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# MEETING REPORT

# Molecular and Cellular Biology of Moderate-Dose (1–10 Gy) Radiation and Potential Mechanisms of Radiation Protection: Report of a Workshop at Bethesda, Maryland, December 17–18, 2001[1]

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**Exposures to doses of radiation of 1–10 Gy, defined in this workshop as moderate-dose radiation, may occur during the course of radiation therapy or as the result of radiation accidents or nuclear/radiological terrorism alone or in conjunction with bioterrorism. The resulting radiation injuries would be due to a series of molecular, cellular, tissue and whole-animal processes. To address the status of research on these issues, a broad-based workshop was convened. The specific recommendations were: (1) *Research*: Identify the key molecular, cellular and tissue pathways that lead from the initial molecular lesions to immediate and delayed injury. The latter is a chronic progressive process for which postexposure treatment may be possible. (2) *Technology*: Develop high-throughput technology for studying gene, protein and other biochemical expression after radiation exposure, and cytogenetic markers of radiation exposure employing rapid and accurate techniques for analyzing multiple samples. (3) *Treatment strategies*: Identify additional biological targets and develop effective treatments for radiation injury. (4) *Ensuring sufficient expertise*: Recruit and train investigators from such fields as radiation biology, cancer biology, molecular biology, cellular biology and wound healing, and encourage collaboration on interdisciplinary research on the mechanisms and treatment of radiation injury. Communicate knowledge of the effects of radiation exposure to the general public and to investigators, policy makers and agencies involved in response to nuclear accidents/events and protection/treatment of the general public.** 

## EXECUTIVE SUMMARY

Normal tissue response and injury after exposure to ionizing radiation are of great importance to patients with cancer, populations potentially subjected to military, accidental or intentional exposure including bioterrorism, and workers in the nuclear power industry. In these situations exposure is likely to include the moderate radiation dose range (1–10 Gy). Exposure of limited tissue volumes to higher doses during cancer treatment has been the subject of research by the National Cancer Institute (NCI), which has also supported research into fundamental radiobiology, DNA damage and repair, and the epidemiology of people exposed to ionizing radiation. The Department of Energy (DOE) is interested in the effects of very low-dose exposure as it relates to protection of the public, as well as workers engaged in the cleanup of contaminated environments resulting from weapons production. NASA addresses the health risks to

[1] A Draft Report was posted on the Radiation Research Program website (http://www3.cancer.gov/rrp/) in February, 2002. All authors have had the opportunity to review and approve this Final Report. It represents the efforts of all participants in the workshop. The conclusions are those of the authors and not those of the individual agencies and institutions.

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astronauts from radiation, which includes low numbers of high-energy heavy particles (cosmic rays) not usually encountered on Earth. Protection of members of the Armed Forces against intentional exposure has been studied by the Department of Defense (DOD) and the Armed Forces Radiobiology Research Institute (AFRRI). Given the wide range of expertise involved, an interdisciplinary scientific workshop was convened to address the recent scientific progress in molecular, cellular and whole-animal radiobiology, biodosimetry, and current and future treatments to prevent or ameliorate radiation damage to normal tissues. This workshop focused on these topics as they pertain to moderate doses, defined as 1–10 Gy, a range that was not addressed in recent scientific workshops on low-dose radiation and radiation oncology. The broad term ''radioprotectors'' was used to include chemical and/or biological treatments that might be administered before or after exposure. Brief summaries of this workshop have been published previously (*1–3*); the present document provides more detail.

Understanding the molecular, cellular and tissue changes that can result from moderate-dose radiation exposure necessitates input from experts in a number of fields including radiation biology, wound healing and clinical medicine. The development of radioprotector strategies for a single radiation exposure will differ from that for radiation oncology, in which treatment is delivered over the course of several days (in brachytherapy) or several weeks (in conventional external-beam radiotherapy), a notable exception being the short course for total-body irradiation for immunosuppression and transplantation. Additionally, in cancer treatment, a radioprotector should not protect the tumor cells from radiation-induced killing to an appreciable extent. Treatment of populations exposed to a single radiation dose requires accurate and rapid biodosimetry to determine an individual's exposure level and risk for morbidity and mortality as a result of the exposure, and the availability of appropriate therapeutic agents and strategies and expertise in treatment.

