.

HHS employs a diverse, balanced portfolio of medical countermeasures to prepare for the threat of radiation exposure. Using a combination of funding mechanisms, including Project BioShield funds, HHS is enhancing the nation's preparedness to respond to the public health threats caused by a radiological or nuclear event. In association with this part of its mission, HHS has recently issued a Request for Proposals (RFP) for treatment of neutropenia associated with acute radiation syndrome (ARS) and a Broad Agency Announcement (BAA) for advanced development leading to therapies for other ARS conditions. HHS anticipates additional solicitations (RFPs and/or BAAs) for various aspects of ARS. Within HHS BARDA has partnered with the National Institute of Allergy and Infectious Diseases (NIAID), an institute of NIH, in Requests for Applications (RFAs) for cutaneous and lung injury associated with ARS. In addition, HHS has a requirement for biodosimetric capabilities, and anticipates releasing a Request for Information (RFI) by mid-2008 to gather information on technologies with potential for meeting this requirement. Subsequently, based on information it receives in response to this RFI, HHS anticipates releasing one or more solicitations (RFP and/or BAA) during fiscal year 2009 for development of biodosimetric capabilities.

Mass Spectrometry of Buccal Mucosa—Biomarkers for Biodosimetry in Radiation Incidents

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The NYU Department of Pharmacology Mass Spectrometry Laboratory is a structural biology/nanochemistry laboratory devoted to the identification and study of tissue and body fluid biomarkers of vascular disease, tumors and putative metabolic pathways of apoptosis triggered by ionizing radiation, reactive oxygen species, ischemia and other insults. The laboratory instrumentation includes, MALDI TOF TOF, LCMS, AFM, and access to 12T FTMS, 900 MHz NMR, and 300 KEV TEM.

In the past year direct mass spectrometry identification of proteins and biomarkers of colorectal carcinoma, ischemic/stroke brain, environmental toxins, and competent *in-vitro* human embryos were reported by the laboratory.

Congruent with the AFFRI research and development goal of "developing methods of rapidly assessing radiation exposure to assure appropriate medical treatment," the laboratory has begun a study of the pre- and post-radiation exposure proteome of murine buccal mucosa.

Recent work has identified a transcription factor, nuclear factor KAPPA B (NF-KB), which induces the TNF-α encoding gene and activates the cyclooxygenase-2 (COX-2) pathway. At 24 hours post irradiation, HIF-1a and COX-2 protein levels were increased. In addition to its well established DNA-damage effects, ionizing radiation induces cell death, and radiation-induced activation of acid sphingomyelinases (ASMases) and the generation of ceramide. Ceramide is generated from sphingomyeline by the action of a neutral or ASMase or by de novo synthesis coordinated through the enzyme ceramidesynthase. Once generated, ceramide may serve as a second messenger molecule in signaling responses to physiologic or environmental stimuli, or it may be converted to a variety of structural or effector molecules. With a single dose of 3 Gy, there is activation of protein kinase B/AKT (PKB/AKT) signaling. Within minutes of irradiation, phosphorylation of the serine/threonin protein kinase PKB/AKT at serine-residue 473 appears. This activation of PKB/AKT contributes to inhibit glycogen synthase kinase-3beta (GSK3beta), which has a clear inhibitory role in endothelial cell survival.

This preliminary study describes the changes in murine buccal mucosa protein profiles when subjected to ionizing radiation in addition to those described above. Tissue sampling is obtained 15 and 30 minutes post exposure. Proteins are extracted from the buccal mucosa with high pressure (Barocycler, Pressure BioSciences, South Easton, MA), the sample divided into two components. One is assigned for trypsin digestion and LCMS analysis (bottoms-up proteomics), and the second one for HPLC protein separation and FTMS analysis (top-down proteomics) to identify post-translational modifications. This preclinical work heralds a clinical translation in head and neck cancer patients, for validation purposes. The long- term aim is the identification of specific profiles that enable reliable associations with dose-exposure, for biodosimetry purposes. If successful, this strategy could result in the development of a self-administered diagnostic test using a buccal mucosa swab.

