Impacts of a 10kt in a Modern US City
Overview of Health Impacts from Nuclear and Radiological Scenarios
PHEMCE MCM Requirements Process
Medical Countermeasure Target Product Profiles (HHS product requirements)
Biodosimetry Target Product Profiles (HHS product requirements)
Product Development under the Animal Rule: Requirements and the Revised Draft Guidance
Countermeasure Product Development Pathway: Laboratory to Licensure
NIAID’s Funding Opportunities for Radiation and Nuclear Medical Countermeasure Product Development and Biodosimetry Tools
Why We Need High-Throughput Radiation Biodosimetry - and Why We Need It Fast
Needs and Challenges for Biodosimetry at the Various Stages of the Response to a Large-Scale Radiation Event
Application of Untargeted and Targeted Metabolomics for Radiation Biodosimetry
MALDI-MSI to Identify Changes in the Lipid Profiles as they Relate to Radiation Damage and Mitigation
Metabolomic Analysis of Urine From Radiation Exposed Immunocompromised Mice: Implications of Genetics in Rapid Identification of Exposed Individuals in a Radiological Event
Development of Transcriptomic Approaches for High-Throughput Minimally Invasive Radiation Biodosimetry
Molecular Mechanisms Regulating the Transcriptional Cascade Induced by Ionizing Irradiation
In Vivo and Ex Vivo EPR Measurements of the Radiation-Induced Signal in Finger/Toe Nails for Rapid Dosimetry
RITN Network and Preparedness for Mass Casualties
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Mitigator Properties and Screening Strategies
Radiation Mitigators GS Nitroxide JP-039 and Bifunctional Sulfoxide MMS35 are Mitochondrial Anti-oxidants
Cutaneous Delivery of JP-039 Using Microneedle Arrays (MNAs) Effectively Mitigates Total Body Irradiation (TBI)
Fibrinogen Coated Nanospheres Prevent Thrombocytopenia-Related Bleeding
Recombinant Human Interleukin-12, but not Filgrastim, Increases Survival after Radiation-induced Myeloablation: Results from Randomized Blinded Placebo-Controlled Study in Rhesus Macaques
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Sequential Administration of Intestinal Crypt Cell Growth Factor (Rspondin1) and Differentiation Factor (ICG-001) Mitigates RIGS
The Synthetic Oleanane Triterpenoid RTA 408 Mitigates Gastrointestinal and Hematopoietic Acute Radiation Syndromes in Mice
Immediate or Delayed Administration of Pleiotrophin Improves the Survival of Irradiated Mice via Activation of Ras
Pulmonary and Systemic Radiation Effects Following Internal Contamination with 137Cs In Neonatal And Adult Mice
Ionizing Radiation Injury Of The Skin: Histologic And Metabolomic Evaluation In The Pig
Wednesday, October 8th

Impacts of a 10kt in a Modern US City
Brooke Buddemeier (Lawrence Livermore)

Overview of Health Impacts from Nuclear and Radiological Scenarios

Nuclear and radiological scenarios have the potential to impact large numbers of people and result in a wide spectrum of complex injuries. Modeling of nuclear detonation scenarios in modern urban cities have indicated that a large number of combined injuries with survivable exposures involving prompt gamma, neutron, and protracted fallout exposures are likely. This assessment is supported by historical data from Hiroshima that indicates 65-70% of injured persons are expected to have acute injuries that involve trauma and/or burn with significant radiation exposure (Geiger 1964). Radiation combined injuries are associated with faster onset of symptoms, exacerbated symptoms, synergistic increases in mortality, and impaired wound healing (Messerschmidt 1965). The impact of combined injury, protracted fallout exposures, inhomogeneous and partial body exposures, and potential cutaneous doses will complicate assessment and treatment of patients. Other scenarios involving radiological dispersal devices (RDDs) and nuclear power plants (NPPs) have the potential to involve more serious cutaneous radiation injuries and internalized radionuclide exposures. A detailed overview of injuries from these scenarios is provided; this overview was developed using insights from historical case studies, along with modeling analysis involving modern urban environments.

PHEMCE MCM Requirements Process
Julio Barrera-Oro, HHS/ASPR/OPP/MCSR

Medical Countermeasure Target Product Profiles (HHS product requirements)
Chad Hrdina, HHS/ASPR/OPP/MCSR

Biodosimetry Target Product Profiles (HHS product requirements)
Lynne Wathen, HHS/ASPR/BARDA

A large-scale chemical, biological, radiological or nuclear (CBRN) incident in a large metropolitan setting would result in an immediate critical need to assess potentially dangerous or pathological exposures received by tens of thousands of individuals to allow for prompt triage and appropriate medical treatment decisions. Measuring the individual exposure levels will require system architecture or a system of platforms that contains diverse, integrated diagnostic and dosimetric tools that are accurate and precise. For large-scale CBRN incidents, precision, rapidity, and ease of screening are essential. The Biomedical Advanced Research and Development Authority (BARDA) within the HHS Office of the Assistant Secretary for Preparedness and Response coordinates and administers programs for the advanced development and acquisition of emergency medical diagnostics. BARDA is currently developing assays and devices for biodosimetry applications in the aftermath of a nuclear or radiological incident. Two types of biodosimetry tools, point of care and high-throughput, are under development and each has distinctly different target product profiles. Their concepts of operation, relevant exposure levels, ease of use, intended use settings, throughput and device characteristics differ greatly. The desired features of each device type will be described and their utility in enhancing the nation's preparedness to ensure effective and appropriate use of medical countermeasures will be presented.

Product Development under the Animal Rule: Requirements and the Revised Draft Guidance
Andrea Powell, FDA/CDER/CTECS

Countermeasure Product Development Pathway: Laboratory to Licensure
David Cassatt, NIH/NIAID/RNCP

NIAID’s Funding Opportunities for Radiation and Nuclear Medical Countermeasure Product Development and Biodosimetry Tools
Francesca Macchiarini, NIAID

The possibility of a radiological or nuclear incident in the United States remains a national security level threat. The gravity of such a scenario would be exacerbated if necessary medical treatments were not available. Unfortunately, there are currently no FDA-approved drugs available in the Strategic National Stockpile that are licensed for the treatment of acute and/or delayed radiation health complications. Since 2004, the National Institute of Allergy and Infectious Diseases’ (NIAID), Radiation and Nuclear Countermeasures Program
(RNCP) has addressed these concerns by building and managing a program for the research and development of safe and efficacious medical countermeasures (MCMs) to mitigate and treat the consequences of radiation exposure. The RNCP’s research priorities are focused on the development of MCMs (with over 150 currently under evaluation), decorporation agents targeting internal radionuclide contamination and biodosimetric tools that measure radiation exposure of victims. Key strategies of the RNCP are to help researchers navigate the challenging path of drug development, while continuing to introduce new initiatives and processes that will enhance the MCM program in years to come. These include an improvement in drug screening programs, as well as exploring areas of unmet needs, such as drug formulations for special populations and qualification of animal models that will advance drug development for human use.

**Why We Need High-Throughput Radiation Biodosimetry - and Why We Need It Fast**
David Brenner (Columbia)

After a large-scale radiological event unless radiation biodosimetry can provide reasonable estimates of individual radiation doses, potential radiation mitigator treatments may be ineffective or may even be harmful. For example, after the Chernobyl accident, 13 individuals were given bone marrow transplants, of which three deaths have since been identified as unnecessary sequelae of the treatment alone - in that the individuals had received radiation doses for which transplants were not indicated.

As well as optimizing treatment for highly exposed individuals, the second role of radiation biodosimetry is triage: Eliminating and reassuring those individuals who do not need medical intervention will be crucial goals in what will be a highly resource-limited and panic-laden scenario.

The third component of radiation biodosimetry refers to individualized predictive assays: When a large number of people are exposed, can a simple blood- or urine-based assay be used to predict which individuals are at risk of early death, or at risk of a serious late effect? This is an extension of biodosimetry which, as well as taking account dose, takes into account individual sensitivity. It now seems likely that, yes one can predict individualized outcome based on blood tests - after exposure, but well before symptoms develop. This opens the door to optimal use of available medical countermeasures.

What emerges is the need for ultra-high throughput biodosimetry – tens of thousands of samples / day. Using standard biodosimetry methodologies, the highest throughput that can be achieved by a single lab is <500 samples / week, and even laboratory networks are expected to have throughputs of at best a few thousand samples / week. While good enough for small-scale events, these numbers would be inadequate if, for example, a major city were targeted.

In the following talks the speakers will address different approaches to achieving high throughput radiation biodosimetry.

**Needs and Challenges for Biodosimetry at the Various Stages of the Response to a Large-Scale Radiation Event**
Hal Swartz (Dartmouth College)

There are 3 different interrelated stages of the initial medical response to a large scale incident involving radiation. Each has different requirements, and the properties of biodosimetry must fit each stage to be most effective. The initial stage requires rapid assessment of radiation exposure, to determine which subjects may have received a dose above a threshold that justifies advancing them into the next stage of the medical response system. For this stage the properties of effective biodosimetry include: the measurements can be done in the field by non experts, should minimize false negatives and the results are immediately available to triage decision makers. The second stage refines those results by removing false positives and advances the estimate of risk, with more precise estimates of the radiation dose and assessments of the homo/heterogeneity of the exposure. Dosimetry in stage two can use reasonably well-organized and specialized facilities and trained staff, with the capability of follow-up monitoring of each subject. High throughput is desirable. The third stage is aimed at guiding medical intervention and will occur in a more advanced treatment facility. Here the dependability of the measurements and their implications for the biological effects will be especially important. High throughput will not be essential and advanced expertise and facilities may be available. All three stages will encounter additional needs and challenges when subjects at risk for radiation injury also have multiple injuries and/or very high stress. There also is a mandate that evaluations at every stage be appropriate to assess all segments of the population, including children and seniors and pregnant women. The CMCRs are
oriented towards meeting these needs and considerable progress has been made. This presentation will summarize the needs and the status of the developments to meet these needs.