The goals of the interdisciplinary workshop were to define the current state of the science and research opportunities. The following are the highlights with additional detail provided in the body of the report.

*1. Research*

The biological changes elicited in the moderate-dose range involve the cells that are irradiated, their nonirradiated neighbors (bystander effect), and the complex interactions among cells, tissues and organs. Research is needed to identify the key molecular, cellular and tissue pathways that lead from the initial molecular lesions to immediate and delayed injury, the latter being a chronic progressive process for which postexposure treatment may now be possible.

In addition to increased support for basic mechanistic studies, consideration should be given to a new program studying radiation toxicology of normal tissues, which involves long-term toxicity and radioprotector studies.

*2. Technology*

High-throughput technology for studying gene, protein and other biochemical expression after irradiation will greatly enhance the discovery of the basic mechanisms of normal tissue injury (for example, a ''normal tissue'' gene and/or protein chip) and, as molecular targets are defined, will identify agents for normal tissue radioprotection for pre- and postirradiation treatment.

Biomarkers of radiation exposure and rapid and accurate techniques for analyzing multiple samples need to be identified and validated to allow for the prompt delivery of the most appropriate treatment.

*3. Treatment strategies*

Prevention and treatment of radiation injury requires accurate and rapid dosimetry and the application of appropriate therapy. At present there are a limited number of pre- and postexposure therapeutic agents. There is a need for research to identify additional biological targets and effective treatments. This is optimally done by collaboration among researchers in academia, industry and governmental agencies. As effective agents are defined, tested and approved for human use, sufficient quantities must be synthesized and distributed throughout the country.

*4. Ensuring sufficient expertise*

Over the last decade or so, the number of investigators studying radiation dosimetry, radiation biology and normal tissue injury has declined substantially. It is critical to maintain an interdisciplinary effort and to train and recruit investigators from such fields as radiation biology, molecular biology, cellular biology and wound healing. Communication of the current state of knowledge of the effects of radiation exposure, of which a great deal is known, is important for the general public and for investigators, policy makers and agencies involved in responding to nuclear accidents/events and protection and treatment of the general public.

## INTRODUCTION

*Goals of the Workshop*

1. Define the state of the science in normal tissue radiobiology, radioprotection and biodosimetry.
2. Describe currently available treatments for preventing and reducing radiation-induced injury.
3. Determine the research opportunities and resources required.
4. Develop a research-action plan for further discussion and implementation.

*Background*

There is an extensive body of research relevant to cancer therapy on radiation exposures higher than those in the range covered in this workshop and also on lower doses of radiation relevant to environmental exposure and specific aspects of nuclear fallout. Normal tissue injury resulting from traditional radiotherapy was the topic of a recent workshop sponsored by the Radiation Research Program of NCI, which has been summarized (Appendix 1). The workshop that is the subject of the present report focused on the moderate-dose range of 1–10 Gy which could be received either in fractionated doses for radiation therapy or in a single dose from accidental or intentional exposure.

Experts with a breadth of scientific expertise (Appendix 2) were invited to discuss the scientific topics of (a) radiation-induced genetic and epigenetic effects in cells and tissues, and whole-body effects; (b) biological dosimetry; and (c) treatment approaches for radiation protection (Appendix 3). Radiogenic DNA repair and effects of radiation damage on the regulation of the cell cycle were touched on in several sessions but were not a main focus at the workshop. The recommendations for research were divided, somewhat arbitrarily, into three groups: immediate, those that could be completed within 1 year; medium term, 1 to 3 years; and longer term, greater than 3 years.

## DEFINING THE EXPOSURES

*Units of Exposure and Dose: Gray (Gy) or Sievert (Sv)*

The Draft Workshop Report used the unit sievert, which is used for radiation protection at low doses. In the final document, the unit gray has been used to indicate radiation dose, as deemed to be the appropriate unit based on ICRU Report 51 (*4*). The unit sievert (Sv), which is a unit of dose equivalent, is defined as dose at a point in tissue multiplied by a quality factor and is defined for use in the low-dose range only, for stochastic effects, and not in the moderate- and high-dose ranges. Radiation effects in any individual, however, will depend on the type of radiation involved (since densely ionizing radiation has a greater relative biological effectiveness than sparsely ionizing radiation), internal uptake and distribution (such as radioactive cesium or iodine from fallout), surface exposure (from fallout), and protection of parts of the body by shielding.

