November 7, 2016

Dr. Cherry Murray
Director, Office of Science
Department of Energy
Washington, DC 20585

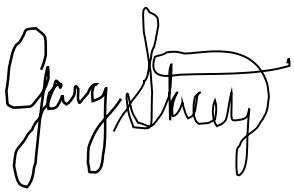
Dear Dr. Murray:

As per request, the BERAC established a subcommittee to address questions relevant to the Low Dose Program as outlined in the letter dated October 8, 2015 and signed by Acting Director, Pat Dehmer. This committee submitted their report to the BERAC during the meeting held Oct. 27-28, 2016. The report was discussed and approved without revision. You will find a copy included with this cover letter.

The report clearly states that further research into this area is unlikely to yield 'conclusive' results. Furthermore, this type of research does not align with current BER priorities Therefore, the BERAC feels strongly that further research on low dose radiation within BER is not warranted.

However, the report does indicate some opportunity to reduce uncertainty in this area. It is our understanding that DOE is already contributing computational resources to a cross-agency effort toward such a goal.

Sincerely,



Gary Stacey, Ph.D.
Chair, BERAC

Final Report
Low Dose Radiation Expert Subcommittee
Biological and Environmental Research Advisory Committee

October 2016

Committee Members

Michael B. Bellamy
Nuclear Engineer
Center for Radiation Protection Knowledge
Oak Ridge National Laboratory
Oak Ridge, TN 37831, USA.

Amy Berrington de González
Branch Chief and Senior Investigator
National Cancer Institute
Div. of Cancer Epidemiology & Genetics
Radiation Epidemiology Branch
9609 Medical Center Drive
Bethesda, Maryland 20892

David G. Hoel
Distinguished University Professor
Public Health Sciences
Medical School of South Carolina
171 Ashley Avenue
Charleston SC 29425SC

Arthur M. Katz (*retired*)
Office Bio. Environ. Research
SC-23, Germantown Building
U.S. Department of Energy
1000 Independence Avenue, SW
Washington, DC 20585-1290
Currently:
2 Stevenage Circle
Rockville, Md 20850

Jerome S. Puskin (retired)
Center for Radiation Protection Division
Environmental Protection Agency
Washington, DC 20460

Judy D. Wall (Chair)
Curators' Distinguished Professor
Biochemistry Division
University of Missouri
Columbia, MO 65211

Zhi-Min Yuan
Professor of Radiobiology
Director, John B. Little Center
Dept. Environmental Health
Dept. Genetics and Complex Disease
Harvard T.H. Chan
School of Public Health
Boston, MA 02115

Biological and Environmental Research, Observers

Sharlene Weatherwax
Associate Director
Todd Anderson
Director, Bio. Sys. Sci. Div.
Tristram West
Senior Technical Advisor

Table of Contents

I. Charge

A. Genesis of Charge

On June 16, 2015, the U.S. Secretary of Energy, Ernest Moniz, queried his Advisory Board (SEAB) for their advice regarding the question of DOE continuing a research program into the low dose radiation exposure to establish whether the "linear no threshold" (LNT) model could be determined to apply. He wanted to know whether specific knowledge gaps existed that could be approached by research efforts that, over time, would lead to an understanding of the health effects of low-level radiation exposure to humans.

In response on June 23, 2015, Dr. John Deutch reported the SEAB recommendation that "DOE continue to sponsor a small, sustained, high quality research program mainly in DOE laboratories but also at centers of excellence on this subject that exist in universities, medical schools and hospitals." However, the SEAB declined from offering guidance on the research program itself and suggested that the Director of the Office of Science (the then Acting Director, Patricia M. Dehmer) should commission a small group of experts to propose a "modest, multi-year, research program in low-level radiation exposure." Dr. Deutch ended with the caveat that Secretary Moniz "should not assume that the results of such a research program would be conclusive."

As a result of this recommendation, Acting Director Dehmer sent a charge letter (Section II, below) to the Biological and Environmental Research Advisory Committee (BERAC) to obtain informed advice on October 8, 2015. From 1998 to 2016, the Low Dose Radiation Research program has been administered through the Biological Systems Science Division (BSSD) of the Biological and Environmental Research (BER) office. The Chair of BERAC, Dr. Gary Stacey, asked Dr. Judy Wall, a member of that committee, to organize a small "expert" subcommittee to address the charge.

With the assistance of Drs. Sharlene Weatherwax, Associate Director of BER, and Todd Anderson, Director of BSSD, a short list of potential experts was assembled. These individuals were invited to participate in a series of teleconferences, beginning May 25, to discuss the controversy about the LNT model, the current status of epidemiological studies and the progress in laboratory research with model tissues and organisms. Drs.Weatherwax and Anderson along with Dr. Tristram West, Senior Technical Advisor for BER, were observers of the teleconference discussions.

B. Charge to the Biological and Environmental Research Advisory Committee

Department of Energy
Office of Science
Washington, DC 20585

October 8, 2015

Dr. Gary Stacey
Associate Director, National Soybean Biotechnology Center
Department of Microbiology and Molecular Immunology
271E Christopher S. Bond Life Sciences Center
University of Missouri
Columbia, MO 65211

Dear Dr. Stacey:

The mission of the Biological and Environmental Research (BER) program is to support fundamental research and scientific user facilities to achieve a predictive understanding of complex biological, climatic, and environmental systems for a secure and sustainable energy future. The program has a history of supporting research on the biological effects of low dose radiation exposure (the Low Dose program) to address DOE's legacy of nuclear weapons development and deployment and the safe production and use of nuclear energy for nuclear workers and the general public. This program was initiated in 1998 and will be completed in 2016. The findings from the Low Dose program have matured our understanding that high dose radiation and low dose radiation affect biological systems in profoundly different ways, and that a variety of complex, systems-wide processes govern gene expression and tissue response to low dose ionizing radiation exposure. While the program has expanded our knowledge of how cells react and adapt to low level radiation exposure (below 0.1 Gy), these molecular-level results cannot be readily extrapolated to assessing the risk of cancer in humans due to low dose radiation. Results in the scientific literature are conflicting, including numerous epidemiological studies that cannot provide conclusive and unambiguous results on which to base a replacement to the linear no-threshold (LNT) model. The Low Dose program will end in 2016 as BER continues to shift its programs towards bioenergy and environmental research.

On June 16, 2015, the Secretary of Energy asked the Secretary of Energy's Advisory Board (SEAB) for

> "…SEAB's perspective on how DOE should pursue research on the question of a 'linear' or 'threshold' low-level radiation exposure model. Should DOE continue its efforts on this subject or leave it to other agencies such as EPA and NIH? Or is there a research effort that over time may lead to knowledge that will resolve the question of health effects of low-level radiation exposure to citizens and workers in the nuclear industry. Has the scientific community identified specific knowledge gaps that would be appropriate research priorities for DOE to pursue?"

The chairperson of SEAB, Dr. John Deutch, responded in a letter on June 23, 2015. He noted that

> "…SEAB recommends DOE continue to sponsor a small, sustained, high quality research program mainly in DOE laboratories but also at centers of excellence on this subject that

exist in universities, medical schools, and hospitals. SEAB does not believe it is the right group to put together such a research program. Low-level radiation exposure is a specialized subject and, experience shows, there is not an obvious research program that will yield decisive results. The Director of DOE's Office of Science should be charged with commissioning a small group of experts (including a couple of smart outsiders to the subject) to propose a modest, multi-year, research program in low-level radiation exposure. If requested, SEAB would review this research plan and make suggestions to the Director of the Office of Science. However, you should not assume that the results of such a research program would be conclusive."

I am now charging BERAC to establish a subcommittee to provide advice that will inform the Office of Science's response to the SEAB recommendation regarding defining a research program that would lead to conclusive results on whether low dose radiation (<0.1Gy) causes cancer in humans. The subcommittee is asked to address:

1. The appropriate research goals for such a program that would lead to conclusive results;
2. DOE and BER mission-relevant goals for such a program;
3. The appropriate scope of a "small, sustained, high quality research program;"
4. Whether conclusive results could be obtained on the cancer risk in humans posed by low dose radiation; and
5. Additional federal agencies or funding bodies with equities in such a research program.

In preparing its response to this charge, the BERAC subcommittee should consider the SEAB response, available public reports and publications, materials prepared by the Low Dose program on prior activities, and that experimental cancer research is beyond the scope of the DOE mission. The subcommittee may also consider the breadth of federal agencies supporting relevant and/or related activities, and the role of fundamental research in informing regulatory requirements. The Low Dose program results are available in peer-reviewed scientific publications and summarized in a history prepared by Dr. Antone Brooks[1], and are available to inform the scientific community and regulatory policy; they have contributed towards National Academy reports[2].

I would like to receive a progress report on this charge at the next meeting in early 2016 and a final report at the summer or fall meeting in 2016. I look forward to what should be a stimulating and useful report. Many thanks for your contributions to this important effort.