**Application of Untargeted and Targeted Metabolomics for Radiation Biodosimetry**
Al Fornace (Georgetown)

**MALDI-MSI to Identify Changes in the Lipid Profiles as they Relate to Radiation Damage and Mitigation**
Maureen Kane (Maryland)

**Metabolomic Analysis of Urine From Radiation Exposed Immunocompromised Mice: Implications of Genetics in Rapid Identification of Exposed Individuals in a Radiological Event**
Evagelia C. Laiakis (Georgetown)

Radiological accidents and nuclear threats have led to increased necessity for development of fast, accurate, and minimally invasive methods for radiation detection. Metabolomics, the comprehensive and quantitative study of small molecules in a biospecimen, has been used extensively to create biosignatures of radiation exposure in urine and blood. Complicating factors, however, in developing a population-based biosignature include genetic variability among others, where 5% or more of the population may be radiosensitive. Previous results have concentrated on wild type (wt) C57Bl/6 mice exposed to ionizing radiation and to lipopolysaccharide, in order to address the specificity of metabolic markers in urine. We have extended our studies to mutant models in order to dissect the genetic differences in response to ionizing radiation. Immunocompromised Prkdc<sup>scid/scid</sup> (scid) mice were exposed to their LD<sub>50/30</sub> dose of 3 Gy of gamma rays and urine was collected at one day post irradiation. Metabolomic profiling through the use UPLC-TOFMS and rigorous statistical analysis revealed a biosignature of the genetic background and markers specific to radiation exposure. Validation of putative ions was conducted through tandem mass spectrometry (MS/MS) with the use of pure standards and verification through MS/MS online databases (i.e. METLIN). While important metabolic differences following radiation exposure are reflected in the urine and have been identified through mass spectrometry, it is imperative to design radiation signatures that will cover a wide group of possible confounders. For this, the use of genetic mutant mouse models remains an important method for designing a comprehensive radiation biosignature.

**Development of Transcriptomic Approaches for High-Throughput Minimally Invasive Radiation Biodosimetry**
Sally Amundson (Columbia)

Transcriptomic signatures have been developed for disease diagnosis and tumor classification, and show promise for personalized medicine. As gene expression is extremely responsive to stresses such as ionizing radiation exposure, the development of transcriptomic signatures to provide radiation biodosimetry for large-scale radiological or nuclear events has been very attractive. Most such efforts have focused on developing signatures in peripheral blood, as this is a tissue that is both transcriptionally very sensitive to radiation exposure, and easily accessible through a finger prick. We have demonstrated the basic ability of post-exposure gene expression signatures to classify samples by exposure dose in both ex vivo and in vivo exposure models. A great potential advantage of such an approach is that it may be able to provide more detailed information about an individual’s exposure than can be obtained from a measurement of physical dose. The transcriptome can carry information about an individual’s response to radiation, or the extent of their specific radiological injury, rather than providing a population average. In more recent work, we have begun to develop gene expression signatures that may ultimately enable treating individual injuries in a radiologic or nuclear event, rather than treating the dose.

**Molecular Mechanisms Regulating the Transcriptional Cascade Induced by Ionizing Irradiation**
Prabhat K Purbey (UCLA)

The threat of widespread radiation exposure due to terrorism or radiation release highlights the importance of developing strategies for mitigating its effects on human health. One fundamental effect of radiation is the activation of a robust transcriptional response in both cycling and non-cycling cells. Multiple pathways have been proposed to contribute to this response, including DNA damage pathways, reactive oxygen species, and various DAMPs acting through pattern-recognition receptors. However, the relationship between each of the proposed radiation-induced pathways and the transcriptional output remains poorly understood. We have taken advantage of the accuracy and quantitative value of RNA sequencing (RNA-seq) to examine the mechanisms regulating the radiation-induced transcriptional cascade in post-mitotic mouse macrophages.
RNA-seq was performed with mRNA from bone marrow-derived macrophages irradiated at different doses for 0, 0.5, 1, 2, 6 and 24-hrs. Seventy-five genes induced by 5-fold or more (p <0.01) were clustered according to their peak transcript abundance into Early (0.5,1 and 2 hr peaks) and Late (24 hr peak) response classes. The transcriptional cascade was unaltered in macrophages from Myd88/-/-Trif/-/- mice, suggesting that the response is not influenced by DAMP signaling. However, macrophages from Atm/-/- and Scid (DNA-PK) mice exhibited greatly diminished responses, with the two mutants affecting distinct subsets of genes. These results reveal that DNA damage-induced pathways are among the most important contributors to the radiation response, and that two major DNA damage-induced pathways make unique contributions. Further studies support the hypothesis that the unfolded protein response (UPR) activates many of the remaining radiation-induced genes that are not activated by ATM and DNA-PK. Finally, a comparison of the transcriptional response to radiation versus the response to other inflammatory stimuli has begun to provide insight into the mechanisms by which different inflammatory stimuli activate overlapping yet distinct sets of genes.

In Vivo and Ex Vivo EPR Measurements of the Radiation-Induced Signal in Finger/Toe Nails for Rapid Dosimetry
Steven G. Swarts (Florida)

Electron Paramagnetic Resonance (EPR) measurement of the radiation-induced signal (RIS) in finger/toe nails is being developed as a method to rapidly and accurately determine individual radiation dose for triage in a radiological/nuclear event. Instrumentation and methodology are being developed for an in vivo nail EPR dosimetry method to directly measure RIS in finger/toe nails in the field. Key components under development are resonators with unique geometries that allow for large sampling volumes but limiting the measurements to the nail plate. Two resonators are under development: the SurfaceArray Resonators (SRA) consisting of parallel elements which limit depth sensitivity of the RIS measurements to within the nail plate, and the Aperture Resonator (AR) which makes use of a high sensitivity cavity resonator modified with an aperture (dimensions of 3.5-5mm length by 0.78-1.0mm wide) for making measurements of the RIS in nails. Initial testing of the SRA and AR prototypes in tissue-equivalent nail models and in vivo nail measurements show that these resonators are approaching the capability of achieving detection sensitivities within the range required for measuring RIS to at the 2 Gy dose triage threshold. As a complement to the in vivo nail dosimetry method, an ex vivo nail method has been developed that uses conventional X-band EPR instrumentation to make precise measurements of the RIS in nail clippings in offsite testing facilities. Sample handling and storage methods are in place to allow for transfer of samples from collection sites to offsite facilities. Instrumental acquisition and spectral fitting modeling techniques have been validated in 50+ donors’ nail sample sets as a means to calculate the RIS from other clipped nail signal components within a dose range of 0-6Gy. Studies are being conducted to understand the factors (e.g., native background signal) that affect the precision of the RIS estimates.

Thursday, October 9th

RITN Network and Preparedness for Mass Casualties
Nelson Chao (Duke)

This presentation will provide an overview of Radiation Injury Treatment Network (RITN), its preparedness activities and capabilities. The RITN is a model for how a collaborative effort can fill a readiness gap, through its network of 71 hospitals, blood donor centers and cord blood banks the RITN is preparing to provide intensive supportive care to 63,000 radiological casualties. A radiological incident or some toxic chemicals such as Mustard agent can destroy a person’s marrow which is the basis of the immune system. RITN’s Cancer Centers deal with purposeful and potentially deadly chemical and radiological exposures daily the resulting complications and necessary treatment to heal the resulting health complications to their patients. The Radiation Injury Treatment Network provides comprehensive evaluation and treatment for victims with marrow toxic injuries. Many of the casualties following an improvised nuclear device detonation with only radiation injuries will be salvageable but require outpatient and/or inpatient care. Recognizing this, the US National Marrow Donor Program (NMDP), US Navy and American Society for Blood and Marrow Transplantation (ASBMT) collaboratively developed RITN, which comprises of medical centers with expertise in the management of bone marrow failure. Since RITN’s inception it has trained and educated over 10,000 hospital staff, coordinated the accomplishment of over 440 exercises, developed treatment guidelines, developed standard operating procedures, and is recognized by the federal government (DHHS-ASPR) as a national response asset.
Paths to the Strategic National Stockpile
Richard Hatchett (DHHS/BARDA)

Mitigator Properties and Screening Strategies
William McBride (UCLA)

High throughput screening (HTS) of chemical libraries of 85,000 drugs resulted in 220 that would mitigate apoptosis of a murine lymphocyte cell line in vitro when added after 2 Gy radiation. Handling this number of data points required establishing an industrial sized database. Maximal common subcluster analysis fortunately could ascribe most to one of 12 clusters based on chemical similarity. The 2 largest clusters were based on quinolin and the 4-nitrophenylsulfonamides scaffolds. We have tested 4 of the former compounds and 5 of the latter in mice and found them all to be mitigators of hematopoietic acute radiation syndrome (hARS) after whole body irradiation (WBI). One 4-nitrophenylsulfonylpiperazine was chosen as a lead because it was effective at a lower dose than most others. Analogs were synthesized to explore structure-activity relationships. The piperazine group seemed essential for low dose activity, but a 4-nitrophenylsulfonamide lacking this group was just as effective at higher dose. During pharmacokinetic analysis a processed metabolite of the 4-nitrophenylsulfonylpiperazine that retains low dose activity, suggesting that processing may be an important step in creating the most effective reagent. This molecule had superior solubility to its parent and may have a better drug-like character. In vivo, the lead compound was able to mitigate against gastrointestinal ARS and radiation pneumonitis and late fibrosis. It has anti-inflammatory properties that may be transmitted through myeloid cells, in particular an immature subpopulation that appears in the bone marrow and other organs very rapidly after WBI. Deletion of this population abolished mitigator activity as does knock out of the anti-oxidant response element that binds Nrf2, which may be a downstream effector anti-inflammatory pathway for this subpopulation. Conceptually, it seems that this and perhaps other mitigators affect the tissue damage response to radiation. This may be by blocking the release of action of damage-associated molecules that trigger acute inflammation resulting in further collateral damage. Alternatively, they may boost the response that controls the initial acute response, or act later during the healing phase by stimulating stem cell proliferation and tissue remodeling.