Abscopal Bone Marrow Stroma Suppression and Acute Death in Gut-Irradiated Mice

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Gastrointestinal (GI) button is often attributed to whole body ionizing irradiation-induced acute death (i.e. within 10 days of irradiation). The mechanisms of GI death, however, are not clear, as the two elements that seem to mediate this acute death, crypt cell damage and GI-derived bacteraemia, have been shown in some reports to be minor players. Here we present evidence that acute GI death is correlated with suppression of un-irradiated bone marrow stroma.

Methods: Ten-week-old male C57BL/6 mice were randomized into three groups, 8–12 mice per group. Mice were positioned on their left side and x-ray irradiation at 0, 15 and 20 Gy was given to the abdominal area only at dose rate of 50 cGy/min. Body weight of the mice was monitored daily and survival rate determined for up to 42 days. Blood and bone marrow were collected from surviving mice at day 8 for bacterial growth and *ex vivo* stromal cell colony formation, respectively. Gut, liver, kidney and the lung were examined for histological abnormality.

Results: Weight loss in both irradiated groups started one day after gut irradiation. The extent of weight loss in the 20-Gy group increased with time throughout the 8-day period. The first death in this group occurred at day 6, and survival rate dropped to 16% by day 8. By contrast, weight loss in the 15-Gy group peaked at day 5, followed by a recovery phase lasting into day 8. All mice in this group survived the 8-day period and the subsequent 6-week follow-up until the experiment was terminated. Gut irradiation resulted in changes in blood bacterial profile, crypt cell death and organ damage in both irradiated groups to a similar extent. *Ex vivo* proliferation capacity of stromal cells in bone marrow from the 20-Gy group was suppressed to less than 10% of the control level. By striking contrast, viability of stromal cells from the 15-Gy group was largely intact.

Conclusions: Our data demonstrate clearly that gut irradiation induces cellular responses in distant organs. These results are consistent with the concept of “abscopal” effects of radiation. Since bone marrow stroma serves both as the source of stem cells as well as the support for these cells to replenish the radiation-damaged cells in the body, we hypothesize that acute GI death following irradiation is mediated by marked suppression of bone marrow stroma.

Optically Stimulated Luminescence (OSL) of Tooth Enamel for Potential Use in Post-Exposure Triage

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An assessment by the Joint Interagency Working Group (JIWG) of the current status of retrospective evaluation of radiation exposure to populations following a radiological or nuclear event highlights the need for new technologies to rapidly triage potential radiation casualties (JIWG, 2005). Notably, the need for biodosimetric methods for estimating radiation exposure to individuals is highlighted. One such potential method is to use optically stimulated luminescence (OSL) from teeth for rapid *in-vivo* dose assessments. We describe in this report progress at Oklahoma State University on the development of methods and instrumentation for *in-vivo* OSL dosimetry of irradiated teeth. The two main areas investigated were: (a) basic OSL properties of human teeth, including stimulation, minimum measurable doses, reproducibility and OSL stability; and (b) development of an instrument for potential *in-vivo* analysis of OSL from human subjects. Following the conclusions we discuss the potential for future research and development. Several OSL measurement modes were investigated and the experiments show that, under optimized conditions, human tooth enamel does emit a measureable optically stimulated luminescence signal after irradiation with doses as small as 2–5 Gy. Although, with the methods used so far, the minimum detectable doses do not yet satisfy the dose requirements for retrospective biophysical radiation dosimetry, the results hold significant promise. In parallel with method development we have also designed and built a prototype portable OSL reader for potential use *in vivo* with human teeth. The OSL instrument will be described and its performance discussed.