*Potential Radiation Exposure during IMRT*

In cancer treatment, exposure of normal tissues to the moderate-dose range is increasingly likely with the use of intensity-modulated radiation therapy (IMRT). IMRT is an evolving radiation therapy technique that allows the radiation oncologist to ''sculpt'' the dose so that there may be a higher dose given to the tumor and a lower dose to nearby normal tissue. Foci of higher doses can also be produced within the tumor, with the theory that the higher dose will improve local tumor control. The implementation of IMRT depends on complex imaging, computerized treatment planning, and treatment delivery. The radiation beam sweeps through large arcs and/or is delivered with multiple fields to focus higher doses within the tumor compared to those achieved with traditional radiotherapy. To accomplish this, the linear accelerator is ''on'' for a longer time and the multiple fields of entry spread out dose delivery to more tissues, resulting in larger volumes of normal tissues receiving some radiation dose, including the accumulation of a higher whole-body dose compared to traditional radiation therapy (*5*).

The dose of radiation to the patient from the linear accelerator depends on the X-ray energy and the technique used. The higher-energy linear accelerators ($>$10 MV and especially $\geq$12 MV) produce neutron contamination that adds to the whole-body equivalent dose (*5–7*). Because of the ''quality factor'' multiplier for neutrons, there would be an increased risk of a patient developing a fatal secondary cancer many years after treatment (*8*). It should be emphasized that lifetime risk estimates of excess cancers with the lower-energy linear accelerators is low, below 2% (*8*). For this reason, IMRT is best performed with machines operating at $\leq$10 MV nominal energy.

The volume of normal tissue treated to a certain dose is limited in radiation therapy by the design of the treatment plan that is aimed at avoiding clinically apparent organ dysfunction. This treatment planning is based on the existing knowledge of organ tolerance, which depends on the organ involved and the dose distribution within that organ, as well as treatment schedule. What is not known is the impact of the dose distributions from IMRT (large volumes at moderate doses) on long-term organ function and susceptibility to damage from other causes years later. Late tissue responses and the development of agents that might reduce latent injury after radiation therapy were the topic of a recent NCI Radiation Research Program workshop entitled ''Modifying Normal Tissue Damage Postirradiation'' (summarized in Appendix 1).

*Acute Effects of Whole-Body Irradiation*

The effects of whole-body exposure to ionizing radiation on animals have been studied in the laboratory. Data on human exposures have been obtained from the Japanese survivors of Hiroshima and Nagasaki and from accidental exposures. Summarizing briefly the extensive literature on whole-body irradiation, there are three general classes of radiation lethality, which depend on dose, exposure rate and quality of radiation (i.e. photons, neutrons or particles) (*9–15*). The syndromes resulting from single-dose exposure are:

1. Cerebrovascular syndrome (CNS syndrome), $>$100 Gy, death within 24–48 h.
2. Gastrointestinal syndrome (GI syndrome), 5–12 Gy (primarily $>$10 Gy), death within 3–10 days; survival possible in lower end of the range.

**TABLE 1**
**Fallout-Area Delayed Effects**

| | Outpatient care patients and worried well | Minimal care patients | Minimal/intensive care patients | Intensive care patients | Lethally exposed patients |
|---|---|---|---|---|---|
| Dose range, Gy | <1.5 | 1.5–3 | 3–5.3 | 5.3–8.3 | >8.3 |
| Casualties, percentage | 47 | 12 | 19 | 14 | 8 |

*Note.* Data provided by Robert Eng, AFRRI.

3. Hematopoietic syndrome (bone marrow syndrome), 2.5 to 8 Gy, death within 1–2 months; survival possible.

The dose range in this workshop encompasses the hematopoietic syndrome and the lower range of the GI syndrome. However, at longer times after exposure in this moderate-dose range, there is also the potential for the expression of injury in other tissues such as the kidney and central nervous system, as well as tumor development. As our ability to deal with the acute effects of moderate-dose exposure improves, the potential for these late effects is of increasing concern.