Radiation Mitigators GS Nitroxide JP4-039 and Bifunctional Sulfoxide MMS35 are Mitochondrial Anti-oxidants
Mike Epperly (Pittsburgh)

GS-nitroxide JP4-039 and the water soluble oxetanyl sulfoxide, MMS350, have been shown to be effective radiation mitigators in vitro and in vivo. We now report the mechanism of action of both drugs is by an anti-apoptotic pathway that stabilizes the mitochondria. Irradiation survival curves were performed with the 32D cl 3 murine hematopoietic progenitor cell line. JP4-039 or MMS350 was added either 1 hr before or 15 min after irradiation. In other experiments, cells were also irradiated to 5 Gy and examined 48 hr later for mitochondrial membrane depolarization, apoptosis, cell cycle arrest, cleavage of PARP and caspase3, and migration of SAP kinase, and JNK kinase to the mitochondria. Incubation of 32D cl 3 cells with JP4-093 or MMS350 before or after irradiation increased the shoulder on the clonogenic survival curves (JP4-039 before 3.9 ± 0.6, JP4-039 after 5.4 ± 0.5, MMS350 before 3.7 ± 0.3 and MMS350 after 6.0 ± 1.9, respectively) compared to 2.0 ± 0.3 (p = 0.0140, 0.0005, 0.0156, or 0.0085, respectively) for control irradiated 32D cl 3 cells. There was a G0/G1 cell cycle arrest for 32D cl 3 cells as well as cells incubated with JP4-039 or MMS350 before or after irradiation. 32D cl 3 cells treated with JP4-039 or MMS350 pre or post irradiation had decreased mitochondrial membrane depolarization and apoptosis compared to irradiated control 32D cl 3 cells. Western analysis of 5 Gy irradiated cells showed decreased cleavage of PARP and caspase 3 and decreased migration of JNK and SAP kinases from nucleus to mitochondria in cells incubated with JP4-039 or MMS350 compared to 32D cl 3 irradiated controls. JP4-039 and MMS350 work at the level of mitochondrial stabilization to mitigate irradiation-induced apoptosis.

Cutaneous Delivery of JP4-039 Using Microneedle Arrays (MNAs) Effectively Mitigates Total Body Irradiation (TBI)
Rhonda Brand (Pittsburgh)

The GS-nitroxide, JP4-039 has been demonstrated to be an effective mitigator of irradiation damage when administered intravenously 24, 48 or 72 hr after irradiation using a F14 emulsion. In the setting of mass casualties, intravenous delivery may not be feasible. The F14-JP4-039 emulsion may not be as stable over long storage intervals as would be dry powder in biodegradable microneedles. The use of a microneedle
arrays (MNAs) for drug delivery has other advantages. The MNAs could be broadly distributed and self-administered, and the drug dose scaled to MNA size for individuals by age and weight. We compared I.V. with MNA JP4-039 delivery at 24 hours after TBI. Each MNA consisted of 100 microneddles containing JP4-039. Needles dissolved in the dermis and released drug reached the circulation for systemic delivery. Ten week old C57BL/6NTac female mice were irradiated to 9.1 Gy and were divided into a control irradiation group, or a group in which MNAs were applied to the ear of mice, or a group receiving drug I.V. in F14 emulsion (n = 15 mice per group). For the ear application, one MNA was placed on the dorsal and one on the ventral side of each ear for a total of 4 MNAs per mouse (equivalent to 10 mg/kg JP4-039). The MNAs were left on for 5 minutes. The mice were followed for development of the hematopoietic syndrome. The 9.1 Gy control was an LD50/30. Mice treated with I.V. JP4-039 or with MNAs containing JP4-039 had a significantly increased survival – LD20/30 or LD7/30 (p = 0.0466 or 0.0172, respectively). These results demonstrate that JP4-039 can be effectively delivered as a TBI radiation mitigator using biodegradable MNAs.

Fibrinogen Coated Nanospheres Prevent Thrombocytopenia-Related Bleeding
Anthony D. Sung (Duke)

Radiation-induced thrombocytopenia may cause severe and life-threatening bleeding. We evaluated the ability of fibrinogen-coated nanospheres (FCN) to prevent thrombocytopenia related bleeding. These nanoparticles are made of human albumin polymerized into a spherical shape and coated with fibrinogen. Administration of FCN (8 mcg/g, i.v., 24 hours, day 5, and day 10) significantly improved survival compared to saline control in both radiation-induced (Fig 1a) and radiation+platelet depleting antibody induced (Fig 1b) models of thrombocytopenia despite no difference in platelet counts (Fig 1c, d, respectively). In particular, the addition of anti-CD41 antibody to 7.0 Gy TBI significantly increased mortality compared to just 7.0 Gy TBI (Fig 1b), suggesting that deaths were related to degree of thrombocytopenia (Fig 1d). Importantly, the majority of these deaths were prevented by FCN (Fig 1b). All deaths were due to gastrointestinal or intracranial hemorrhage (Fig 1e) and hemoglobin of moribund mice ranged from 3-5 g/dL. Furthermore, FCN shortened bleeding times in a platelet-concentration dependent manner in a murine model of antibody-induced thrombocytopenia (Fig 1f). There were no clinical signs of thrombosis or laboratory signs of disseminated intravascular coagulation after FCN (Table 1). Fluorescent microscopy imaging studies suggest that FCN bind to platelets upon platelet activation (data not shown). Interestingly, no differences in platelet aggregation or clot strength were detectable on light aggregometry (PAP-8E), impedance aggregometry (Multiplate), or thromboelastography (TEG, ROTEM) (data not shown). Nor were differences seen in fibrin formation (Fig 2a). However, addition of FCN significantly inhibited clot lysis in a dosedependent manner (Fig 2b). FCN may prevent thrombocytopenia-related bleeding by interacting with platelets and inhibiting clot lysis. FCN may be helpful in many disorders including primary (immune thrombocytopenia) as well as secondary thrombocytopenia (cancer, chemotherapy, trauma, radiation injury), potentially reducing platelet transfusions, particularly in situations where platelets may not be readily available.

| Table 1. Coagulation studies 24 and 48 hours after saline or FCN |
|-----------------|--------|--------|--------|--------|
| PT (s)          | 11.4   | 11.7   | 11.5   | 11.1   |
| aPTT (s)        | 23.6   | 23.9   | 21.5   | 24.7   |
| TT (s)          | 20.1   | 20.4   | 18.5   | 16.9   |
| Fibrinogen (mg/dL) | 153     | 116     | 116    | 173    |
| D-dimer (mcg/ml) | 0.22   | 0.22   | 0.22   | 0.22   |
Recombinant Human Interleukin-12, but not Filgrastim, Increases Survival after Radiation-induced Myeloablation: Results from Randomized Blinded Placebo-Controlled Study in Rhesus Macaques

Zoya Gluzman-Poltorak (Neumedicines Inc., Pasadena)

rHuIL-12 is being developed for mitigation of HSARS under the FDA Animal Rule using a NHP model of HSARS. Our previous randomized, blinded, placebo-controlled GLP study in the NHP showed that a single subcutaneous injection of rHuIL-12 (50ng/kg-500 ng/kg) 24-25 hours after irradiation significantly improved overall survival without the use of antibiotics, fluids or blood transfusions.

Here we report the results from our second GLP randomized blinded placebo-controlled NHP study that was designed to compare rHuIL-12 to filgrastim (rHuG-CSF). Animals were irradiated (700 cGy) and treated with a
single subcutaneous injection of vehicle or rHuIL-12 175ng/kg 24-25 hours after irradiation, daily subcutaneous injections of rHuG-CSF at 10µg/kg/d for 18 days starting 24-25 hours after irradiation, or combination of a single rHuIL-12 and 18 rHuG-CSF injections (26-36 animals/group) without supportive care.

rHuIL-12 increased survival compared to rHuG-CSF (56% vs 31%, Logrank test p<0.05) while rHuG-CSF did not provide any survival benefit over the control group (36%). Combination of rHuIL-12 and rHuG-CSF was similar to the rHuIL-12 monotherapy group (58% survival).

rHuIL-12 significantly decreased incidence of severe neutropenia and thrombocytopenia compared to vehicle and rHuG-CSF. Combination of rHuIL-12 with rHuG-CSF further improved neutrophil and platelet counts. rHuIL-12 also decreased incidence of severe infections, while rHuG-CSF increased incidence of infections and mucositis.