Sincerely,

Patricia M. Dehmer

Patricia M. Dehmer
Acting Director, Office of Science

---

[1] http://lowdose.energy.gov/pdf/albRoughDraft/doeHistoryComplete09262012.pdf
[2] E.g http://www.nap.edu/catalog/18732/research-on-health-effects-of-low-level-ionizing-radiation-exposure and http://www.nap.edu/catalog/11340/health-risks-from-exposure-to-low-levels-of-ionizing-radiation

II. Responses to Charge questions

1) *The appropriate research goals for such a [research] program that would lead to conclusive results [on whether low dose radiation (<0.1 Gy) causes cancer in humans]*.

Even if statistical significance is relatively strong, it is unlikely that a single area of research will provide data considered conclusive about the health effects of ionizing radiation exposures at low dose/dose rate. The current model shaping policy decisions is that the risk of exposure to ionizing radiation extrapolates linearly. The ability to refute an accepted hypothesis requires a body of research that is confirmed by alternative methods over time. A functional goal for a targeted research program would be realistically to decrease uncertainty.

Epidemiological studies – on the whole – appear to be consistent with the Linear No Threshold model. In contrast, accumulated data from laboratory experiments have identified DNA repair systems, protein loci apparently associated with repair complexes, tumor suppressing proteins, and an increasing list of mutant tumor suppressor genes associated with specific cancers. These findings along with the "adaptive response" point to the possibility of a non-linear extrapolation.

Epidemiological data and biological research have not been functionally meshed. In epidemiological studies, the statistical strength of events at low dose radiation is only now approaching a level needed for confident declarations. Confounding factors occurring in the former collections of epidemiological data weakened the conclusions drawn. However great progress has been made in the design of epidemiological studies through the identification of confounding factors in earlier studies that can now be avoided. Given the improvements in record keeping, dosimetry and access to data that does not necessitate active participation of the cohort, there is optimism that studies currently in progress or planned will provide high quality data that will impact the understanding of health effects of low dose/dose rate ionizing radiation.

As for laboratory experiments, the irradiated model systems often do not closely mimic whole animal or human biology and the long time spans of interest are not achievable in the current research environment. Models are improving to accommodate the known influence of surrounding tissue and the importance of limiting the oxygen exposure of irradiated cells to a level determined to be found naturally within tissues. The progress in studies aimed at understanding the molecular and cellular events occurring in low dose irradiated tissues has been remarkable, much of it derived from research supported by the DOE BER program. The advent of new molecular tools, or those not previously well developed, offer the promise of providing unexpected advances in understanding of connections between DNA damage, tissue responses and cancer development. More insights are likely to result from the creative application of the CRISPR cas9 system for developmental tracking of cells; new catalogs of human genome sequences and SNP identification in disease states; and systems biology approaches to complex events occurring following radiation exposure, transformation and cancer development. New

tools for imagining in whole animals and developments in immunological regulation may also provide cancer models with more predictive power for humans.

2) *DOE and BER mission-relevant goals for such a program.*

   a) Understand why DNA damage repair systems are not evident in epidemiological data.
   b) Improve confidence in the shape of the dose response curve at low dose radiation.
   c) Establish whether differential transcription of genes, synthesis of proteins and/or metabolites in human cells occur at low versus high doses of radiation when administered at low or high rates.
   d) Understand the molecular factors affecting the development of cancer from transformed cells and whether those factors can be detected in cells exposed to low dose/dose rate of ionizing radiation.
   e) Determine whether apoptosis can be manipulated to remove cells damaged by ionizing radiation.
   f) Explore drugs or chemicals that might stimulate repair following an acute exposure to radiation, a.k.a., a Fukushima event, that would provide insights into the biology and mechanism of radiation response.
   g) Obtain bioindicators for radiation etiology. H2AX foci appear as an indicator of non-homologous end joining repair of double-strand breaks. Other protein/nucleic acid foci may form that indicate other processes of damage.
   h) Determine the conservation of mechanisms of response to radiation between animal models and humans.
   i) Use genome resequencing to establish whether there is a progression of DNA changes occurring following ionizing radiation exposure.

3) *The appropriate scope of a "small, sustained, high quality research program;"*

   Should a decision be made to pursue continued DOE support of research into the health effects of ionizing radiation at low doses and low dose rates, this committee recommends that one or more workshops be convened with experts in both systems biology and epidemiology to formulate a specific research program. At such a workshop, suggestions like those below could be part of the discussion.

   a) Determine transcriptional, proteomic and metabolic responses to low and high doses of radiation given at low rates. In addition, follow these omic responses to a low dose of radiation given at low and high rates. Determine whether differential transcription or splicing is observed and whether these changes are predictive of the dose or dose rate. Transcription of key genes or splicing events that are responsive to dose or dose rate might be identified that may be used for the development of bioindicators for low dose ionizing radiation.
   b) Apply the Collaborative Cross to explore genes involved in responses to low dose ionizing radiation in this specially generated population of inbred mice. Identify markers (omics) for increased radiation sensitivity and those that can relate immunological and cell matrix behavior to low dose responses that could be of

interest. This collection of genetically marked mice is designed to identify genes contributing to multi-loci diseases.

c) Explore the development of biomarkers for dose level that are specific to radiation rather than general oxidative damage.
d) Continue to support the Million Man Study in collaboration with other federal agencies. Alternatively, establish an epidemiological study based on available medical records that will decrease or eliminate the bias of confounding attrition.

4) *Whether conclusive results could be obtained on the cancer risk in humans posed by low dose radiation;*

It is highly unlikely that conclusive results will be obtained by a single focused program. However, results are likely to decrease the uncertainty in predicting cancer risk.

5) *Additional federal agencies or funding bodies with equities in such a research program.*

NRC, NIH, NCI, EPA, DHS, DOD and NASA could all benefit from the reduction in uncertainty obtained by this research.

III. Historical perspective of the Low Dose Radiation Program of BER

Exposure of large numbers of the world's population to radiation could occur through "dirty bombs" instigated by terrorist actions, critical accidents at nuclear power plants such as that at Fukushima or through therapeutic treatments. After initial phases of such events as these, continued and more widespread exposure to low dose/dose rate radiation will be the challenge. In addition, exposures from medical diagnostic applications or from flying too frequently must also be considered when predicting health outcomes of low dose radiation. These events result in low dose/dose rate radiation exposures that are generally defined as <100 mGy and <5 mGy/h, respectively (Kendall et al., 2013). The magnitude of the health risk imposed by exposure to low linear energy transfer (LET) radiation (gamma rays and X-rays) at these levels and rates remains uncertain and subject to much debate. The outcome is of significant health and economic importance for implementing public health standards.

At the time of the first atomic bombs, researchers knew the short-term effects of acute radiation doses. Long term consequences, however, and the effect of low doses of radiation were unknown and cause for concern. Epidemiological studies of exposed populations such as uranium miners, soldiers and radium-dial painters provided valuable insights into the nature of radiation effects. These studies were limited in their ability to provide a mechanistic understanding of radiation carcinogenesis and failed to provide information in the low-dose region of the radiation dose-response curve.

To bridge this gap, the Department of Energy turned to extensive animal studies that, during the course of the program, expanded our understanding of the distribution, retention and health consequences of internal emitters. Several significant discoveries were made such as the

vulnerability of mammalian embryos to radiation induced health effects. Additionally, during this period significant advances in radiation dosimetry were made including the development of the film-badge, thermoluminescent dosimetry, biomarker dosimeters along with solid state and scintillation detectors.

Scientific attention shifted to the DNA molecule after a link between the lack of DNA repair and carcinogenesis was found revealing that X-rays, gamma rays and chemical carcinogens can all cause cancer in the same way- by inducing damage in the DNA molecule. This newly discovered link motivated further investigation on the possibility that radiation may induce changes in the DNA of an exposed individual's progeny. Focus was placed on genetics research in the form on the Human Genome Project which gave us an unprecedented look into nature's genetic blueprint for building a human being.

Radiation biology in the DOE Low Dose Program played an important role in increasing our mechanistic understanding of molecular and cellular responses to ionizing radiation. Under this program, several new phenomena related to the effects of low levels of radiation on biological systems were discovered. Among these discoveries, the most significant include radiogenic genomic instability, bystander effects and adaptive response. Hundreds of journal articles have been published on these subject areas (DOE, 2012).

Genomic instability describes a phenomenon where radiation-induced genetic changes continue to occur both in the progeny of the irradiated cell and also in the progeny of bystander cells. This effect can be observed after several 'normal' cell divisions where the progeny has an increased rate of genetic alterations. The endpoint associated with the loss of genetic stability is diverse and includes gene mutations, amplification of DNA regions and delayed reproductive cell death (Morgan et al., 1996). The mechanisms that lead from the initial radiation damage to the subsequent manifestation of genomic instability are unknown.