Spatially Resolved Biodosimetry Based on Electron Paramagnetic Resonance of Teeth and Fingernails

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There is growing awareness of the need for methodology for retrospective or "after-the-fact" dosimetry to carry out triage after an event in which large numbers of people have potentially received clinically significant doses of ionizing radiation. Although some very promising approaches are being developed using biologically based parameters such as changes in DNA, gene activation, etc., there also is recognition that such measurements have the potential to be confounded by other factors that also can affect these parameters. These approaches are especially potentially problematical under circumstances where there may be high levels of biological perturbations and reactions due to wounds, burns, and extreme psychological stress. These are factors that are likely to be quite prominent in the same scenarios that are likely to lead to potential widespread exposures to ionizing radiation. Therefore there is a high need for other complementary dosimetric methods that will not be affected by these potential confounders, which would enable the biologically based measurements to be used more effectively. It is increasingly recognized that EPR dosimetry has the potential for providing the needed complementary information. The EPR measurements are based on physical changes in tissues whose magnitudes are not affected by the factors that can confound biologically based assessments. The EPR methods are based on the generation of stable free radicals, whose magnitude is proportional to the total dose of radiation received by the tissue, thereby using these tissues as endogenous physical dosimeters. Both *in vivo* tooth measurements using L-band (1.2 GHz) spectroscopy and measurements of fingernail clippings at X-Band (9.5 GHz) appear to be suitable for EPR-based dosimetry. Both types of EPR dosimetry share several very desirable characteristics that make them especially well suited to be part of the general methodology to be used for estimating radiation dose for triage, including independence from confounding biologic factors, non-invasive measurement procedure (excluding fingernail clipping), capability to make measurements at any time after the event (immediately after the exposure and indefinitely afterward for the methods based on teeth and likely for up to several weeks using fingernails), and the developing ability to perform measurements with non-expert users in the field at the site of an event. EPR dosimetry can be used to provide quantitative estimates of heterogeneous absorbed dose distributions in cases of partial-body irradiation through the combination of measurements of several teeth and/or fingernail clippings from both hands and feet. The ability of *in vivo* tooth dosimetry to provide estimates of absorbed dose has been established through a series of experiments using unirradiated volunteers with specifically irradiated teeth placed *in situ* within gaps in their dentition [1–4] and in patients who have completed courses of radiation therapy for head and neck cancers. *In vivo* measurements have been performed using molar, premolar, and canine teeth and multiple measurements in individual patients demonstrate the expected heterogeneous dose distributions. Dose response curves have been generated using both populations and, using the current methodology and instrument, the standard error of prediction is approximately 150 cGy based on 4.5-min measurements. Averaging of independent measurements can reduce this error significantly. While such averaging may not be practical when deployed in the field, this result provides us with important insights as to the factors that need to be improved and that, with these improvements, the technique would be appropriate for effective triage. The development of the methodology for reliable fingernail-based EPR dosimetry is underway and results indicate that there are quantifiable radiation induced

signals in fingernails that can be detected at doses of 100 cGy or less [5, 6]. These results also show that the potentially interfering signals induced by clipping of the fingernails can be reduced by a factor of ten by the simple treatment of soaking the clippings in water prior to the measurements [7, 8]. Variation in intensity with time after irradiation can be minimized by keeping the fingernails in a humidified atmosphere. Clippings that have been treated with water (to restore them to that state prior to clipping), irradiated, and then treated again with water (to simulate the situation for real use, in which clippings will be taken after irradiation) retain a radiation-induced signal in a dose-dependent manner. In summary, it seems plausible that the EPR dosimetry techniques will have an important role in after-the-fact dosimetry for both homogeneous and inhomogeneous exposures involving large numbers of individuals.

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Broncho-Alveolar Lavage Analysis for Studying Early Inflammatory Responses following Plutonium Pulmonary Contamination

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Pulmonary pathologies, mainly tumors of epithelial origin, represent the major risk following exposure to actinide particles. Alveolar macrophages are key elements in the clearance of particles after phagocytosis and, in addition, represent one of the main actors in inflammatory reactions. Moderately soluble Pu compounds, such as nitrate forms are also stored in macrophages. Broncho alveolar lavages (BAL) represent a commonly used source of diagnosis biomarkers for lung pathologies in man and thus BAL analysis could be an interesting approach to evaluate lung damage following actinide contamination.