During the highest mortality period (Days 10-18) rHuIL-12 improved neutrophil, platelet, lymphocyte, red blood cell, and reticulocyte counts. rHuG-CSF increased neutrophils and platelets starting from day 14 only, had no effect on reticulocytes, and negative impact on lymphocytes and red blood cells.

In conclusion, single subcutaneous injection of rHuIL-12 alone reproducibly improves survival in the NHP model of HSARS at dose levels shown to be safe in healthy humans, while rHuG-CSF has no survival benefit in this model. These results support further development of rHuIL-12 as a medical countermeasure for HSARS.

Evaluation of EntericSorb, a Non-absorbable Anti-Inflammatory Oral Therapy, in a Mouse Model of Gastrointestinal Acute Radiation Syndrome
Humayra Ali (CytoSorbents Corporation, NJ) and Catherine Booth (Epistem, Ltd.)

Risk of radiological catastrophe underscores the need for effective medical countermeasures. Acute radiation syndrome (ARS) first manifests in the gastrointestinal tract (GI-ARS). In the mouse, GI-ARS manifest at doses typically >12Gy.

EntericSorb is a novel orally-administered topical anti-inflammatory therapy for the GI tract that uses a non-absorbable, highly-porous polymeric sorbent. EntericSorb sequesters intra-luminal cytokines, bacterial toxins and other mediators based on pore capture and surface adsorption and excretes them from the body. EntericSorb has previously demonstrated anti-inflammatory activity in a mouse model of TNBS-induced inflammatory bowel disease. We hypothesized, EntericSorb may also reduce the risk of death in GI-ARS by reducing intestinal inflammation, gut permeability, the systemic inflammatory response syndrome and septicemia.

Two groups of 20 adult C57BL/6 male mice were exposed to 13.5Gy partial-body irradiation (PBI), delivered at 0.812Gy/minute, using a 300kV X-ray source, shielding 5% of the bone marrow. This is the LD50 dose in control mice. Mice were orally gavaged twice daily with water (control) or a slurry of EntericSorb (treatment), each followed by a second water gavage, starting 24-hours post-irradiation. Animal survival and weights were monitored for 15 days.

EntericSorb improved mean survival time by 1 day during GI-ARS, with an absolute improvement in survival on Day 8 of 30% (85% vs. 55% control), and 20% on Day 10 (35% vs. 15% control). During GI-ARS, more EntericSorb-treated animals retained at least 75% of initial weight each day, compared to controls; 40% vs. 15% control animals on Day 8, and 25% vs. 5% control animals on Day 10, met this criteria. EntericSorb also provided greater symptomatic improvement in the severity and duration of diarrhea than control.

Further studies are planned using total-body irradiation vs. PBI at different doses of irradiation. We will also explore dose optimization and the mechanisms of action (specific cytokine and enteric toxin sequestration).

Sequential Administration of Intestinal Crypt Cell Growth Factor (Rspondin1) and Differentiation Factor (ICG-001) Mitigates RIGS
Bhanji, Guha (Albert Einstein)

The Synthetic Oleanane Triterpenoid RTA 408 Mitigates Gastrointestinal and Hematopoietic Acute Radiation Syndromes in Mice
Ulrich Rodeck (Jefferson)

High dose whole body exposure to ionizing radiation leads to life threatening complications due to catastrophic damage to fast proliferating cell types, primarily the gastrointestinal and hemopoietic systems. Currently, no
small molecule agents are approved that rescue either gastrointestinal or hematopoietic syndromes when administered after radiation exposure. Here, we report effective mitigation of acute gastrointestinal and hematopoietic radiation syndromes in mice by several thiol modifying compounds and, in particular, the synthetic oleanane triterpenoid, RTA 408. The administration of a brief course of RTA 408 treatment beginning 24 h after bone marrow lethal doses of radiation significantly increased overall survival after radiation doses ranging from 7 to 8 Gy. RTA 408 led to full recovery of steady-state hematopoiesis with normalization of the frequency of hematopoietic stem and progenitor cells. Moreover, hematopoietic stem cells from RTA 408-mitigated mice showed lineage-balanced, long-term, multilineage potential in serial transplantation assays, indicative of their normal self-renewal activity. Importantly, RTA 408 also preserved the integrity of the mucosal lining of the GI tract and maintained crypt stem cell survival and proliferation after lethal radiation doses. The composite mitigating effects of RTA 408 are mechanistically linked as RTA 408-dependent rescue of gastrointestinal damage depends on recruitment and reprogramming of bone marrow-derived myeloid cells. The potency of RTA 408 in mitigating both, radiation-induced bone marrow suppression and gastrointestinal syndrome makes it an attractive candidate to treat radiation injury caused by whole body radiation exposure.

Immediate or Delayed Administration of Pleiotrophin Improves the Survival of Irradiated Mice via Activation of Ras
Heather Himburg (UCLA)

Hematopoietic stem cells (HSCs) are highly susceptible to ionizing radiation-mediated (IR-mediated) death via induction of ROS, DNA double strand breaks, and apoptotic pathways. The development of therapeutics capable of mitigating IR-induced hematopoietic toxicities could benefit both victims of acute radiation sickness and patients undergoing hematopoietic cell transplantation. Unfortunately, therapies capable of accelerating hematopoietic reconstitution following lethal radiation exposure have remained elusive. Here, we found that systemic administration of pleiotrophin (PTN), a protein that is secreted by bone marrow endothelial cells (BM ECs), substantially increases the survival of mice following radiation exposure and after myeloablative BM transplantation. In both models, PTN increased survival by accelerating the recovery of BM hematopoietic stem and progenitor cells in vivo. PTN promoted HSC regeneration via activation of the RAS pathway in mice which expressed protein tyrosine phosphatase receptor-zeta (PTPRZ), whereas PTN treatment did not induce RAS signaling in PTPRZ-deficient mice, suggesting that PTN-mediated activation of Ras was dependent upon signaling through PTPRZ. PTN strongly inhibited HSC cycling following irradiation, whereas RAS inhibition abrogated PTN-mediated induction of HSC quiescence, blocked PTN-mediated recovery of hematopoietic stem and progenitor cells (HSPCs), and abolished PTN-mediated survival of irradiated mice. These studies demonstrate the therapeutic potential of PTN to improve survival after myeloablation and suggest that PTN-mediated hematopoietic regeneration occurs in a RAS-dependent manner.

Pulmonary and Systemic Radiation Effects Following Internal Contamination with 137Cs In Neonatal And Adult Mice
Carl J. Johnston (Rochester)

Internal contamination with radioactive material can result either from the detonation of a “dirty bomb” packed with material or through accidental release as a result of a nuclear event such as seen by recent nuclear power incidents. Such exposure can lead to internal contamination and systemic delivery of long-lived isotopes. The chronic effects of such exposure are not well characterized. In particular, significant differences exist between the physiology of the immature, neonate compared to the adult, which may affect acute and late response to irradiation. Identifying these differences would be critical in developing successful mitigation strategies for this special population. Our current hypothesis is that systemic radiation in a young animal will lead to enhance pulmonary effects through a vascular exposure paradigm. C57BL/6J mice, 14 or 56 days of age, mice either received a 5 Gy TBI dose or received a single 50 or 100 uCi I.P. injection of 137Cs. Mice were measured daily the for first 2 weeks and twice weekly thereafter for internal activity. Body Weights were recorded weekly. Mice were examined at 12, 20 and 26 weeks post injection. Pulmonary response was determined by lavage and histological examination. Analysis of epithelial and inflammatory markers and gene expression were done by immunohistochemical and mRNA analysis. Neonatal mice demonstrated slower clearance kinetics as compared to adult mice. Furthermore significant differences in injury were measured in neonatal mice that received systemic exposure as compared to those mice that received external exposure. These results demonstrate that early life systemic radiation injury affects the lung’s response differentially as compared to the mature lung. Furthermore these results demonstrate that systemic radiation induces unique responses as
compared to external irradiation injury.

**Ionizing Radiation Injury Of The Skin: Histologic And Metabolomic Evaluation In The Pig**

Michael Tytell (Wake Forest)

Destruction of the epidermis and loss of barrier function after exposure to ionizing radiation is a major concern following radiological accidents. Current treatments are mainly palliative and do not reduce damage or promote repair. We have developed a pig model (Sus scrofa) to assess skin responses to a range of β and x-ray radiation doses (18-48 Gy) over time. For β-ray radiation, a custom device was designed using strontium-90. For x-ray radiation, a conventional 300 kV orthovoltage x-ray device was modified to provide localized doses. Radiation was delivered to six, 4 cm diameter circular sites on each flank of ~30 kg female pigs (12 sites/pig). Doses and treatments were randomized on each pig, visually graded and photographed every 3 or 4 days for up to 70 days. Pigs were euthanized on post-irradiation days 15, 35 or 70. From each site, four 1-cm biopsies were collected for histological evaluation and one 5-mm biopsy was frozen for metabolomic analysis. Visual grading revealed dose-related early and late erythema and moist desquamation. These observations correlated with histologic changes that included epidermal atrophy and dermal inflammation, edema, and vascular pathology. Abnormal regenerative changes occurred at higher radiation doses and longer post-irradiation intervals. Metabolomic analysis revealed dose-related increases in oxidative damage, prostaglandin-related molecules, mitochondrial breakdown, and bacterial penetration. These observations provide a greater understanding of the skin’s response to ionizing radiation and serve as a foundation for potential treatment testing.