Even after substantial investigation, identifying the important molecules related to genomic instability poses difficulties for researchers. The role of radiation in this process is also unknown as there is no conclusive evidence that radiation induced DNA damage is the initiator of genomic instability. Some evidence points to DNA double strand breaks as a potential initial cause of genomic instability but other studies suggest that double strand breaks are not the true initiator (Stoler et al., 1992, Wojcik et al., 1996, Morgan, 2011). Genomic instability was a significant discovery in the field of radiation biology because genomic instability is now accepted as an important part of carcinogenesis (Cheng & Loeb, 1993).

The bystander effect has been revealed as a phenomenon where cells may sustain radiogenic damage even though no radiation tracks pass through them (Nagasawa & Little, 1992). This is in contradiction to many years of scientific consensus during which it was thought that cells and the DNA within cells are damaged through either direct ionization or through interactions with the free radicals generated by indirect ionization. However, for a variety of biological endpoints,

studies have consistently shown that cells not directly irradiated can sustain damage (Sedelnikova et al., 2007; Blyth & Sykes, 2011).

Endpoints of the bystander effect include cell killing, micronucleus induction, mutation induction and variation in the expression of genes (Lehnert & Goodwin, 1997; Iyer & Lehnert, 2000). This effect has been observed between cells separated by various distances and is seen both *in vitro* and *in vivo*. The nature of the communication mechanism that exists between the irradiated cells with its neighbors is not fully understood, but it is speculated that cell-cell bystander interactions are different from interactions mediated by the messenger molecules released into the growth media or blood. Based on the results of broadbeam and microbeam experiments, the bystander effect may be the dominant effect at doses below 200 mGy (Brenner et al., 2001). Therefore, the bystander effect may potentially be of greatest concern in the low dose region.

Adaptive response research was a significant research area in the DOE low dose program. Adaptive response refers the production of any potentially beneficial effect caused by a low dose of radiation. The existence of hormesis (a theoretical phenomenon in which exposure to a low dose of a chemical agent or environmental factor has beneficial effects that at higher concentrations is harmful) is the major topic for debate in the radiation protection and radiation biology circles. Adaptive responses have been experimentally validated for *in vitro* tissue cultures (Azzam et al., 1996; Redpath et al., 2001; Calabrese, 2003; Redpath et al., 2003; Redpath, 2007).

With regard to adaptive responses in humans, small priming doses of ionizing radiation have been shown to lessen the damaging effects of a subsequent high dose of radiation. This priming dose effect has been seen for a variety of endpoints including apoptosis, micronuclei, and mutations (Wolff, 1998). The number of chromatin aberrations following a large dose of radiation was reported to be reduced if the exposed cells were first irradiated with a much smaller dose of X-rays (Olivieri et al., 1984). The mitigating effect of the priming dose was found to become fully active after about 5 hours. While there is evidence that a low dose of radiation lessens the effect of subsequent exposures to high radiation dose, there are no reported studies showing that a low dose of radiation is protective relative to the zero exposure case.

The main research goal of the DOE low dose research program was to further our understanding of the mechanisms of molecular and cellular responses to radiation and improve the scientific underpinning for estimating risks from these exposures. Under this program significant advances were made in our understanding of reactive oxygen species, the role of DNA repair and the immune system, the nature of the bystander effect, adaptive responses and genomic instability. Furthermore, many new scientific techniques and tools were developed under this program such as flow cytometry, gene chips genomics, proteomics, metabolomics and microbeams. In the excellent summary written by Antone Brooks (Brooks, 2015), one can read the techniques and advances made under the US DOE Low Dose Radiation Research Program. Also, all of the

journal articles and publications filed under the Low dose program are available online (DOE, 2012).

IV. Information from epidemiology studies

The Life Span Study (LSS) of Japanese A-bomb survivors shows a statistically significant increase in risk for doses above about 0.2 Gy. Conducting epidemiology studies at lower doses poses serious challenges with respect to statistical power and possible sources of bias. Nevertheless, there is now a substantial body of epidemiological data obtained at low doses and dose rates suggesting that the cancer risks extrapolated from the LSS data do not greatly overestimate risks for exposures commonly received diagnostically, occupationally, or environmentally. The strongest evidence is for radiogenic leukemia and, to a lesser extent, breast cancer.

Cohort studies of female patients receiving repeated diagnostic X-rays to monitor medical treatment for tuberculosis and for scoliosis found elevated incidences of breast cancer positively correlated with accumulated radiation doses to the breast (Boice et al., 1988; Howe & McLaughlin, 1996; Doody et al., 2000). In particular, it was found that the dose-response for breast cancer in the TB patients was similar to that expected based on the LSS data although the average dose from each exam was only about 8 mGy corresponding to no more than a few electron tracks per cell nucleus. The exams were conducted weeks apart, a time period believed to be adequate for completion of DNA repair processes.

More recently, a large study of UK residents undergoing CT scans as children has shown apparent increases in leukemia with accumulated estimated dose to the bone marrow that are in close agreement with the central estimate of slope determined from the LSS (Pearce et al., 2012). The increase in risk was statistically significant for doses above about 50 mGy. Likewise an increase in brain cancers was associated with radiation doses to the brain, an increase somewhat higher than projected from the LSS data. Two issues with the CT study are: (1) lack of detailed individual dose estimates for the patients and (2) possible confounding factors relating to selection biases, where patients receiving more CT scans might either already have cancer or are predisposed to getting it.

Numerous studies of occupational cohorts exposed to chronic radiation exposures have been published. Recent pooled analyses of data on nuclear workers from the US, UK, and France indicate statistically significant dose-dependent increases in leukemia and solid cancers, in reasonable agreement with LSS predictions (Leuraud et al., 2015; Richardson et al., 2015). Statistically significant increases could only be observed at doses above about 0.3 Gy; nevertheless, because the exposures were at a low dose rate the studies lend support to the hypothesis that risks from very low doses can be summed to produce a significant cancer risk, as predicted from the LNT model. A major issue with occupational studies is possible confounding by other occupational exposures or lifestyle factors, including smoking. However, smoking is not

expected to be a major confounder for leukemia incidence. Moreover, the increased risk of solid cancers remained even after removing data on lung and pleural cancers (Richardson et al., 2015).

One important study of chronic radiation exposure is that on a cohort of people who lived along the radioactively contaminated Techa River, downstream from the Mayak Plant in the eastern Urals. A massive effort, sponsored by the U.S. DOE, has been undertaken to estimate the radiation doses received by individuals in the population and to correlate those dose estimates with the incidence of cancer. Results from preliminary analyses again show statistically significant increases in leukemia (Krestinina et al., 2009) and solid cancers (Davis et al., 2015), consistent with extrapolations from the LSS based on the LNT model. The data on solid cancers is somewhat less convincing than on leukemia, particularly when considering individual types of cancer.

A number of studies have also been carried out on exposures to natural background radiation (NBR). Most of these have shown no dose-dependent differences in cancer incidence or mortality (reviewed in Hendry et al. 2009). A carefully designed cohort study in Kerala, India, also did not show an excess cancer risk from terrestrial gamma radiation (Nair et al., 2009). However, many NBR studies were "ecological studies": i.e., they simply compared cancer rates in populations living in different NBR environments (Tao et al., 2000). Even if the doses and population sizes provide adequate statistical power, such studies are often plagued with potential biases and confounding factors that are difficult or impossible to correct during analysis. Thus, such ecological studies tend to be ambiguous.

Results from a recent study in Great Britain based on registry data indicate a positive correlation between childhood leukemia incidence and NBR (Kendall et al., 2013). Although the range of doses was rather narrow, adequate statistical power was achieved by employing a very large database. It remains unclear, however, as to whether the small signal observed might be due to an unaccounted-for bias. A cohort study of a population living in a high NBR area in Kerala, India, is also underway, which may yield a useful test of LNT (Boice et al., 2010).

Overall, the evidence for risk at low doses and dose rates is most compelling for leukemia. That evidence includes data on: cohorts of nuclear workers, CT patients, members of the Techa River Cohort, residents of Taiwanese buildings constructed with radioactively contaminated steel (Hwang et al., 2008), as well as children exposed to various levels of NBR in Great Britain and in Switzerland (Boice et al., 2010; Spycher et al., 2015). A 2011 meta-analysis of studies on chronic radiation exposures (some data from which were also incorporated into the analysis of health outcomes of nuclear workers in the US, UK and France discussed above; Leuraud et al., 2015) found that 21 of 23 studies yielded a positive correlation between radiation dose and leukemia risk (Daniels et al., 2011). The estimated dose-response derived from the meta-analysis was statistically significant and in reasonable agreement with that derived from the LSS.