The goal of this study is to evaluate the early consequences of plutonium oxide (PuO_2) inhalation or Pu nitrate intratracheal administration in rats, by the analysis of BAL content. Sprague-Dawley rats were exposed to either PuO_2 particles or Pu nitrate and were euthanized 3 days to 6 weeks post-contamination, and BAL carried out.

First, the distribution of activity in the different compartments of the lungs was evaluated. Total α activity was assessed in cellular or lipoproteic fraction of BAL by scintillation counting, and the percentage of Pu-associated macrophages was determined by autoradiography studies. These parameters reflected the initial deposit in lungs, as well as the activity within whole lungs, both after contamination with oxide and nitrate forms. However, proportions of activity between cellular and acellular fraction of BAL varied with time and solubility of the Pu compound.

Second, cellular composition and total protein concentration of BAL were evaluated as a marker of lung inflammatory reaction. Percentages of lymphocytes and granulocytes in the BAL increased with time in PuO_2-contaminated rats as compared to the sham animals, although they remained unchanged after Pu nitrate. Morphological alterations were also observed in alveolar macrophages from PuO_2-contaminated rats (increase in cell size, appearance of binucleated cells), which were dependent upon initial deposit. Total protein concentration increased in both groups of Pu-contaminated rats, as compared to sham-contaminated animals.

Third, production of the cytokine TNF-α and chemokines (MCP-1, CINC-1 and MIP-2) was measured 24 h after plating of alveolar macrophages obtained from BAL of contaminated animals, as a measurement of cell activation. Results showed an increase in inflammatory mediator production as early as 3 days post PuO_2 inhalation, as compared to macrophages isolated from sham animals. The production of inflammatory mediators was dependent upon the initial lung deposit. A similar increase was observed following lung contamination with Pu nitrate. Macrophage activation preceded histological evidences of lung damage, observed 6 weeks post-contamination.

These results show that BAL represent a good reflection of lung clearance of activity and early lung damage following Pu contamination. Thus, the dose-dependent functional changes observed in alveolar macrophages after PuO_2 inhalation or Pu nitrate exposure could represent biomarkers for actinide exposure and should be considered for a risk evaluation.

Treatment of PuO_2 Lung Contamination Using a Dry Powder Formulation of DTPA

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Lung contamination can result from accidental release of transuranic actinides such as Pu and Am. The therapeutic approach to reduce the effective radiation dose is to remove the α-emitting radionuclides from the body by promoting their decorporation. Diethylene triamine pentaacetic acid (DTPA) is the most commonly used treatment of internal contamination by plutonium, and represents the only available ligand for *in vivo* chelation of this actinide. The present work investigates the decorporation efficacy of a dry powder formulation of $CaNa_3$-DTPA on a pulmonary contamination with the insoluble physicochemical form of Pu, PuO_2. Adult male Sprague-Dawley rats were exposed to PuO_2 aerosols generated from an industrial powder (47.3% ^{241}Am of α activity equivalent to 3.3% of mass). Two hours after contamination, rats received an intratracheal insufflation of $CaNa_3$-DTPA (18.2 ± 1.4 µmol DTPA/kg) formulated into porous particles of a dry powder. Urines were collected daily for 7 days. Initial lung deposit (ILD) was determined by x-ray spectrometry counting 7 days post-inhalation. Fourteen days post-inhalation, rats were euthanized, liver, femurs and lungs were collected and broncho alveolar lavages (BAL) were carried out. The total α activity of samples was measured by liquid scintillation counting, in BAL, BAL cells and BAL fluids, isolated from alveolar immune cells by centrifugation.

The ILDs of contaminated untreated rats and contaminated DTPA-treated rats were respectively 15.6 ± 2.3 kBq and 13.6 ± 2.3 kBq. The cumulative activity urinary excretion over 7 days was 7-fold higher after DTPA administration as compared to untreated rats, and represented approximately 7% of the ILD for DTPA-treated animals. In the main retention tissues, liver and skeleton, the deposit of activity in DTPA-treated rats was less than 5% of the one of untreated animals (1.13% of ILD in liver of untreated rats vs. 0.05% in DTPA-treated rats; 2.75% in skeleton vs. 0.1%).