**Changes in Pulmonary Macrophage Subsets Following Irradiation**

Angela Groves (Rochester)

One long-term complication of exposure to radiation is the development of pulmonary fibrosis. Responding to microenvironmental signals, macrophages become phenotypically polarized to orchestrate inflammatory responses. Alternatively activated macrophages may contribute to the development of fibrosis in the lung. The role of these cells in radiation induced pulmonary fibrosis was investigated. Fibrosis prone C57 or pneumonitis prone C3H mice were exposed to 0 or 12.5 Gy whole lung irradiation and lung digests were collected at various time points following exposure. CD45+ leukocytes were characterized by flow cytometry. Alveolar macrophages (AM’s, CD11b low, CD11c+), interstitial macrophages (IM’s, CD11b+, CD11c+), and infiltrating macrophages (CD11b+, CD11c-) were identified. Expression of F4/80, present on mature macrophages, Ly6C, expressed by pro-inflammatory monocytes and macrophages, and mannose receptor (CD206), an alternative activation marker, were assessed in each population.

Lung irradiation decreased macrophages at early time points. Identification of discrete populations showed that in both C57 and C3H mice, the proportion of AM’s decreased but IM’s increased by 3 wk following irradiation. AMs exhibited decreased expression of F4/80 and CD206, whereas in IM’s the expression of Ly6C was increased. In C57 mice, AM’s were not similar to 0 Gy values until 26 wk, whereas this occurred more quickly in C3H mice. IM’s began to return to unirradiated proportions by 8 wk in both C57 and C3H mice. An increase in infiltrating macrophages was observed 3 wks following irradiation in all mice. In C57 mice this is followed by a decrease in the proportion of these cells compared to unirradiated values. Differences in the responses of these discrete macrophage populations in fibrosis prone and resistant mice suggest a contribution of these cells to radiation-induced fibrosis.

**Novel Radiation Mitigators and Anticancer Drugs**

Robert Schiestl (UCLA)

The possibility of a radiation disaster from a nuclear detonation or accident has existed for over 50 years and spawned much of the basic research in radiobiology in the 1950-60s. The recent Fukushima accident was yet another reminder that there remains a dire need to develop novel therapies against radiation-induced toxicities. Here we report on the development of two novel radiation countermeasure therapies: Yel001 and Yel002. These small, biologically active, drug-like molecules were uncovered in the DEL high throughput assay reducing radiation-induced cyto- and geno-toxicity in yeast. Further, Yel compounds increases survival to 100% in vivo following an LD100/30 dose of ionizing radiation (IR) with the first therapeutic injection administered 24 hours post exposure followed by injections at 48,72,96, and 120 hours. Additionally, treatment with Yel001 and Yel002 compounds reduces radiation-induced leukemia from 90% to 50% and 40% respectively. Of note, treatment with either Yel001 or Yel002 reduced spontaneous leukemia rate from 10% to
0%. Treatment with Yel002 following IR accelerates the recovery of the hematopoietic cells after sub-lethal exposures. In addition, treatment with Yel002 reduces EMS, MMS, UV, cigarette smoke extract as well as nitrogen mustard induced toxicity as well as genotoxicity showing a broad application spectrum. It also prolongs live of cells in a senescence assay. In addition, Yel002 complements a zebrafish model of Diamond Blackfan Anemia. It works in yeast, CHO cells, different human cells, mice and zebrafish. Toxicity has not been observed in neither in vitro or in vivo administrations. Overall, Yel compounds have much potential as stockpile therapies for radiation-induced lethality and cancer: they are highly effective when administered up to 24 hours post exposure, they reduce radiation-induce sequelae such as leukemia, and appear to have an acceptable toxicity profile.

**Life Time Memory of Genotoxicity by Dormant Senescence-Prone Cells (DSPC): Radiomitigation and Biodosimetry Implications**

Andrei V. Gudkov (Rosewall Park)

Systemic genotoxic stress (e.g., exposure to UV or ionizing radiation, treatment with chemotherapeutic drugs, etc.) results in DNA damage that remains unrecognized in mesenchymal cells and can stay unrepaired for the entire life of the organism. An attempt to enter the cell cycle leads to activation of DNA damage response and conversion of such cells, in a p53-dependent manner, into senescence. Abundance of such dormant senescence-prone cells (DSPC) in tissues reflects radiation dose and remains unchanged during the entire mouse life. We consider DSPC as a natural memory mechanism that “records” all genotoxic events that happened with the organism during its life-time. Role of DSPC in long-term pathological consequences of irradiation and biodosimetry will be discussed.

Friday October 10th

**After ARS – The Need for Late Effect Mitigation**

Jacqueline Williams (University of Rochester)

**Acute and Long Term Immune Responses to Radiation and Mitigation**

Genhong Cheng (UCLA)

One important aspect of radiation injury is the release of both endogenous damage associated molecular patterns (DAMPs) from the damaged tissues and pathogen associated molecular patterns (PAMPs) from the gastrointestinal system. These DAMPs and PAMPs released after irradiation interact with common pattern recognition receptors such as Toll-Like Receptors (TLR) and activate overlapping gene programs to regulate innate and adaptive immune responses as well as tissue damage and repair. Based on our results from multiple tissue damage models, we have recently hypothesized that over reactive innate immune responses to DAMPs and PAMPs released after irradiation can further trigger secondary tissue damages by proinflammatory cytokines and autoantibodies. We have further developed a widely available and easily deliverable DAMP blocking compound, glycyrrhizic acid (GA), as a potent radiation mitigator. In addition, while GCSF has been used as a standard mitigator, we have developed a bivalent GCSF (Bi-GCSF) as a more potent and stable radiation mitigator. Interestingly, although different mitigators can effectively rescue lethally irradiated mice during acute radiation syndrome (ARS), many rescued mice often develop delayed effects of acute radiation exposure (DEARE) exhibiting chronic diseases such as heart and kidney failures around a year after WBI. Our preliminary studies indicate that these chronic diseases are associated with proinflammatory responses. Based on these studies, we hypothesize that balanced immune and inflammatory responses are critical not only for rescuing acute phase tissue damages but also for preventing chronic diseases caused by radiation.

**Strategies for Detection of Organ-Specific Biomarkers of Delayed and Late Effects of Acute Radiation Exposure (DEARE)**

Julian Whitelegge (UCLA)

The recent efforts of the countermeasures community have focused on the development of mitigator compounds to be administered at least 24 hours after radiation exposure, with survival accessed after 30 days. In the human scenario survival beyond 30 days will be critical and thus biomarkers that predict delayed and late effects of acute radiation exposure are of significant current interest, especially if they can provide an individualized signature of tissue/organ damage to guide treatment strategies.
We are currently performing discovery proteomics mass spectrometry experiments on blood from irradiated mice in order to provide biodosimetry information and potentially organ-specific signatures of damage. Focus to date has been on 2- and 5-day time points post whole body irradiation and we will report a panel of ten candidate biomarkers.

Blood from mice that survived a LD70 dose of whole body irradiation (WBI; 7.75 Gy) plus or minus mitigator (UCLA 512) were collected 3 months after radiation exposure. In another experiment, blood from mice that survived 4 months after lung thoracic irradiation (LTI; 14 or 18 Gy) plus or minus mitigator (UCLA 512) were collected. These samples are being compared to controls using our discovery proteomics mass spectrometry protocols. The presentation will summarize our current strategies for discovery of organ-specific biomarkers of DEARE.

**Developing Biomarkers to Predict Radiation Lung Injury**

Meetha Medhora (Wisconsin)

**Introduction:** Radiation to the lung induces lethal pneumonitis after a latent period of 6 weeks. One class of mitigators, angiotensin converting enzyme (ACE) inhibitors improve survival even if started at 5 weeks after radiation.

**Goal:** To identify noninvasive assays to predict lethal lung injury by 4 weeks after a single fraction of ionizing irradiation that is relevant to a radio-nuclear terrorism event.

**Methods and Results:** Rats (female WAG/RijCmcr at 9-10 weeks of age) were randomized into: (i) no irradiation (control) (ii) a lethal dose of 15 Gy X-rays to the whole thorax. The lungs were imaged in vivo by SPECT after 1, 2, 3 and 4 weeks using 99mTc-duramycin (DU) and 99mTc-macroaggregated albumin (MAA) as probes to detect apoptosis and loss of vasculature respectively. Lung sections were also stained with cleaved caspase 3 antibody to confirm increase in apoptosis.

MicroRNA libraries by next-generation sequencing of blood, plasma and exosomes were generated using Illumina TruSeq. The Illumina analysis tool CASAVA was used to identify candidate markers which were confirmed by qPCR. Serum miRNA were compared by a hybridization based nanoString assay.

A lethal 15 Gy dose to the lungs increased the DU SPECT signal in the thorax by 75% at 2 and 39% at 3 weeks (n>3 rats/group), indicating the role of apoptosis in radiation-induced lung injury. This was accompanied by a decrease in the MAA SPECT signal in irradiated lungs. MicroRNA levels were altered at 2 weeks after radiation in blood, plasma and exosomes, showing alteration of gene expression as a mechanistic biomarker.