The similarity between risk estimates derived from the LSS and from the TB patients receiving highly fractionated doses tends to support the LNT model in the case of breast cancer as well. Results from the Taiwanese building study also suggest a risk of breast cancer from chronic exposures, but the results are of borderline statistical significance (Hwang et al., 2008).

Overall, the results from epidemiological studies are generally supportive of the LNT model, but the evidence cannot yet be regarded as definitive because of inherent limitations in such studies. In general, to be definitive, either the observed relative risk in epidemiological studies has to be very high, as in the case of lung cancer caused by cigarette smoke or mesothelioma caused by inhaled asbestos fibers, or the findings must be in concordance with a mechanistic understanding of the carcinogenesis process. Given the low relative risks involved in low-dose studies and current gaps in our understanding of mechanisms of cancer development, further epidemiological and radiobiological data are required before the weight of evidence can be regarded as conclusive one way or the other.

V. Ideal epidemiological study and cohort

As described in section IV the limitations of many of the existing studies of low-dose radiation exposure and subsequent cancer risk are that they are underpowered, or that confounding or other biases cannot be ruled out. To increase statistical power while minimizing the threat of biases concurrently is best achieved in the situation where there is a high relative risk. The highest relative risks observed to date have been for the risk of leukemia following childhood radiation exposure (Kendall et al., 2013). The excess relative risk (calculated as the rate of disease in an exposed population divided by the rate of disease in an unexposed population minus 1.0; BEIR VII, 2006) per Gy of accumulated exposure to infants is increased 50-fold compared to the background occurrence at age 20 yr. There are few other established causes of childhood leukemia and few known confounders, although that does not rule out the possibility of unknown confounders. They would have to be correlated with radiation dose, however, as well as leukemia. Inaccurate dose estimates also reduce the statistical power of a study. Random error tends to bias the risk estimates towards the null. Finally, low participation or follow-up rates with the cohort can introduce bias. Record-linkage studies that are not dependent on personal contact with the cohort members eliminate participation rate bias, but incomplete passive follow-up could potentially still introduce bias. Therefore, settings with complete cancer registries and vital status data are required. A retrospective study would provide a much more timely result than a prospective study where it might take decades to accrue sufficient cases.

Considering the problems and limitations encountered in previous work, the ideal epidemiological study would be a record-linkage study of childhood leukemia following low-dose radiation exposure in a setting where doses can be measured accurately. A sufficient sample size to achieve adequate statistical power would be needed, ideally in a setting with reliable cancer registrations going back several decades to enable a retrospective study design.

To achieve the criteria listed above, Little et al. (2010) developed methods to estimate the required sample size for a cohort or case-control study of childhood leukemia and natural background radiation. A case-control study with five matched controls per case had similar power to a cohort study. Fewer study subjects in total is an advantage in terms of cost if there is individual subject dosimetry. For their calculations, Little et al. (2010) used the UK childhood leukemia rates and assessed how many years of follow-up would be required to achieve 80% power for a 1-sided 5% significance test. With 5 controls per case and background radiation dose distributions typical of the UK, they estimated that 7800 cases would be required which would be achieved with 17 years follow-up. In a retrospective case-control study this means that you need at least 17 years of retrospective data.

The UK study that was developed on the basis of these calculations included 9000 childhood leukemia cases and 12,000 controls selected from the same birth register as the case (Kendall et al., 2013). As reported in section VI, they found a significant dose-response relationship for leukemia and background gamma radiation. Dose estimates were based on the mother's residence and birth mapped to national survey maps. Socio-economic status was the major known potential confounder (with more affluent families having a higher incidence) and adjustment was made with the Carstairs deprivation index from the mother's residence at birth. Adjustment did not materially alter the risk estimates, however. The main limitations of the study were the dose estimates from exposures for medical reasons, which were not fully individualized and also were only at one point in time. These dose errors would most likely reduce the power and bias results towards the null. Correction therefore may increase the risk estimate and the statistical significance. As the study was entirely records based there is no response bias. The UK national cancer registry captures about 90% of cancer diagnoses (http://www.ncin.org.uk/collecting_and_using_data/national_cancer_data_repository/).

The study is now being expanded, further back in time, to include more cases and dosimetry is being refined (Chernyavskiy et al.,2016). Kendall et al. (2013) concluded that their results support models of predicted risk at low doses of radiation that are extrapolations from moderate and high dose/dose rate observations.

Ideally we should have a replication study in another country using, to the extent possible, a comparable design. However, data from the U.S. are not available as there is no national cancer registry.

VI. Laboratory studies of low dose/dose rate radiation

A. Cellular environment

It is well recognized that human data should be the chief source for risk assessment of radiation exposure on humans, with studies from model systems providing supporting evidence. However, experiments to determine radiation effects on humans are unethical. Especially for low levels of radiation exposure, there are only limited human data. Therefore, it is necessary to use molecular, cellular and animal data in facilitating low dose radiation (LDR) risk assessment. An

appropriate experimental model system under physiologically relevant conditions is of great importance for the investigation of the effects of LDR.

Biological tissues consist of approximately 75% water by weight. As a result, a major fraction of ionizing radiation (IR) exposure induces hydrolysis resulting in different types of reactive oxygen species (ROS) (Hall & Giaccia, 2006). IR induces the production of ROS proportional to its dose. High-dose IR induces an excess amount of ROS that can overwhelm the cellular antioxidant capacity causing oxidative stress and damage to nucleic acids, proteins, and lipids (Hall & Giaccia, 2006). When mildly increased, ROS however, can function as second messengers transducing signals that alter cellular physiology (Finkel, 2012; Bissell & Radisky, 2001). Evidence indicates that ROS-mediated signaling plays a critical role in facilitating cellular stress adaptation in a context dependent manner (Bissell & Radisky, 2001). Considering the fact that the principle mode of IR action is through induction of ROS (Hall & Giaccia, 2006), it is not surprising that the effects of LDR, mediated by a moderate level of ROS, can be profoundly affected by oxygen concentration. Consistent with this notion is our finding that maintaining cell cultures at low O_2, 5%, is necessary to detect many LDR-induced responses (Lall et al., 2014).

Cells exist in a tightly regulated microenvironment where homeostatic processes dictate whether a given cell remains quiescent, proliferates, differentiates, or undergoes apoptosis (Bissell & Radisky, 2001). Like biological behaviors, cellular responsiveness to LDR can also be affected by the tissue microenvironment. Perturbation of the tissue microenvironment could result in deregulated cell/cell and cell/extracellular matrix (ECM) interactions, which may lead to disruption of tissue homeostasis affecting cellular response to LDR. Being reproducible and easy to manipulate, monotypic cell cultures have been widely used in study of radiation-induced cellular responses, which however lack the *in vivo* tissue context. To bridge the gap, a 3D cell coculture system has been developed to mimic the physiologic microenvironment for investigating cell-cell and cell-ECM interactions *in vitro* (Schmeichel & Bissell, 2003). This system employs either primary or immortalized human cells and natural or synthetic hydrogels (e.g., collagen I, reconstituted basement membranes, or carboxymethylcellulose) to examine cell behavior in the context of an organized tissue structure in vitro. The 3D coculture system allows recapitulation of cell-adhesion dependent tissue architectures and interactions of cells with their neighbors in a tissue-like surrounding. Cells within multicellular spheroids exhibit reduced rates of metabolism and oxygen consumption, lower levels of mitochondrial respiration, and require smaller amounts of glucose to sustain viability (Freyer, 1998). Such an effect is mediated through modulation of energy metabolism via altered expression of genes. Indeed, it was reported that the expression of genes in cells involved in the stimulation of cell growth and division are markedly higher in 2D than in 3D (Birgersdotter et al., 2005). Interestingly, cells in 3D spheroids rapidly acquire resistance to IR (Sminia et al., 2003). However, when fibroblasts, the primary stromal cell type that produces the ECM, were included, cocultured epithelial cells became considerably sensitized to IR treatment (Tsai et al., 2005). Thus the use of 3D coculture

can aid in studies of LDR-induced cellular response in the context of tissues by taking into consideration the interactions of different cell types with their neighbors and their surroundings.

B. Bioindicators of ionizing radiation

Ample data suggest that when treated with the same total dose, low dose/dose rate radiations are much less effective in inducing changes than high dose rates. Multiple readouts, such as DNA damage and repair, gene expression, cell cycle progression, metabolic response, epigenetic modification, have been used to assess the radiation dose-rate effects. Some of the data support a considerable dose-rate effect. In a study by Ishizaki et al. (2004), the number of γH2AX foci/cell (a marker for double-strand breaks) was used to assess radiation-induced damage and repair. A complete dose response was observed with both high and low dose rate radiation treatment. Exposure to high dose rates induced a linear increase in γH2AX over a range of doses. Remarkably, when the same dose was delivered at low dose rates, there was little increase in the number of γH2AX above the background level. The observed difference was evident up to an accumulated dose of 5Gy, revealing a marked dose-rate effect. In a recent study by Cao et al (2014), human fibroblasts were exposed to chronic γ-irradiation and monitored with an automated fluorescence microscope. With the same accumulated dose of 5Gy, only the dose-rate at 0.694mGy/min but not 0, 0.069, or 0.347 mGy/min, induced 53BP1-foci, a surrogate marker of DNA damage, revealing a threshold dose-rate of γ-irradiation around 0.694 mGy/min necessary to induce accumulation of DNA damage in cultured human fibroblasts.