Distribution of α activity within lungs of treated or untreated rats was determined. Alpha activity recovered in the BAL fluids from DTPA-treated rats was 7.3-times lower than in BAL fluids from non-treated animals. However, although the activity associated with BAL cells (mainly alveolar macrophages) tended to decrease, the difference between treated and non-treated animals remained non significant, suggesting that pulmonary surfactant and/or serum-derived proteins represented the major accessible lung compartment for DTPA decorporation. However, no significant decrease in whole lung activity was obtained.

Our study shows the efficacy of a dry DTPA powder administered directly to the lungs on Pu decorporation. By inhibiting actinide deposit in skeleton and liver, a limitation of the dose delivered to these tissues is expected, thus limiting the risks for radiation-induced diseases. In addition, DTPA treatment modified distribution of activity within lungs. It is generally admitted that soluble compounds leading to more homogeneous irradiation of the lungs cause higher lung damage than insoluble forms, trapped in macrophages. The decorporation of the most soluble fraction of radionuclide present in the acellular fraction of BAL, could thus also limit lung damage. Finally, direct pulmonary administration of DTPA offers the potential for needle-free treatment, which would be convenient in case of several contaminated people at the same time as a first pass emergency treatment.

Prevention of Irradiation-Induced Salivary Hypofunction by Microvessel Protection in Mouse Salivary Glands

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Treatment of most head and neck cancers includes radiotherapy. Salivary glands (SGs) in the irradiation (IR) field are irreversibly damaged resulting in severe hyposalivation. We evaluated the importance of SG endothelial cells to this IR-induced injury, and whether serotype 5 adenoviral (Ad5) vector mediated transfer of basic fibroblast growth factor (AdbFGF) or vascular endothelial growth factor (AdVEGF) cDNAs would afford radioprotection. Four hours after IR, microvessels density (MVD) in SGs decreased by ~45%. However, if mice were pretreated with either AdVEGF or AdbFGF 48 hours before IR, the loss in MVD was significantly reduced. An irrelevant vector, AdLacZ, encoding E. Coli β-galactosidase, was without effect. After 8 weeks, IR reduced salivary flow ~65% in untreated mice. Mice pretreated (5×10^9 particles/gland 48h prior to IR) with AdLacZ exhibited a reduction in salivary flow similar to untreated mice receiving IR. However, irradiated mice pretreated with AdbFGF or AdVEGF showed a significant improvement in their salivary flow, to ~70% ($p<0.01$) and 80% ($p<0.01$), respectively, of unirradiated control mice. These results are consistent with the notion that injury to the adjacent microvasculature may play an important role in SG radiation damage. Furthermore, our results suggest that a local transient treatment directed at protecting SG endothelial cells may be beneficial for patients undergoing IR for head and neck cancer.

Inhibition of Caspase-Dependent Apoptosis by Inactivating the iNOS Pathway Protects Human T Cells against Gamma Radiation Injury

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Exposure to ionizing radiation results in DNA breaks that activate the ATM and CHK2 pathways. NF-kB/p53/CDC25 are then activated, which arrests the cell cycle (Houtgraaf et al., 2006). This in turn leads to acute radiation syndrome (ARS) followed by multiple organ dysfunction syndrome (MODS) and multiple organ failure (MOF). iNOS expression and NO production increase after radiation exposure (Inano and Onoda, 2005; Zhong et al., 2004; Chang et al., 2003; Chung et al., 2003). We previously showed that inhibiting iNOS expression prevents injury induced by hemorrhage (Kiang et al., 2004, 2006, 2007a, 2007b) and hypoxia (Kiang et al., 2008). We, therefore, investigated whether gamma radiation-induced activation of the iNOS pathway is associated with increased caspase activity and apoptosis. Furthermore, we studied whether agents inactivating the iNOS pathway limited the caspase-dependent apoptosis induced by gamma radiation.