The effects of an accidental or intentional nuclear event are complex interactions of the immediate blast and the radiation. To place whole-body exposure in context for scientific discussion, data regarding a potential nuclear event were reviewed. The consequences for this scenario were partitioned into what are called ''blast-prompt'' and ''fallout-area delayed'' effects. These casualties in the fallout area were stratified into several categories of medical care and dose range (Table 1), recognizing that age or concomitant illness could have a significant impact on a particular individual's outcome.

The $LD_{50}$, used to quantify mortality in a population, is defined for radiation as the dose of radiation that will cause death in half (50%) of the people (or animals) exposed. The time of death depends on the dose, as noted above, being within hours for the CNS syndrome, approximately 3–10 days for the GI syndrome, and 30–60 days for the hematopoietic syndrome. Therefore, the term for hematopoietic death is the $LD_{50/30}$ (it is also known as $LD_{50/60}$, because death from marrow failure may occur at up to 60 days in humans). $LD_{50/60}$ values for humans are estimated to be about 4.5 Gy (approximate range of 3–6 Gy) based on the experience of the Japanese atomic bomb survivors and other studies (*13*, *14*).

Medical interventions such as blood cell replacements, antibiotics, cytokines and, in high-dose cases, hematopoietic stem cell transplants could increase survival to the extent of doubling the $LD_{50}$ value (*9–11*, *16*). The largest proportion of people (47% in Table 1) would represent both worried-well patients (no radiation exposure) and individuals exposed to nonlethal radiation doses (i.e. $\leq$1.5 Gy). In the other extreme, some 22% of people (Table 1) would include both those lethally exposed and those requiring intensive care. The ability to identify and triage people exposed to intermediate doses (1.5–5.3 Gy), which represent 31% of this casualty component, can result in reductions in acute casualties and possibly in a reduction in cancer incidence in these survivors should effective treatments be developed and used. To optimize treatment, biodosimetry is essential. For triage of a large number of individuals, preliminary biodosimetry should be rapid with a low false negative rate, followed by secondary biodosimetry for determining treatment, which may require the use of different, more accurate technology and methodology.

The radius and range of significant injuries from a nuclear event depend on the yield (*9*). The 4-Gy dose is within the moderate-dose range of this workshop (Table 2). For a ''dirty bomb'', exposure will depend on many factors such as the radioactive material, the type of blast, the location and other environmental conditions.[3]

**TABLE 2**
**Expected Ranges for Injuries from Nuclear Weapons of Various Sizes**

| Yield (kiloton) | Range for 50% mortality from air blast (m) | Range for 50% mortality from thermal burns (m) | Range for 4 Gy initial nuclear radiation (m) | Range for 4 Gy fallout in first hour after blast (m) |
|---|---|---|---|---|
| 0.01 | 60 | 60 | 250 | 1,270 |
| 0.1 | 130 | 200 | 460 | 2,750 |
| 1 | 275 | 610 | 790 | 5,500 |
| 10 | 590 | 1,800 | 1,200 | 9,600 |

*Notes.* Reproduced with permission from the NCRP. Note that meteorological conditions such as wind and precipitation will affect the pattern of deposition of radioactive materials from fallout (*9*).

## MOLECULAR AND CELLULAR BIOLOGY AND DETECTION OF RADIATION DAMAGE

### *Summary of Critical Information*

Classical radiobiology is based on the paradigm that cell death results from DNA damage that occurs both directly in the form of DNA strand breaks and indirectly as a result of oxidative reactions (*15*). Loss of clonogenic capacity may occur through a number of mechanisms including apoptosis, mitotic catastrophe, terminal differentiation and necrosis (*15*). In cells that survive, there is the potential for DNA mutations and chromosomal aberrations (*17–20*). Mu-

[3] For a description of a ''dirty bomb,'' see the CDC website: http://www.cdc.gov/nceh/radiation/db.htm.

tations, and to some extent chromosomal alterations, can be characterized at the molecular level, although their mechanisms of formation after radiation exposure remain to be fully defined. New techniques, especially those based on fluorescence *in situ* hybridization (FISH) (*18, 20*), allow for a more complete assessment of the genomic changes after radiation exposure. In addition, FISH should allow for the identification of informative biomarkers after exposure (*20*).