A number of biomarkers have been used in assessment of the effects of low dose of radiation exposure. Among them, DNA damage markers, such as γH2AX and 53BP1, gene expression, cell cycle checkpoints, metabolic response, epigenetic modification, have been used to measure the responses to low doses of radiation exposure. We recently showed that cellular metabolism is exquisitely sensitive to the treatment of low doses of radiation (Lall et al., 2014), which revealed a cluster of highly sensitive metabolic biomarkers. Consistent with a close interaction between cellular metabolism and epigenetic modification, a group of epigenetic markers were found to be equally sensitive in assessing the effects of low doses of radiation exposure.

C. Future research

Most information on low dose rate effects is derived from either animal or cellular studies and human data are very limited. Animal model results cannot be conclusive because it is not possible to have certainty in the correctness of extrapolation to humans. Further investigations are necessary to determine the confidence with which data generated from animal and cellular studies may be applied to assessing health risk of low dose/rate radiation exposure in humans.

VII. Technology for research on low dose radiation health effects

A. Impact of technology development

Historical accounts underscore the importance of the development of new technologies in the advancement of knowledge about the effects of low dose radiation. A good example is the microbeam that provided the ability to deliver a controlled radiation dose to a single cell within

the context of neighboring cells. From these studies, the response of neighboring cells or "bystanders" was described causing a paradigm shift in understanding that radiation effects were detected in non-radiated cells adjacent to the target cells and in cells not even directly adjacent (Wilson et al., 2001; Schettino et al., 2003). Coupling fluorescently labeled radiation-induced protein markers with the microbeam radiation allowed real-time spatiotemporal documentation of responses following radiation doses as low as 0.05 Gy (Blakely et al., 2006). Signal was detectable within 10 min and remained unresolved up to three hours.

Tools for the determination of sites of DNA damage became quite rigorous with the availability of the human genome sequence, fluorescent *in situ* hybridization and flow cytometry (Cornforth, 2006). The types of damage and kinetics of their repair could be studied as a function of dose and dose rate.

Over the last decade a variety of powerful biological (e.g., genomics), analytical (proteomic, metabolomics, transcriptomics) (Vasdekis & Stephanopoulos, 2015; Xue et al., 2015), imaging (Sydor et al., 2015; Hell, 2007) and bioinformatics tools (Altrock et al., 2015) have improved or have been developed, enhancing the potential approaches that could extend the range of possible studies in low dose radiation health effects. The challenge is to adapt and integrate these tools to produce studies of consequence that further illuminate low dose radiation health effects.

There are three potential components that are required to obtain predictive data from these studies. First, and critical, is to have a realistic model biological system – most desirably associated with human responses to radiation. However, lacking that, alternative three-dimensional tissue and animal models with the inevitable caveats of these systems have been developed. Second, the tools to make the appropriate measurements of key biological processes are required, and finally, the application of bioinformatics tools to analyze data and illuminate relationships.

B. Model system

The challenge is to perform ethical studies if we want to try to use human beings – which often is not possible. The alternative is to identify or create a model system that mimics wholly or partially the dynamics of human biology. The differences in key biological systems and pathways between the human and animal models are problematic.

What has emerged in recent years are more credible three dimensional tissue models such as organoids that use human stem cells (Fatehullah et al., 2016; Sokol et al., 2016). The results are attractive since they can mimic at least some human biology characteristics, although effects of the immune system are still lacking in current 3D models. In addition, the application of technologies such as CRISPR cas9 can introduce genetic changes that make it possible to compare biological differences between model systems with great precision (Gong et al., 2005; Jinek et al. 2012; Overballe-Petersen et al., 2013).

Also possible is the creation of models that might provide insights into issues such as the sources of radiation hypersensitivity in populations. The Collaborative Cross (Churchill et al., 2004; Threadgill et al., 2002, Mao et al., 2015; Gralinski et al., 2015) is based on a cohort of mouse strains with widely divergent genetic compositions with a population structure that randomizes existing genetic variation. This variation provides the power needed to assign causality and thus to understand complex traits and diseases with multifaceted etiologies. With CRISPR Cas9 and gene drive techniques, targeted changes could be introduced in models systems to test hypotheses of causality. These approaches may be useful in pursuing genetic pathways to radiation-induced cancers.

C. Genes, proteins, metabolites, and imaging

Since the first sequencing of the human genome, the increase in speed and accuracy of sequencing enabled genomic analysis on a scale heretofore not contemplated. Moreover, the very recent introduction of single cell analysis/sequencing, highly accurate proteomics, metabolomics technology, and CRISPR cas9, gene drive, have made possible a systems biology approach than can observe changes in pathways, and components, such as molecular machines, that could reveal critical changes important to the development of cancer. For example, attempting to track the evolutionary path of cells precisely to identify those forming a cancer can now be considered through the identification of unique changes serving as barcodes by a set of genomic cassettes introduced with the CRISPR cas9 technology (Dominguez et al., 2016, Kupferschmidt, 2016; Perli et al., 2016). There are early indications that this approach could produce the equivalent of a phylogenic tree tracing the lineage and evolution of particular cells. The ability to examine single cells within a tissue complex could reveal insights into the steps in the transformation of normal cells to cancer or other aberrations. With cell-level imaging techniques currently available and with expected improvements in the future, it should be possible to examine the dynamics of system biology revealing critical interactions among biological components in time and space.

The maturation of the “omics” technologies that move the research field from studies of one gene or protein at a time to genome wide queries has been a revolution in the examination of complex biological systems. Gene expression changes in response to dose and dose rate revealed apparent differences in gene expression changes in cells challenged at low versus high doses (Amundson & Fornace, 2003). Even with these fine-tuned data, conflicting results have been reported that transcriptional responses to different ionizing radiation doses were similar (McDonald et al., 2014). *In vitro* radiation of peripheral blood mononuclear cells at 0.1 versus 1.0 Gy at a dose rate of 0.26 Gy/min showed that p53 regulated genes were spliced differentially at the two dose levels (Macaeva et al., 2016). In addition, the use of qRT-PCR on the exon level could distinguish the radiation dose, an approach that holds promise for radiation biodosimetry (Macaeva et al., 2016).

The advances in data analyses both in terms of techniques and scale of data processing has introduced an ability to probe and tease apart previously unobservable biological changes and

processes. Newer techniques allow the integration of data from multiple sources e.g. proteomics, genomics, metabolomics, in a coherent manner (Platig et al., 2016). Subtle changes in groups of associated genes in single or multiple pathways can be observed with the emerging analytical and computational capabilities. In some instances it may be possible to see significant changes in biological networks that could not be identified by simply looking at changes in single genes with approaches such as Genome –Wide Association Studies (GWAS) or expression quantitative trait loci studies (eQTL) or protein-protein interaction studies (PPI) (Glass et al., 2015; 2014).

Finally, the scale of accessible human medical data is also changing, with the potential of examining the biology, or at least, the genetics of human disease at an unprecedented level of detail and differentiation into categories that have statistical significance. Initiatives around the world are emerging, such as the U.S.'s Precision Medicine Initiative program, which aims to enlist a million volunteers to provide their medical data. In Canada, the University of British Columbia has a Personalized Medicine Initiative with similar goals that has already spawned ten companies creating their own databases. The challenge will be designing meaningful studies that identify questions that can be answered with the available data, securing and particularly validating the data and, potentially most demanding, finding a mechanism where the data can be shared domestically and internationally. For the low dose radiation health research finding a way to utilize this potential effectively would be very valuable.

Currently the elements needed to enhance our ability to explore the difficult biology of low dose radiation and human health effects are becoming available. The combination of emerging model systems, analytical tools and computation/bioinformatics power are now in place to move research forward.

VIII. References

Altrock PM, LL Liu, F Michor. 2015. The mathematics of cancer: integrating quantitative models. Nat Rev Cancer **15**:730-745.

Amundson SA, AJ Fornace. 2003. Monitoring human radiation exposure by gene expression profiling: Possibilities and pitfalls. Health Phys **85**(1): 36-42.

Azzam EI, SM de Toledo, GP Raaphorst, REG Mitchel. 1996. Low-dose ionizing radiation decreases the frequency of neoplastic transformation to a level below the spontaneous rate in C3H 10T1/2 cells. Radiat Res. **146**:369–373.