Human Jurkat T cells were exposed to gamma radiation (4 Gy). The irradiated cells were collected at different times after irradiation. In these cells we measured cell viability first. Then, cell lysates were prepared to measure protein levels of KLF6, KLF4, NF-kB, iNOS, p53, Bcl-2, and Bax, NO production, lipid peroxidation, apoptosome formed by cytochrome c, caspase-9, and Apaf-1, and caspase-3 enzymatic activity. To evaluate the relationship between iNOS and the caspases, we inhibited iNOS expression by treating cells with the iNOS inhibitor 17-DMAG or iNOS siRNA.

Gamma radiation exposure increased iNOS expression by increasing levels of its transcription factors, NF-kB-p50 and KLF6 within 4 hr after irradiation. Twenty-four hours after irradiation, cell viability was reduced by 35%. In these cells, nitrate (representing NO production) and MDA (representing lipid peroxidation) increased in a radiation dose-dependent and post-radiation time-dependent manner. Apoptosome formation (complex of caspase-9, cytochrome c, and Apaf-1, Jiang and Wang, 2004, Kiang, 2006) and caspaspe-3 enzymatic activity were significantly elevated, suggesting gamma radiation-induced apoptosis is mediated by the intrinsic caspase-dependent pathway. Treatment with iNOS inhibitor 17-DMAG 24 hr prior to gamma radiation significantly limited these biomolecular changes and increased the cell viability. Treatment with iNOS siRNA to silence the iNOS gene produced similar results, further confirming the correlation between the iNOS pathway and the radiation-induced apoptosis.

These results suggest gamma radiation activates the iNOS pathway, which leads to caspase-3-dependent apoptosis. NO, MDA, and caspase-3 are potential biomolecules responding to irradiation. Agents including 17-DMAG that inhibit the iNOS pathway may prove useful for treating radiation injury. (Supported by AFRRI RAB2CF)

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Partial-Body Cutaneous Radiation Injury: Liposomal Glutathione Treatment and Monitoring by Optical Reflectance Spectroscopy

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Recent events have highlighted that terrorist actions may be intended to set up a nuclear explosion or disseminate radioactive materials using a radiological dispersal device. Under these conditions, significant whole body radiation exposures are likely to be accompanied by local cutaneous radiation injury. The skin response to high-dose ionizing radiation involves multiple inflammatory and necrotic reactions. Recent information on molecular and cellular mechanisms of skin radiation injury suggests that adhesion molecules defining cell surface structure, cellular signalling processes, and alteration of the redox status play an important role in both injury and healing. The cascade of effects is initiated by the formation of free radicals following exposure to ionizing radiation. These include DNA damage, protein oxidation, and lipid peroxidation leading to apoptotic cell death, confusion of the cell signalling pathways, arrest of the cell cycle, and NFkB-related inflammation. Inflammation with the concomitant generation of reactive oxygen and reactive nitrogen species (ROS/RNS), and activation of multiple signalling pathways is associated with a reduction in the antioxidant capacity of the irradiated tissue. It has been suggested that the collapse of skin antioxidant status interferes directly with wound healing in the cutaneous radiation injury.

Tissue levels of glutathione, an antioxidant that is found in almost every cell, depend on the ability of the liver to produce and excrete glutathione into the circulation, as well as the ability of tissues to synthesize the peptide intracellularly. Gamma radiation has been shown to deplete the function of glutathione reductase and decrease glutathione. The depletion of glutathione can occur systemically or locally in affected tissues. Oxidative stress, which accompanies low glutathione can result in peroxidation of red blood cell membranes with increased levels of malondialdehyde, as well as increased formation of 3-nitrotyrosine in tissues modulating ROS/RNS and interfering with the healing process. We hypothesized that combined administration of topical and systemic glutathione would reduce the severity of cutaneous radiation injury and accelerate healing. A stable liposomal encapsulation of glutathione that can be orally and topically administered has recently become available. Liposomal glutathione has been demonstrated to exhibit the antioxidant and antiatherogenic properties relevant to the cutaneous radiation injury. In this report we will describe the effect of topical and oral treatment with liposomal glutathione on skin injury induced by gamma radiation exposure in Fisher F344 rats. As part of this study, we evaluated the potential of using optical spectroscopy for non-invasive evaluation of the severity, progression, and effect of glutathione treatment on cutaneous radiation injury. This approach has the potential for conducting non-invasive *in vivo* biodosimetry in partial body radiation exposures. For this, an ultra-violet/visible (UV-vis) spectrometer coupled fiber-optically with a reflectance/backscattering probe was used to analyze the functional characteristics of radiation-exposed leg tissue from day 1 to day 40 post-exposure. A principal component analysis (PCA) of the data was successful in differentiating between levels of exposure (0, 20, and 40 Gy) as well as between treated and control animals.