**Conclusion:** Using a combination of assays we are developing a specific injury profile to predict pulmonary lethality as early as 2 weeks after exposure. This time frame is well suited to a mass casualty event and will permit effective mitigation by ACE inhibitors.

**CTGF expression is increased in Irradiated Non-Human Primate Lung Tissue**

Pei Zhang (Maryland)

**Introduction:** Radiation-induced pulmonary fibrosis is a serious health threat after high dose radiation exposure to the thoracic region. Connective tissue growth factor (CTGF) has been shown to be a central mediator of tissue remodeling and was found to be required for the development of persistent fibrosis in murine models. CTGF expression was shown to be changed in a time-dependent manner in a fibrosis-prone murine strain after thoracic irradiation. We investigated the expression of CTGF in pulmonary tissue from non-human primates (NHP) exposed to whole thorax lung irradiation (WTLI).

**Methods:** NHP were exposed to WTLI at doses from 9.0 Gy to 12.0 Gy. All animals were followed and provided with supportive care (including dexamethasone) for a planned in-life study of 180 days. Lung tissue was procured from NHP that met euthanasia criteria or reached end of the study and was prepared for molecular and histological analysis.

**Results:** CTGF mRNA levels were elevated in NHP exposed to WTLI in a dose- and time-dependent manner. Higher doses of radiation were associated with higher levels of CTGF mRNA expression. The elevated expression was more significant at early time points (NHP that were euthanatized between day 40 and 100) during the 180d time course. At later time points (>100d) CTGF levels diminished, but were still higher than the average baseline levels. CTGF protein levels followed a similar trend, increasing at early time points and then diminishing toward baseline values. Immunohistochemical staining of CTGF showed higher expression of...
CTGF in type 2 alveolar cells and inflammatory cells in irradiated lungs. Treatment with dexamethasone may affect CTGF expression.

**Conclusion:** CTGF expression was elevated on both mRNA and protein levels after thoracic irradiation in NHP. Suppressing the elevated CTGF may be a promising therapeutic method to treat radiation-induced pulmonary fibrosis.

**Persistent Immune Imbalance in Long-term ARS Survivors**
Dorthe Schau (UCLA)

This study focused on mice that had survived near lethal whole body irradiation (WBI) and progressed to become long-term survivors of acute hematopoietic radiation syndrome (ARS). The variety and persistence of organ-specific dysfunction that followed, such as heart and lung fibrosis, exceeded any site-specific damage one would expect if the same radiation dose had been given locally. Much of the disease remained subclinical but there was increased mortality. The nature of the findings, combined with evidence of sporadic lymphoid cell infiltration strongly suggests immune involvement in orchestrating these tissue-specific late effects. We therefore performed immune phenotyping and functional profiling of spleen and bone marrow as well as on immune infiltrates in affected organs in C3H and C57BL/6 mice that survived WBI at LD70/30 with or without the help of pharmaceutical mitigation. Antibody staining and multi-color flow cytometry revealed splenic immune profiles that had higher than normal suppressor cell levels, both in the lymphoid and in the myeloid compartment while cells of the B lymphocyte lineage were greatly diminished. Similarly, the bone marrow niche in ARS survivors seemed to have severely depressed levels of hematopoietic stem and progenitor cells (KSL), which was partially reversible by 512 mitigation. Based on Enzyme-linked immunosorbent spot (ELISPOT) assay data and broad cytokine profiling, the immune system in long-term survivors seems functionally geared towards a proinflammatory, Th1 type cytokines involved in hematopoiesis. The cause of the persistent hematopoietic insufficiencies, immune imbalances, and organ dysfunction needs to be better understood if we are to find agents that efficiently mitigate this consequential late radiation damage.

**Long Term Morbidity in Irradiated Nonhuman Primates**
Mark Cline (Wake Forest)

**Introduction/Methods:** The major burden of radiation injury among survivors lies in long-term effects, including organ failure, fibrosis, and neoplasia. We present here the clinical and pathologic long-term adverse effects of single dose whole body exposure at 3.5 to 8.4 Gy in 49 rhesus monkeys exposed at a median age of 2.8 years (range 1.2-10.3 years) and observed for a median 5.2 years (range 1.2 to 7.2 years) after exposure. Radiation was administered by linear accelerator at 60 cGy/min in two opposed fields. Observations include clinical examinations, imaging (CT and MRI scans), hematology, clinical chemistry, and necropsy/histopathology.

**Results:** Major disease processes identified include (1) chronic pulmonary disease including pulmonary fibrosis, bronchopulmonary epithelial hyperplasia and dysplasia; (2) type II diabetes mellitus associated with islet hyperplasia and amyloidosis and increased peripheral insulin resistance; (3) infections including skin and wound infections, bronchopneumonia, pericarditis, and sepsis; (4) cardiac abnormalities including loss of ventricular function and myocardial fibrosis; (5) osteopenia; and (6) various neoplasms including myelodysplasia, renal carcinoma, cutaneous squamous cell carcinoma, neurofibroma, leiomyosarcoma, a heart base paraganglioma, and a Merkel cell tumor. Multiple disorders in the same animal were common, with diabetes being the most common co-morbid condition.

**Comments:** Our growing body of data indicates that a substantial burden of disease is present in long-term survivor non-human primates, including complex patterns of co-morbidity, immunosuppression, and disorders involving both loss of functional tissue as well as cytoregulatory disorders (hyperplastic and neoplastic lesions).

**Internal Cesium Exposure Synergizes with External Irradiation Acutely and Causes Late Injury to the Hematopoietic Stem Cell Compartment**
Jim Palis (Rochester)

A bioterrorist attack or nuclear accident could lead not only to acute external, but also to internal, radiation exposure through inhalation/ingestion of radioactive particulates. We have developed a C57BL/6 mouse model to investigate acute and late effects of internal cesium on the hematopoietic system. 100µCi soluble 137Cs
delivered IP leads to widespread organ distribution with an accumulated dose estimated at 2.5Gy TBI over 7 weeks. Interestingly, concomitant addition of external 6Gy TBI resulted in delayed excretion of internal 137Cs, resulting in profound peripheral cytopenias and 40% mortality at 30 days from acute hematopoietic syndrome. To further interrogate the outcome of combined internal and external radiation on the hematopoietic system, we compared the late effects of 100μCi soluble 137Cs to 6Gy TBI, as well as the combination of the two at 12 and 26 weeks after injury. At 12 weeks, peripheral blood counts and erythroid and myeloid progenitor numbers in the marrow were similar to age-matched controls. However, internal radiation exposure decreased phenotypic hematopoietic stem cell (HSC) numbers, particularly short-term HSCs. Surprisingly, HSC function, assayed by competitive repopulation, was also compromised by internal, as well as external, radiation exposure. At 26 weeks, hematopoietic defects were evident in the peripheral blood, with anemia following TBI and combined internal/external exposure. Persistent decreases in short-term HSC occurred in all irradiated mice, along with a drastic decline in HSC repopulation capacity. Finally, late radiation-dependent changes were evident in the marrow microenvironment, with differential regional distribution of adipocytes. Taken together, our data indicate that internal contamination with sublethal doses of long-lived, soluble radioactive isotopes damages long-term HSC and significantly alters the marrow microenvironment. We also conclude that a nuclear accident or bioterrorist event, where individuals may be at risk of both external radiation exposure and internal contamination, can lead to unexpected acute and late hematological consequences.

Poster Session – Thursday, October 9th

GS-Nitroxide, JP4-039, is an Effective Mitigator of Combined Injury (Irradiation Plus Bone Injury)

Michael W. Epperly1, Julie Glowacki2, and Joel S. Greenberger1 (1Department of Radiation Oncology, University of Pittsburgh Cancer Institute, Pittsburgh, PA 15213; 2Department of Orthopedic Surgery, Brigham and Women's Hospital and Harvard Medical School, Boston, MA)

Uncortical bone wounding has been demonstrated to be an effective model for the measurement of the timing and effectiveness of bone healing. Uncortical 2-mm diameter bone wounds were drilled in the proximal tibia of C57BL/6NHsd mice and observed by orthovoltage x-ray to completely heal within 35 days. Mice with bilateral tibial uncortical wounds were irradiated to one leg immediately after bone drilling. Subgroups of mice received I.P. JP4-039 at 10 mg/kg, and the time of bone wounding and time to complete healing was quantified. Irradiation to 10, 20, or 30 Gy showed a dose dependent increase of the time to bone wound healing. Mice receiving JP4-039 in F14 emulsion I.P. (10 mg/kg) 24 hours after irradiation showed significant amelioration of the delay in bone wound healing by day 21 with the diameter of the bone wound being 1.06 ± 0.04 mm for the 20 Gy irradiated leg compared with 0.52 ± 0.01 mm for the JP4-039 treated, 20 Gy irradiated leg (p < 0.0001). JP4-039/F14 shortened the time to bone wound healing in non-irradiated limbs at day 21 from 0.72 ± 0.02 to 0.46 ± 0.01 mm for the JP4-039 treated mice (p < 0.0001). Orthotopic 3LL tumors in the right hind leg were not protected from irradiation by JP4-039. The GS-nitroxide radiation mitigator is also effective in the setting of combined injury involving bone fracture.