BEIR VII Phase 2. 2006. Health risks from exposure to low levels of ionizing radiation. The National Research Council, Washington, DC: The National Academies Press. ISBN 0-309-09156-X.

Birgersdotter A, R Sandberg, I Ernberg. 2005. Gene expression perturbation in vitro—a growing case for three-dimensional (3D) culture systems. Semin Cancer Biol **15**:405-12.

Bissell MJ, D Radisky. 2001. Putting tumors in context. Nat Rev Cancer **1**:46-54.

Blakely EA, AC Thompson, RI Schwarz, P Chang, K Bjornstad, C Rosen, C Wisnewski, D Mocherla, B Parvin. 2006. Radiation-induced bystander effects with a 12.5 keV X-ray microbeam. Radiat Res **166**(4): 684-684.

Blyth BJ, PJ Sykes. 2011. Radiation-induced bystander effects: what are they, and how relevant are they to human radiation exposures? Radiat Res **176**:139–157.

Boice JD, Jr., D Preston, FG Davis, RR Monson. 1988. Frequent chest X-ray fluoroscopy and breast cancer incidence among tuberculosis patients in Massachusetts. Radiat Res **125:** 214-222.

Boice, JD, Jr., JH Hendry, N Nakamura, O Niwa, S Nakamura, K Yoshida. 2010. Low-dose-rate epidemiology of high background radiation areas. Radiat Res **173**: 849-854.

Brenner DJ, JB Little, RK Sachs. 2001. The bystander effect in radiation oncogenesis: II. A quantitative model. Radiat Res **155**(3):402-408.

Brooks AL. 2015. A commentary on: "A history of the United States Department of Energy (DOE) Low Dose Radiation Research Program: 1998-2008". Radiat Res **183**(4):375-381.

Calabrese EJ. 2003. The maturing of hormesis as a credible dose-response model. Nonlinearity Biol Toxicol Med. **1**(3):319-43

Cao L, H Kawai, M Sasatani, D Iizuka, Y Masuda, T Inaba, K Suzuki, A Ootsuyama, T Umata, K Kamiya, F Suzuki. 2014. A novel ATM/TP53/p21-mediated checkpoint only activated by chronic γ-irradiation. PLoS One. **9**(8):e104279.

Cheng KC, LA Loeb. 1993. Genomic instability and tumor progression: mechanistic considerations. Adv Cancer Res. **60**:121-156.

Chernyavskiy P, GM Kendall, R Wakeford, MP Little. 2016. Spatial prediction of naturally occurring gamma radiation in Great Britain. J Environ Radioact 164:300-311. doi: 10.1016/j.jenvrad.2016.07.029.

Churchill GA, DC Airey, H Allayee, JM Angel, AD Attie, et al. 2004. The Collaborative Cross, a community resource for the genetic analysis of complex traits. Nat Genet **36**:1133 – 1137. doi:10.1038/ng1104-1133.

Cornforth MN. 2006. Perspectives on the formation of radiation-induced exchange aberrations. DNA Repair **5**(9-10): 1182-1191.

Daniels RD, MK Schubauer-Berigan. 2011. A meta-analysis of leukaemia risk from protracted exposure to low-dose gamma radiation. Occup Environ Med **68**: 457-464.

Davis FG, KL Yu, D Preston, S Epifanova, M Degteva, AV Akleyev. 2015. Solid cancer incidence in the Techa River incidence cohort: 1956–2007. Radiat Res **184**: 56-65.

DOE. 2012. Office of Science, Low Dose Radiation Research Program, Program Publications. [archived website], Published online: http://science.energy.gov/ber/research/bssd/low-dose-radiation . Accessed on 9-28-2016

Dominguez AA, WA Lim, LS Qi. 2016. Beyond editing: repurposing CRISPR–Cas9 for precision genome regulation and interrogation. Nat Rev Mol Cell Bio **17**(1):5–15

Doody MM, JE Lonstein, M Stovall, DG Hacker, N Luckyanov, CE Land. 2000. Breast cancer mortality after diagnostic radiography: Findings from the U.S. Scoliosis Cohort Study. Spine **25**: 2052-63.

Fatehullah A, SH Tan, N Barker. 2016. Organoids as an *in vitro* model of human development and disease. Nat Cell Bio **18**(3):246-254. doi:10.1038/ncb3312

Finkel T. 2012. Signal transduction by mitochondrial oxidants. J. Biol. Chem. **287**:4434-4440.

Freyer JP. 1998. Decreased mitochondrial function in quiescent cells isolated from multicellular tumor spheroids. J Cell Physiol **176**:138-49.

Glass K, J Quackenbush , EK Silverman, B Celli, SI Rennard , GC Yuan, DL DeMeo. 2014. Sexually-dimorphic targeting of functionally-related genes in COPD. MC Syst Biol. **8**:118. doi: 10.1186/s12918-014-0118-y.

Glass K, J Quackenbush, D Spentzos, B Haibe-Kains, GC Yuan. 2015. A network model for angiogenesis in ovarian cancer. BMC Bioinform.16:115-DOI 10.1186/s12859-015-0551-y

Gong C, P Bongiorno, A Martins, NC Stephanou, H Zhu, S Shuman, MS Glickman. 2005. Mechanism of nonhomologous end-joining in mycobacteria: a low-fidelity repair system driven by Ku, ligase D and ligase C. Nat Struc Mol Bio **12**:304 - 312 doi:10.1038/nsmb915.

Gralinski LE, MT Ferris, DL Aylor, AC Whitmore, R Green, MB Frieman, D Deming, VD Menachery1, DR Miller, RJ Buus,TA Bell, GA Churchill, DW Threadgill, MG Katze, L McMillan. 2015. Genome wide identification of SARS-CoV susceptibility loci using the collaborative cross. PLOS Gen | DOI:10.1371/journal.pgen.1005504

Hall E, AJ Giaccia. 2006. *Radiobiology for the Radiobiologist*. Lippincott, Williams & Wilkins.

Hell SW. 2007. Far -field optical nanoscopy. Science **316** (5828), 1153-1158.

Hendry JH, SL Simon, A Wojcik, M Sohrabi, W Bufkart, E Cardis, D Laurier, M Tirmarche, I Hayata. 2009. Human exposure to high natural background radiation: what can it teach us about radiation risks? J Radiol Prot **29**(0):A29-A42.

Howe GR, J McLaughlin. 1996. Breast cancer mortality between 1950 and 1987 after exposure to fractionated moderate-dose-rate ionizing radiation in the Canadian fluoroscopy cohort study. Radiat Res **145**: 694-707.

Hwang SL, JS Hwang, YT Yang, WA Hsieh, TC Chang, et al. 2008. Estimates of relative risks for cancers in a population after prolonged low-dose rate radiation exposure: A follow-up assessment from 1983 to 2005. Radiat Res **170**: 143-8.

Ishizaki K, Y Hayashi, H Nakamura, Y Yasui, K Komatsu, A Tachibana. 2004. No indication of p53 phosphorylation and few focus formation of phosphorylated H2AX suggest efficient repair of DNA damage during chronic low-dose-rate irradiation in human cells. J Radiat Res (Tokyo) **45**:521–525.

Iyer R, BE Lehnert. 2000. Factors underlying the cell growth-related bystander responses to alpha particles. Cancer Res **60**:1290–1298.

Jinek M, K Chylinski, I Fonfara, M Hauer, JA Doudna, E Charpentier. 2012. A programmable dual-RNA–guided DNA endonuclease in adaptive bacterial immunity. Science **337**(6096):816-821.

Kendall GM, MP Little, R Wakeford, KJ Bunch, JCH Miles et al. 2013. A record-based case–control study of natural background radiation and the incidence of childhood leukaemia and other cancers in Great Britain during 1980–2006. Leukemia **27:** 3–9.

Krestinina L, DL Preston, FG Davis, S Epifanova, E Ostroumova et al. 2009. Leukemia incidence among people exposed to chronic radiation from the contaminated Techa River, 1953–2005. Radiat Environ Biophys. **49**: 195–201. DOI 10.1007/s00411-009-0257-5.

Kupferschmidt K. 2016. CRISPR views of embryos and cells. Science **352(**6290):1156-1158

Lall R, S Ganapathy, M Yang, S Xiao, T Xu, H Su, M Shadfan, JM Asara, I Ben-Sahra, BD Manning, JB Little, Z-M Yuan. 2014. Low-dose radiation exposure induces a HIF-1-mediated adaptive and protective metabolic response. Cell Death Differ **4**:1–9.

Lehnert BE, EH Goodwin. 1997. Extracellular factor(s) following exposure to a particles can cause sister chromatid exchanges in normal human cells. Cancer Res **57**:2164-2171.