Genetic Molecular Markers for Radiation Exposure: Applications of the Gene Expression Bioassay

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Gene expression changes represent an early bioindicator of radiation exposure. Our work aims to decipher mechanisms by which cells detect damage to DNA from ionizing radiation (IR) and oxidative stress and then signal DNA repair and cell-cycle delay. These studies permit us to identify sentinel radiation-responsive gene targets that we then validate for biodosimetry applications. It is clear from work in many laboratories that genes involved in cell-cycle checkpoints, together with DNA repair and apoptosis, are integrated into a circuitry that determines the ultimate cellular response to oxidative damage caused by IR. Changes in radiation-responsive gene expression reflect the overall health status of the organism, comprising differences in genetic determinants, prior exposures to genotoxic agents, therapeutic treatments (pharmaceuticals, nutraceuticals, anti-oxidants, immune modulators, etc.), and other epigenetic determinants (i.e., imprinting, gene-silencing, X-chromosome inactivation, maternal effects, and the progress of carcinogenesis). MicroRNAs (miRNAs) are a recently discovered family of highly conserved, small non-protein-coding RNAs known to negatively regulate expression of protein-coding genes. At least one third of the human genes may be regulated by miRNAs (Lewis et al., 2004, Lim et al., Lewis et al., 2005). MiRNAs appear to show tissue and organ specificity and represent another mechanism of epigenetic control. Radiation was reported to cause no change in expression of miRNAs in human lymphoblastoid cells (Marsit et al. 2006), but an increase in expression of miRNAs using murine embryonic stem cells (Ishii and Saito, 2006) and lung cancer cells (Weidhass et al. 2007).

In order to elucidate the quantitative and qualitative changes in a gene expression-DNA damage response to IR, we developed a four-prong approach to (1) identify radiation-responsive miRNAs by microarray (Amundson *et al.* 2004), (2) measure sentinel gene expression biomarkers by QRT-PCR assay (Grace *et al.* 2002, 2003), (3) screen DNA damage/repair capacity of individual cells by immuno-fluorescent detection of damage-induced foci of phosphorylated histone H2AX (γH2AX) at specific sites of DNA double-strand breaks (DSBs) and eroded telomeres using fluorescence-activated cell sorting (FACS), and (4) assess an individual's inherent radiation sensitivity with a genotoxic challenge assay where whole blood samples are irradiated *ex vivo* or treated with a radiomimetic agent followed by QRT-PCR and γH2AX assays.

Our findings using the human *ex vivo* blood radiation model demonstrate meaningful IR dose responses for multiple targets (i.e., *gadd45a*, *cdkn1a*, *ddb-2*, *bax*, *bcl-2*, and the ratio of *bax*:*bcl2*) and are derived from i) three healthy donors over a broad dose range (0–3 Gy) and ii) 20 healthy donors at two doses (0.25 and 2.5 Gy) (Grace and Blakely, 2007). Results using nonhuman primate and mouse *in vivo* radiation models show that several gene expression changes in apoptotic, DNA repair, and cell-cycle pathways are dose-dependent, although these are limited data sets that vary in the shapes of dose- and time-response curves. Countermeasure agents such as genistein (Grace et al. 2007) and 5-AED (submitted for publication) can influence the levels of selected radiation responsive gene targets. Expression profiles of 1500 miRNA molecules were screened by quadruplicate microarray hybridizations using pooled blood samples from BALB/c mice at 0, 3.5, and 6 Gy. Differential miRNA expression patterns were observed temporally, coinciding with our radiation-responsive gene targets. The type of information generated by these studies provides a promising foundation for developing mechanism-based gene expression signatures of radiation dose and injury that correlate with the time of absorbed dose and level of radiation injury.