Mitochondrial Targeted Radiation Mitigator, GS-Nitroxide, JP4-039, is Effective in DNA Damage Response Deficient Fanconi Anemia (Fancd2-/) Mice

Ashwin Shinde1, Hebist Berhane1, Michael W. Epperly1, Kalindi Parmar2, Eva Guinan2, Song Li3, Peter Wipf4 and Joel S. Greenberger1 (1Department of Radiation Oncology, 2School of Pharmacy, and 4Department of Chemistry and Center for Chemical Methodologies and Library Development, University of Pittsburgh, Pittsburgh, PA; 3Dana Farber Cancer Institute, Harvard Medical School, Boston, MA)

The distribution of radiation mitigator agents to victims in a setting of mass casualties from a radiation terrorist event will include individuals with reduced levels of intrinsic DNA damage response capacity, and, therefore, increased radiosensitivity. To determine whether a known mitochondrial targeted radiation mitigator, JP4-039, was effective in radiosensitive populations, Fancd2-/- mice derived from each of two genetic mouse strain backgrounds (C57BL/6, and FVB/N) were irradiated to the head and neck and oral cavity/oropharyngeal mucositis was quantitated at day 5. Subgroups of mice received intraoral JP4-039/F15 emulsion, non-mitochondrial targeted Tempol/F15, F15 emulsion alone, or no drug therapy immediately prior to irradiation. In C57BL/6 mice, fractionated irradiation of 8 Gy x 4 was administered with each drug therapy delivered immediately prior to each radiation fraction. In all studies, heterozygote Fancd2+/+ and control Fancd2+/+ mice were also tested. There was a significant decrease in irradiation-induced ulceration in JP4-039/F15 treated, but not Tempol/F15, or F15 treated mice compared to irradiated control mice. Following 28 Gy, wild type mice of the FVB/N background had 60.0 ± 5.0% ulceration of the oral cavity compared to 13.0 ± 4.0% (p = 0.0047)
for mice treated with JP4-039/F15. Wild type mice of the C57BL/6 background irradiated to 28 Gy had 62.8 ± 15.5 % ulceration compared to 12.2 ± 1.0 (p = 0.0049) for mice treated with JP4-039/F15 before irradiation. Following 8 Gy X 4, wild type C57BL/6 mice had 72.3 ± 17.2% ulceration which was decreased to 16.9 ± 12.6% (p < 0.0001) with JP4-039/F15 pretreatment. With Fancd2/-/- mice, a decrease in radiation damage was observed by JP4-039/F15 treatment with both background mouse strains. After 28 Gy FVB/N FancD2/-/- mice had 73.3 ± 3.3% ulceration compared to 11.1 ± 2.5% (p = 0.0171) for mice pretreated with JP4-039/F15. Similarly, C57BL/6 FancD2 -/- mice after 28 Gy had 81.7 ± 2.7% ulceration compared to 47.8 ± 3.2% (p < 0.0001) for FancD2 -/- pretreated with JP4-039/F15. C57BL/6 FancD2/-/- mice following 8 Gy X 4 had 68.0 ± 6.0% oral cavity ulceration compared to 11.0 ± 3.0 for JP4-039/F15 treated mice (p < 0.0001). Treatment with F15 alone or Tempol/F15 had no significant effect on irradiation induced oral cavity ulceration of any groups. JP-039 preserved intracellular antioxidant stores and ameliorated irradiation-induced stress response gene and cytokine RNA transcripts in the oral cavity tissues in vivo. In conclusion, the mitochondrial targeted JP4-039 facilitates effective reduction of irradiation damage in vivo in the setting of a defective DNA damage response pathway.

Radiation Induces Accelerated Senescence in Lung Stromal Cell Populations

Tyler Beach (University of Rochester School Of Medicine and Dentistry)

Pulmonary radiation injury may induce physiological changes, most notably pulmonary pneumonitis or chronic fibrosis resulting from changes in cell populations and disruption in immune function and cell signaling. Radiation can induce apoptosis, and has been theorized to induce senescence among the surviving cell population. Changes in immune cell populations correlated with alterations in cytokine levels as a response to radiation have been reported. Recently, our lab has shown a decline in Club cell populations in radiation treated mice, while other groups have indicated changes in populations of Type 2 alveolar epithelia. An extension of this is to explore the role of senescence in population changes. A model consisting of 18 month aged C57BL/6J mice exposed 12.5 Gy whole lung irradiation via a Cesium137 gamma radiation source were compared with non-irradiated age matched controls. Stromal cell populations were assessed in whole lung tissue by flow cytometry. OCT inflated frozen lung sections were fixed for histological examination. Surfactant Protein C (SPC), Club Cell Secretory Protein (CCSP), and CD31 staining was employed to assess changes in stromal cell populations, with p21 and beta-galactosidase staining for senescence indication. The irradiated group displays altered lung morphology as evident in Hematolxyn and Eosin stained sections. Whole lung tissue rt-PCR results have indicated changes in p21 mRNA abundance. Beta-galactosidase staining is also suggestive of changes among the stromal population when compared to the age matched control. Expression of p21 and beta-galactosidase is potentially linked to change in stromal cell subtypes within the lung. Ultimately this research may lend insight to the role senescence plays in the disruption of cell signaling, and yield insight into therapeutic interventions for radiation induced lung injury.

EUK is a Potential Pulmonary Mitigator for Late Radiation Effects

Carl J. Johnston (Department of Pediatrics University of Rochester School of Medicine)

The pulmonary complications that can follow both localized and total body irradiation can affect survivors of the acute radiation syndrome resulting from a nuclear or radiological event, leading to significant morbidity and mortality. The acknowledged radiation sensitivity of the lung also suggests that personnel exposed to relatively low (sub-lethal) total body doses and who may, therefore, not necessarily be treated in the immediate aftermath of an event, may similarly be susceptible to these outcomes. EUK-207 operates through catalytic scavenging of reactive oxygen and nitrogen species (ROS/RNS). EUK-207 is proposed to decrease oxidative stress in the mitochondria and elsewhere. The oxidative stress is hypothesized to occur chronically following irradiation and to cause delayed irradiation injury. C57BL/6J mice 56 days of age, received a 5 GyTBI dose followed immediately by a 10 Gy lung only dose. Mice received 30 or 60 Mg/Kg subcutaneously or by inhalation beginning either: 72 hours or 4 or 8 weeks post irradiation. Mice were treated daily for 4 or 8 weeks. Body weights were recorded weekly. Mice were examined at 20, 26, 28 and 30 weeks post irradiation. Pulmonary response was determined by lavage and histological examination. Analysis of epithelial and inflammatory markers and gene expression were done by immunohistochemical and mRNA analysis. Treatments, which began 4 weeks post irradiation, had the greatest effect in suppressing macrophage infiltration and minimal loss of airspace associated with radiation injury. These results demonstrate an inhibition of macrophage infiltration associated with radiation injury. Macrophage infiltration has been associated with matrix deposition suggesting that EUK may help prevent fibrosis development associated with radiation
damage. Furthermore, the timing of EUK delivery, 4 weeks post injury, appears to demonstrate the greatest efficacy.

**Novel Model of Combined Injury to Investigate the Effect of Skin Damage and Repair on Radiation Toxicity**
Sade Fridy (Department of Dermatology University of Rochester)

A sub-lethal total body dose of γ-radiation (TBI) combined with a subsequent skin injury (combined injury), such as a burn or wound, has been reported to increase mortality over either event alone. It is our hypothesis that skin damage correlates with morbidity in the combined injury group. Consequently, agents that repair epidermal barrier defect might mitigate much of the toxic effects observed from combined injury. In order to test this hypothesis, we proposed a novel combined injury model, which utilizes sub-lethal TBI and ultraviolet B-radiation (UVB) exposure as the secondary injury. Seven days post-TBI (6Gy) mice (BALB/c) were exposed to 360 mJ/cm2UVB (20 minutes sun exposure in Rochester, NY in July). Clinical outcomes and skin immune profile were evaluated. Profound suppression of white blood cell (WBC) and platelet counts were observed after TBI ± UVB. Importantly, the recovery was significantly delayed in the combined injury groups compared to the 6Gy alone (n=3; p<0.001, 91 days post-TBI). Petechial rash was more severe in combined injury mice than in those that received only TBI (n=3; p<0.05, 11 days post-TBI). A striking finding from our study was that the UVB induced skin inflammation was largely abrogated in the combined injury groups. We performed flow cytometric analysis on skin samples at 12 days post-TBI, when the lesion were clearly visible in mice that received UVB only. A marked increase of several T-cells subtypes (e.g. Th2, Th17, Treg and CD8+ cells) was observed after UVB. While very few T-cells were detected in the skin of mice that received TBI±UVB, suggesting a loss of ability to mount a cutaneous inflammatory response post-TBI. Altogether these preliminary findings suggested that this novel model of combined injury might prove a useful tool to further investigate the effect of skin damage on radiation toxicity as well as to test mitigators.