Leuraud K, DB Richardson, E Cardis, RD Daniels, M Gillies et al. 2015. Ionising radiation and risk of death from leukaemia and lymphoma in radiation-monitored workers (INWORKS): an international cohort study. www.thelancet.com/haematology Published online: http://dx.doi.org/10.1016/S2352-3026(15)00094-0

Little MP, R Wakeford, JH Lubin, GM Kendall. 2010. The statistical power of epidemiological studies analyzing the relationship between exposure to ionizing radiation and cancer, with special reference to childhood leukemia and natural background radiation. Radiat Res. **174**(3):387-402. doi: 10.1667/RR2110.1.

Macaeva, E, Y Saeys, K Tabary, A Janssen, A Michaux, et al. 2016. Radiation-induced alternative transcription and splicing events and their applicability to practical biodosimetry. Sci Rep **6**:19251. doi:10.1038/srep19251.

Mao J-H, SA Langley , Y Huang , M Hang , KE Bouchard, SE Celniker , JB Brown, JK Jansson, GH Karpen, AM Snijders. 2015. Identification of genetic factors that modify motor performance and body weight using Collaborative Cross mice Sci Rep **5**: Article number: 16247 doi:10.1038/srep16247.

McDonald, JT, C. Briggs, H Szalag, M Peluso, D Schneider, A Perepletchikov, GL Klement, I Tuerk, L Hlatky. 2014. Chronic low dose-rate radiation down-regulates transcription related to mitosis and chromosomal movement similar to acute high dose in prostate cells. Int J Radiat Biol **90**(3):231-240.

Morgan WF. 2011. Radiation-induced genomic instability. Health Phys **100**(3): 280-281.

Morgan WF, JP Day, MI Kaplan, EM McGhee, CL Limoli. 1996. Genomic instability induced by ionizing radiation. Radiat Res **146**(3): 247-258.

Nagasawa H, JB Little. 1992. Induction of sister chromatid exchanges by extremely low doses of alpha-particles. Cancer Res **52** (22): 6394–6396.

Nair RRK, B Rajan, S Akiba, P Jayalekshmi, MK Nair, P Gangadharan, T Koga, H Morishima,S Nakamura, T. Sugahara. 2009. Background radiation and cancer incidence in Kerala, India-Karanagappally cohort study. Health Phys 96(1):55-66.

Olivieri G, J Bodycote, S Wolff. 1984. Adaptive response of human-lymphocytes to low concentrations of radioactive thymidine. Science **223**(4636): 594-597.

Overballe-Petersen S, K Harms, LAA Orlando, JVMoreno Mayar, S Rasmussen, TW Dahl, MT Rosing, AM Poole, T Sicheritz-Ponten, S Brunak, S Inselmann, J de Vries, W Wackernagel, OG Pybus, R Nielsen, PJ Johnsen, KM Nielsen, E Willerslev. 2013. Bacterial natural transformation by highly fragmented and damaged DNA. Proc Natl Acad Sci USA **110** (49) 19860-19865 doi:10.1073/pnas.1315278110

Pearce MS, JA Salotti, MP Little, K McHugh, C Lee et al. 2012. Radiation exposure from CT scans in childhood and subsequent risk of leukaemia and brain tumours: a retrospective cohort study. Lancet. **380**(9840):499-505. doi: 10.1016/S0140-6736(12)60815-0.

Perli SD, CH Cui, TK Lu. 2016. Continuous genetic recording with self-targeting CRISPR-Cas in human cells. Science **353**:(6304), 1115.

Platig J, PJ. Castaldi, D DeMeo, J Quackenbush. 2016. Bipartite community structure of eQTLs, published online, 12 Sep, PLOS Comp Bio http://dx.doi.org/10.1371/journal.pcbi.1005033

Redpath JL. 2007. In vitro radiation-induced neoplastic transformation:suppressive effects at low doses. Radiat Res. **167**:345–346.

Redpath JL, D Liang, TH Taylor, C Christie, E Elmore. 2001. The shape of the dose-response curve for radiation-induced neoplastic transformation *in vitro*: evidence for an adaptive response against neoplastic transformation at low doses of low-LET radiation. Radiat Res.**156**:700–707.

Redpath JL, Q Lu, X Lao, S Molloi, E Elmore. 2003. Low doses of diagnostic energy X-rays protect against neoplastic transformation *in vitro*. Int J Radiat Biol. **79**:235–240.

Richardson DB, E Cardis, RD Daniels, M Gillies, JA O'Hagan et al. 2015. Risk of cancer from occupational exposure to ionizing radiation: retrospective cohort study of workers in France, the United Kingdom, and the United States (INWORKS). BMJ 2015 **351**:h5359. Correction BMJ 2015 **351**:h6634

Schettino G, M Folkard, KM Prise, B Vojnovic, KD Held, BD Michael. 2003. Low-dose studies of bystander cell killing with targeted soft X rays. Radiat Res **160**(5): 505-511.

Schmeichel KL, MJ Bissell. 2003. Modeling tissue-specific signaling and organ function in three dimensions. J Cell Sci **116**:2377-88.

Sedelnikova OA, Nakamura A, Kovalchuk O, et al. 2007. DNA double-strand breaks form in bystander cells after microbeam irradiation of three-dimensional human tissue models. Cancer Res **67** (9):4295–4302

Sminia P, H Acker, HP Eikesdal, P Kaaijk, Pø Enger, B Slotman, R Bjerkvig. 2003. Oxygenation and response to irradiation of organotypic multicellular spheroids of human glioma. Anticancer Res **23**:1461-6.

Sokol ES, DH Miller, A Breggia, KC Spencer, LM Arendt, PB. 2016. Growth of human breast tissues from patient cells in 3D hydrogel scaffolds. Breast Can Res **18**:19 DOI: 10.1186/s13058-016-0677-5

Spycher BD, JE Lupatsch, M Zwahlen, M Röösli, F Niggli et al. 2015. Background ionizing radiation and the risk of childhood cancer: A census-based nationwide cohort study. Environ Health Perspect **123:** 622-8.

Stoler DL, GR Anderson, C A Russo, AM Spina and TA Beerman. 1992. Anoxia-induced endonuclease activity as a potential basis for the genomic instability of cancer cells. Cancer Res **52**:4372-4378.

Sydor AM, KJ Czymmek, EM Puchner, V Mennella. 2015. Super-resolution microscopy: from single molecules to supramolecular assemblies. Trends Cell Biol **25**(12): 730-48 http://dx.doi.org/10.1016/j.tcb.2015.10.004

Tao ZF, YG Zha, S Akiba, QF Sun, JM Zou, J Li, YS Liu, H Kato, TT Sugahara, L Wei. 2000. Cancer mortality in the high background radiation areas of Yangjiang, China during the period between 1979 and 1995. J Radiat Res **41**:Suppl. 31-41.

Threadgill, D W, KW Hunter, RW Williams. 2002. Genetic dissection of complex and quantitative traits: from fantasy to reality via a community effort. Mamm Genome. **13**:175-8.

Tsai KK, EY Chuang, JB Little, ZM Yuan. 2005. Cellular mechanisms for low-dose ionizing radiation-induced perturbation of the breast tissue microenvironment. Cancer Res. **65**(15):6734-44

Vasdekis AE, G Stephanopoulos. 2015. Review of methods to probe single cell metabolism and bioenergetics. Metabolic Eng **27:**115–135

Wilson WE, DJ Lynch, K Wei, LA Braby. 2001. Microdosimetry of a 25 keV electron microbeam. Radiat Res **155**(1): 89-94.

Wojcik A, K Bonk, WU Miller, G Obe and C Streffer. 1996. Do DNA double-strand breaks induced by Alu I lead to development of novel aberrations in the second and third post-treatment mitoses? Radiat Res **145**:119-127.

Wolff S. 1998. The adaptive response in radiobiology: Evolving insights and implications. Environ Health Perspect **106**:277-283.

Xue M, W Wei, Y Su, J Kim, YS Shin, WX. Mai, DA. Nathanson, J R Heath. 2015. Chemical methods for the simultaneous quantitation of metabolites and proteins from single cells. J Am Chem Soc. **137**: 4066−4069. DOI: 10.1021/jacs.5b00944

IX. Biographies of participants

A. Subcommittee members

Dr. Michael Bellamy emigrated from the Republic of Trinidad and Tobago to complete a double major in physics and math at Morehouse College. He is now a scientist at Oak Ridge National Laboratory primarily interested in the Radiation Protection research. Dr. Bellamy is currently focused on calculating human health risks associated with environmental and occupational exposure to radionuclides. He completed a double major in physics and math from Morehouse College and, after earning a Master's Degree at Georgia Tech, obtained his Ph.D in Nuclear Engineering under Dr. Keith Eckerman. Dr. Bellamy helped to develop mathematical models to better quantify the interaction of radiation with DNA. He serves as a corresponding member of the International Commission on Radiation Protection by helping to develop the next generation of computational human phantoms and environmental radiation dose coefficients. Dr. Bellamy now devotes much of his time to the estimating the risk of cancer due to the inhalation and ingestion of radionuclides. Outside of his life as a researcher, Michael is a board member for the Oak Ridge Civic Music Association and the chair of the ORNL Early Career Professionals.