While our multiparametric approach is currently at an early stage of development for applications to triage for mass casualties, it is a potentially useful approach that might include bioassays applicable to partial-body exposure scenarios. Gene expression signatures in isolated cells or tissues can provide organ specific diagnostic information. Further studies are clearly needed to achieve the in-depth understanding of the complete dynamics of miRNAome patterns in regulating cellular differentiation, proliferation, and apoptosis, as well as bystander effects and tissue specificities, particularly useful for partial-body exposure scenarios. The genotoxic challenge assay appears potentially very informative for monitoring populations for previous or chronic genotoxic exposures. Concomitant analysis of gene expression changes in DNA damage effector target pathways (i.e., cell-cycle, DNA repair, apoptosis, etc.) by QRT-PCR assays, with rapid screening of DNA damage/repair capacity by γH2AX foci and/or micronuclei as reference targets, can be used as complementary methods to gauge potential exposure levels to IR.

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In Vivo Dose-Response Calibration Curves for Early-Response Exposure Assessment Using Multiple Radiation-Responsive Blood Protein Biomarkers

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The present need to rapidly identify severely irradiated individuals who require prompt medical treatment in mass-casualty incidents, as well as exposed vs. non-exposed individuals in population-monitoring radiation scenarios, prompted a murine *in vivo* dose- and time course-dependent study to evaluate the potential utility to use radiation-responsive blood protein biomarkers for exposure assessment purposes. Protein targets were measured by enzyme linked immunosorbent assay (ELISA) in male BALB/c mice (6–8 weeks old) blood plasma after whole-body ^{60}Co γ-exposure (10 cGy/min) to a broad dose range (0–7 Gy) and time-points (4–96 h).

Our research strategy involves the use of human, non-human primate, and murine models involving *ex vivo* and *in vivo* radiation exposure to identify and validate radiation-responsive protein biomarkers. Using an *ex vivo* model of human peripheral blood lymphocytes as well as an *in vivo* murine model, we earlier reported radiation-responsive changes in the expression of proteins *ras*-p21, *raf*-1, GADD45a, p53, and p21WAF1/CIP1, IL-6, each with a progressive time- and radiation dose-dependent increase. These results also revealed dose-dependent correlations among this subset of protein biomarkers, demonstrating their utility to identify potentially exposed individuals during the early assessment of radiation exposure. In addition, we recently presented similar data from non-human primates exposed to whole-body 6-Gy 250-kVp x-irradiation and 6.5-Gy ^{60}Co γ-irradiation. Data analyzed with use of multivariate discriminant analysis established very successful separation of animal groups before and after irradiation.

Here we present results from on-going murine *in vivo* studies demonstrating time- and dose-dependent increases in multiple blood protein biomarkers (i.e., GADD45a, IL-6, serum amyloid A or SAA). The use of multiple protein targets was evaluated using multiple regression analysis to provide dose-response calibration curves to enhance radiation sensitivity. Our efforts show for the first time the proof-of-concept that protein expression profile can be developed not only to predict radiation exposure in mice but also to distinguish the level of radiation exposure, ranging from 1 to 7 Gy.

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14. ABSTRACT
In May 2008, the Armed Forces Radiobiology Research Institute (AFRRI) held the Partial-Body Radiation Diagnostic Biomarkers and Medical Management of Radiation Injury Workshop to 1) comprehend the current state of knowledge about partial-body exposures, 2) identify current approaches and available biomarkers, 3) review the impact of partial-body biodosimetry on treatment, 4) identify research gaps, and 5) propose future directions. Twenty-nine experts, including 17 speakers and 12 poster presenters, reviewed the state-of-the-science in a variety of areas.

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