**Microdosimetric and Biological Effects of Photon Irradiation at Different Energies in Bone Marrow**
Julian Down (Duke University)

To ensure reliability and reproducibility of radiobiological data, it is necessary to standardize dosimetry practices across all research institutions. The photoelectric effect that dominates at low energy and in high atomic number materials such as bone can lead to increased dose deposition in adjacent soft tissue due to secondary radiation particles and deviate from higher energy radiation that best model exposures from clinical radiotherapy or nuclear incidences. Past theoretical considerations have indicated that this process would impact on radiation exposure of neighboring bone marrow (BM) and account for reported differences in relative biological effectiveness (RBE) for hematopoietic lethality in rodents. The purpose of our study is to provide a more definitive estimation of dose distribution and biological effectiveness within the BM compartment for 137Cs g-rays and 320 kVp x-rays at two levels of filtration (1 and 4mm-Cu HVL). We performed (i) Monte Carlo simulations on 5µm resolution micro-CT images of mouse vertebrae, (ii) in-vitro biological experiments irradiating BM cells plated directly on the surface of a bone-equivalent material (BEM) and (iii) an in-vivo study in live mice that is planned to follow. Simulation results showed that the relative dose increase averaged over the entire BM volume was 1.33, 1.08, and 0.98 and at 5µm distance from cortical bone surfaces (representative of 10% of total BM) the relative dose increase was 1.6, 1.15, and 0.99 for the 1mm-Cu, 4mm-Cu HVL, and 137Cs respectively. In-vitro exposure results following 6 Gy showed relative cell killing of hematopoietic progenitors (CFU-C) that significantly increased for the 1mm-Cu HVL X-rays when the cells were located on BEM and in accordance with Monte Carlo simulations. Thus applying X-rays of highest kVp and filtration are needed in studies involving effects on the hematopoietic system so as to avoid the consequences of artificial high doses to functional BM.

**Mechanism Study of Actions and Development of Novel Radiomitigators**
Gang Deng (UCLA Chemistry & Biochemistry)

Over 30 phenylsulfonamides compounds were prepared and tested for their radiomitigatory activities. The most potent units, namely, R2 = N,N-diethylamide, 4-phenylpiperazine, 4-(4-fluorophenyl)piperazine, 4-(3-chlorophenyl)piperazine, and 4-cinnamylpiperazine, were retained, while the 4-nitro group, which was present in the most potent radiomitigators to date, was replaced with other strongly electron-withdrawing groups such as carboxylic acid at the C-2, C-3, or C-4 position. We also tested compounds with a nitro group at the C-2 position of the phenyl ring. Most of these analogs show good activity in the ATP viability assay in Til-1 cells.
Compounds with the carboxylic acid moiety at the C-2 position show greater potency than our original lead compounds, the 4-nitrophenylsulfonamides. Some of them have great drug-like potency as radiomitigators.

A Comparative Analysis Between Whole-Body Acute and Low Dose Rate Protracted Radiation Exposure in Mice Using a Metabolomic Approach
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Mass spectrometry (MS) has served as the desired platform for high-throughput monitoring of even subtle changes in the concentration of metabolites in complex biological samples. Thus, we employed MS to explore the effects of dose rate, rather than dose, on the urinary excretion levels of metabolites 2 days after exposure. A wide variety of statistical tools were employed to further focus on metabolites, which showed responses specific to low dose-rate (LDR) radiation exposure (0.00309 Gy/min) distinguishable from high dose rate (HDR) responses. From a total of 709 detected mass spectral features, 100 were determined to be statistically significant when comparing urine from 1.1 Gy irradiated mice to that of sham-irradiated mice 2 days post exposure. These metabolites were then subjected to different comparative analyses to identify which ones showed dose rate-specific behaviors post exposure. The results of this study show that LDR and HDR exposures perturb many of the similar pathways. However, the individual metabolite targets of LDR and HDR in a given pathway may be different. Establishing specific metabolomic responses to LDR and HDR may help determine an individual’s exposure in case of a radiobiological event.

In Vivo EPR Tooth Dosimetry Development and Validation for ARS Triage
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In vivo EPR tooth dosimetry instrumentation and procedures have been developed to provide accurate and precise estimates of absorbed dose to aid in emergency triage and medical decision making following suspected mass exposures. Noninvasive measurements of the upper incisor teeth can be performed and repeated at any time to provide an immediate estimate of the cumulative absorbed dose. The resulting physical dose estimates are not affected by concomitant injury. A transportable EPR tooth dosimeter and procedures for in vivo measurements have been developed using continuous-wave EPR at 1.2GHz. EPR tooth dosimetry has been verified in a series of in vitro and in vivo tests. An in vitro study was carried out using anatomic mouth models constructed using upper incisors collected from 5 individual subjects. Both central incisors were measured prior to irradiation and at cumulative doses of 1Gy, 2Gy, 4Gy, 6Gy, and 10Gy using a standard protocol with 6 minutes of data acquisition per measurement. Data were analyzed, after adjusting for variable tooth sizes, demonstrating a linear dose response with a standard error of inverse dose prediction (SEIP) of 0.49Gy. A subsequent in vivo study was carried out with 10 unirradiated subjects where both incisors were measured four times, each measurement acquiring 10 minutes of data. A number of ergonomic, instrumental, and procedural refinements were instituted for this study. After adjusting for age, gender, and tooth size, an SEIP of 0.66Gy was estimated. Improved precision of dose estimation, increased throughput, and further miniaturization of the device are feasible through use of higher RF frequencies and pulse-mode EPR. Prototype systems employing these technologies have been developed and initial measurements confirm feasibility and conferred benefits. Based on these results and unique abilities of this approach, EPR tooth dosimetry is poised to play an important role in radiation emergency management.

Ex-RAD (Recilisib) for Post-Radiation Exposure Mitigation of Hematopoietic Toxicity
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Background: The potential of a radiation disaster from a nuclear detonation or accident has existed for over 60 years. A recent Japanese nuclear power plant disaster and potential terrorist threats from a radiation dispersal device have heightened interest in research and development of radiation injury countermeasures for prophylaxis and mitigation of harmful effects of radiation exposure on the general population and emergency workers.

Ex-RAD™ (recilisib sodium), a novel chlorobenzylstyrlysufone, is currently under development by Onconova Therapeutics, Inc. as both an oral and subcutaneously administered radiation countermeasure. Prophylactic recilisib (given prior to irradiation) has been reported to significantly (P < 0.001) protect mice and rabbits from total body irradiation (TBI) mortality in exposures up to 8Gy.

Mitigation: Suman recently reported that post-exposure subcutaneously-administered recilisib treatment (24 and 36 hours post-irradiation) significantly mitigated radiation-induced hematopoietic toxicity in mice. Mitigation was evaluated by peripheral white blood cell (WBC) and platelet counts at 3,7,21 and 28 days after exposure. Granulocyte macrophage colony forming units (GM-CFU) assay using isolated bone marrow (BM) cells and BM histopathology, including terminal deoxynucleotidyl transferase dUTP nick end-labeling (TUNEL), were performed at 7 days post-exposure. The DNA damage response (DDR) pathway was investigated by western blot for ATM and p53.

Results: Compared to vehicle, recilisib-treated animals exhibited accelerated recovery of peripheral WBC and platelet counts and higher BM GM-CFU (P < 0.05). Both phospho-ATM and phospho-p53 were significantly lower in the BM of treated animals, suggesting attenuation of the ATM-p53-dependent DDR in bone marrow cells.

Safety of recilisib has been demonstrated in multiple animal species and in two first-in-man randomized, placebo-controlled ascending-dose trials of recilisib in healthy volunteers that found the agent to be well-tolerated, without clinically significant drug-related systemic toxicity. Further investigation of the mitigation potential for recilisib is warranted.

Development and Performance of a Rapid, High Throughput, Gene Expression Based Biodosimetric Assay

Following a mass scale nuclear event caused by detonation of a nuclear weapon or a nuclear accident, the rapid and accurate biodosimetry of thousands of potentially affected individuals will be essential for effective medical management of the crisis. Here, we describe the development and performance of a genomic assay suitable for the high-throughput determination of individualized levels of radiation exposure for up to 7 days post-exposure. The multi-gene assay was developed by cross-correlating the time dependent (6 hours to 7 days post exposure) RNA gene expression of peripheral blood collected from in-vivo irradiated non-human primates (NHPs), ex vivo irradiated human and NHP peripheral blood, and human cancer patients undergoing total body irradiation (TBI) in preparation for bone marrow transplantation. Genes that showed similar response profiles to radiation exposure in all both species were determined and used to develop a 29-gene predictor of radiation exposure. The 29-gene predictor was transferred to a high throughput system capable of rapid analysis of stabilized blood samples without requiring isolation of RNA. The performance of the Chemical Ligation-dependent Probe Amplification Radiation Exposure Test (CLPA-RET) has been evaluated in pre-clinical and blinded studies involving irradiated NHPs (up to 7 days post-exposure), human TBI patients, and more than 1,000 non-irradiated human subjects with potentially confounding diseases and mixed ethnicities.

Long-Term Effects Of Radiation Exposure On Immune Response To Challenge

Gayle Boxx (UCLA Microbiology, Immunology and Molecular Genetics)
It is well established that prior exposure to infectious diseases skews the production of innate cytokines and chemokines in the short term and establishes long term immunological memory, however the long term effects of radiation exposure on the immune system is not well understood. To investigate the response of radiation survivors to an immunological challenge, mice were subcutaneously co-injected with a peptide of chicken ovalbumin, OVA, and either Poly I:C, a TLR3 agonist or CpG B, a TLR9 agonist. We found that prior to immunization, survivor mice had altered serum cytokine levels, and that this alteration correlated with the use and type of radiation mitigator. Following immunization, mice treated with certain mitigators showed enhanced cytokine response compared with unirradiated or irradiated only mice, yet mitigator treated mice were unable to generate a sustained OVA specific antibody response. This suggests that serum cytokine may be indicative of the degree of immune competence, thus serve as a biomarker. Furthermore, this study demonstrates that mitigators confer distinct signatures to the immune system that shape the immune response when challenged.