Dr. Amy Berrington de González received a D.Phil. in Cancer Epidemiology from the University of Oxford. She was on the faculty at the University of Oxford and then Johns Hopkins Bloomberg School of Public Health before moving to the Radiation Epidemiology Branch at NCI in 2008. She was awarded NIH scientific tenure in 2012, and was promoted to Branch Chief in 2014. Dr. Berrington de González is currently serving on two radiation risk committees for the National Academy of Science and previously served on the UK Health Protection Agency's Advisory Group on Ionising Radiation, and the UK Breast Screening Programme's Advisory Group.

Dr. David G. Hoel is Distinguished University Professor in the Department of Medicine at the Medical University of South Carolina. He was at the NIH National Institute of Environmental Health Sciences for more than 20 years and served as director of its Division of Environmental Risk Assessment. Dr. Hoel has particular interest in estimating the health effects of radiation exposures and spent three years working at the Radiation Effects Research Foundation in Hiroshima, Japan, as one of its program directors. His activities also include service on U.S. Environmental Protection Agency, International Atomic Energy Agency, and World Health Organization advisory committees. Dr. Hoel has been on several National Academies committees that addressed radiation exposure and other risk-assessment topics and was a member of the NRC Nuclear and Radiation Studies Board. He earned a Ph.D. in mathematical statistics from the University of North Carolina at Chapel Hill and completed postdoctoral training in preventive medicine at Stanford University. Dr. Hoel is a member of the IOM and a fellow of the American Association for the Advancement of Science.

Dr. Arthur M. Katz, recently retired, worked almost 40 years in the U.S. Department of Energy and predecessor agencies dealing with a wide range of energy and science topics including advanced nuclear reactors, planning and international collaboration in fusion energy, and fundamental biological and environmental research. In the Office of Biological and Environmental Research (OBER) he was responsible for managing projects that were part of DOE's contribution to the Human Genome Program, research into low dose radiation health

effects, and a variety of molecular imaging and structural biology projects. He has a PhD in chemistry (University of Rochester 1969) and a M.S. in meteorology (MIT 1974).

Dr. Jerome S. Puskin is recently retired from the directorship of the Center of Science and Technology in the Radiation Protection Division, U.S. Environmental Protection Agency (EPA). He was at the EPA from 1985 to 2016, heading a group with responsibility for developing models for EPA's assessment of radiation doses and risks. From 1982-1998, he worked on similar issues at the U.S. Nuclear Regulatory Commission (NRC). Prior to this, Dr. Puskin was a Postdoctoral Fellow and then a faculty member in the Department of Radiation Biology and Biophysics at the University of Rochester, where he performed research on ion transport into mitochondria and ion binding to phospholipid membranes. His academic degrees include a BA from Johns Hopkins and a PhD from Harvard, both in Physics. He represented EPA on a number of interagency committees, including a committee established to advise on health protection measures for the U.S. population after Chernobyl and the Executive Committees for the committee on Interagency Radiation Research and Policy Coordination and the Joint Coordinating Committee for Radiation Effects Research. Dr. Puskin served on the scientific committee for NCRP Report No. 160, Ionizing Radiation Exposure of the Population of the United States and is currently a member of the NCRP SC 1-20, which is addressing the issue of enhanced relative biological effectiveness for low-energy photons and electrons.

Dr. Judy D. Wall is a Curators' Distinguished Professor in the Biochemistry Division at the University of Missouri-Columbia. She received her bachelor's degree in chemistry from the University of North Carolina at Greensboro (1967) and her Ph.D. at Duke University (1974) in Biochemistry. Her postdoctoral studies at Indiana University introduced her to environmental microbes that have been the focus of her research for over 40 years with an emphasis on microbial interactions with toxic metals. She has been honored as a Fellow of American Association for the Advancement of Science (1994), Fellow of American Academy of Microbiology (1998), Byler Distinguished Professor Award (1999), the Tribute to MU Women Award (2005), the University of Missouri Curators' Professorship (2013) and the MU Southeastern Conference Faculty Achievement Award (2014). She has been a career-long member of the American Society for Microbiology, serving as Editor in Chief of *Applied and Environmental Microbiology,* for six years. Since 2009, she has served on the DOE's Office of Biological and Environmental Research Advisory Committee. In that capacity, she was asked to serve as the organizer of this expert subcommittee for Low Dose Radiation research considerations.

Dr. Zhi-Min Yuan is a Morningside Professor of Radiobiology and the Director of the John B. Little Center for Radiation Science and has appointments in both the Department of Environmental Health and Genetics and Complex Diseases in the Harvard T.H. Chan School of Public Health. Dr. Yuan's research focuses on elucidation of signaling mechanisms that regulate cellular stress responses and on examining how stress signals affect cell behaviors in the context of cancer. He received his Ph.D training from University of Maryland in 1993 and his postdoctoral training at the Dana-Farber Cancer Institute, Harvard Medical School. He began his academic career at Harvard School of Public Health as an Assistant Professor in 1998. In 2008, Dr. Yuan joined the University of Texas Health Science Center at San Antonio becoming

Professor and Chief of the Division of Radiation Biology in the Department of Radiation Oncology before returning to Harvard in 2012.

B. Expert witnesses

Sally A. Amundson, Sc.D., obtained her Sc.D. degree in Cancer Biology from Harvard University, School of Public Health in 1992. She carried out postdoctoral research in Radiation Biology at Los Alamos National Laboratory, 1992 – 1995, and in Cancer Biology at the National Cancer Institute from 1995 to 1998. She was awarded the Michael Fry Research Award from the Radiation Research Society in 2004. She is currently a member of the National Council on Radiation Protection and Measurements and has been Associate Editor of Radiation Research Journal from 2007-present. In addition, she is on the Scientific Advisory Council of the Radiation Effects Research Foundation (RERF), Hiroshima, Japan. She currently holds a position as Associate Professor of Radiation Oncology in the Center for Radiological Research at Columbia University Medical Center.

Dr. Mary Helen Barcellos-Hoff received an undergraduate degree from the University of Chicago and earned a doctoral degree in experimental pathology from the University of California, San Francisco. Her postdoctoral research on extracellular matrix mediated functional differentiation was conducted with Mina Bissell at the Lawrence Berkeley National Laboratory (LBNL). Dr. Barcellos-Hoff joined LBNL as a staff scientist in 1988 and left in 2008 as Senior Scientist and Associate Director of the Life Sciences Division. She then joined the faculty of New York University School of Medicine as Director of Radiation Biology in the Department of Radiation Oncology and, in 2015, returned to UCSF as Professor and Vice Chair of Research in the Department of Radiation Oncology. She is a member of AAAS, the Radiation Research Society, and the American Association of Cancer Research. Dr. Barcellos-Hoff's research focuses on how tissues integrate information across scales of organization and she uses this information to identify critical events in terms of effects on cell phenotype and tissue interaction during radiation carcinogenesis. She discovered that transforming growth factor β (TGFβ) is activated by radiation and mediates the DNA damage response, as well as the composition of the tumor microenvironment, particularly immune cell phenotypes.

X. Acronyms

BEIR	Biological Effects of Ionizing Radiation
BER	Biological and Environmental Research
BSSD	Biological Systems Science Division
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
CT	Computed Tomography
DHS	Department of Homeland Security
DOD	Department of Defense
DOE	U.S. Department of Energy
ECM	Extracellular Matrix
EPA	Environmental Protection Agency
eQTL	Expression Quantitative Trait Loci
ERR	Excess Relative Risk
GWAS	Genome-Wide Association Studies
Gy	Gray, actual physical dose of radiation, 1Gy = 1J/Kg
IOM	Institute of Medicine
IR	Ionizing Radiation
LDR	Low Dose Radiation
LET	Linear Energy Transfer
LNT	Linear No Threshold
LSS	Life Span Study
NASA	National Aeronautics and Space Administration
NBR	Natural Background Radiation
NCI	National Cancer Institute
NCRP	National Council on Radiation Protection and Measurements
NIH	National Institutes of Health
NRC	Nuclear Regulatory Commission
OBER	Office of Biological and Environmental Research
PPI	Protein-Protein Interactions
qRT-PCR	quantitative Real Time-Polymerase Chain Reaction
RBM	Red Bone Marrow
RERF	Radiation Effects Research Foundation
ROS	Reactive Oxygen Species
Sv	Sievert, equivalent and effective dose, depends on the radiation type and biological context. For gamma radiation, Sv ≈ Gy.
TB	Tuberculosis
UK	United Kingdom
US	United States