Radiation induces a variety of additional effects that can be expressed at cellular and tissue levels. These effects include the generation of oxidative stress (*21, 22*), alterations in gene transcription (*23*), changes in signal transduction (*24*), and a number of epigenetic phenomena (*25*). The latter, to be described in more detail below, involve alterations in cells and tissues not directly related to a change in the structure of the DNA itself. Although a wide variety of events occur, their specific role in tissue radioresponse requires further investigation using a variety of model systems ranging from single-cell mechanisms to complex multicellular models to *in vivo* organ and whole-animal studies.

In addition to contributing to the fundamental understanding of radiation effects within tissue, evaluation of specific changes in gene expression or protein profiles in irradiated cells will likely provide a practical means of defining tissue exposure (*26, 27*). Such information may identify sentinel genes or proteins that can serve as *in vivo* biodosimeters. This type of research is in its infancy. However, its advancement would likely provide a powerful tool for the accurate assessment of the risk to individuals within an exposed population and determination of appropriate pre- and postexposure interventions.

To clearly understand noncarcinogenic and carcinogenic radiation effects, it is necessary to understand multiple response pathways, including cell–cell and cell–microenvironment interactions. Although less is known about epigenetically mediated responses, it is becoming clear that there are complex sets of cell–cell interactions so that irradiating one cell may induce transformation, mutation and transcriptional activation in neighboring unirradiated cells, a phenomenon known as the bystander effect (*28*). This effect enlarges the population of affected cells from that predicted by physical dose distribution. Thus the bystander effect, discussed in more detail below, can be expected to contribute to tissue-level response.

These types of cell–cell interactions again serve to highlight the need to address radiation responses at the level of the tissue and whole animal in addition to that of single cells. An understanding of each level of response along with the translation of research from *in vitro* systems to *in vivo* and clinical studies will be needed to predict adverse health outcomes after radiation exposure and to develop interventions to prevent and ameliorate injury.

### *Chromosomal Damage*

Chromosomal aberrations are important indicators of radiation exposure and have been used extensively to investigate the mechanisms of radiation action; they can also serve as a sensitive biodosimeter (*17, 29, 30*). Aneuploidy, mutagenesis and carcinogenesis are significant outcomes from chromosomal damage. Chromosomal abnormalities can be assessed by classical scoring of Giemsa-stained metaphase cells or by the use of FISH (*17, 18*), multiplex FISH (mFISH) (*19*), or spectral karyotypic analysis (SKY) (*31*). Symmetrical exchanges, which by definition are considered to be relatively stable, do not involve the production of acentric fragments and therefore are not usually lethal to cells. Such abnormalities are generally cumulative over a lifetime. The use of mFISH has demonstrated that with $\gamma$-ray exposure in the 1–4-Gy dose range, up to 25–30% of abnormalities are complex, i.e. involve three or more breakpoints in two or more chromosomes (*19*). For densely ionizing radiation such as charged particles, a much higher proportion of aberrations are complex (*20*). Better understanding of mechanisms of formation of chromosomal aberrations will help elucidate the pathways involved in mutagenesis and carcinogenesis. Methods are needed for analyzing chromosomal aberrations in cells from tissues other than blood.

In addition to scoring of aberrations in metaphase cells, another sensitive methodology for measuring radiation damage is induction of premature chromosome condensation (PCC) in interphase cells (*29, 32–40*). It is now possible to induce high yields of prematurely condensed chromosomes in proliferating cells. For example, a recently developed alternative PCC technique employs a phosphatase inhibitor (e.g. okadaic acid or calyculin A) combined with p34 (CDC2)/cyclin B kinase to induce high yields of prematurely condensed chromosomes in resting human peripheral blood lymphocytes, producing spreads suitable for biodosimetry applications (*30, 38–40*). Detection of cells with translocations by specific chromosome painting allows evaluation over a broad range of radiation doses using automated cytological systems.

### *Mutagenesis and Carcinogenesis*

Ionizing radiation in the range of 1–10 Gy causes mutagenesis and carcinogenesis. Cancer has been associated with exposures in the 1-Gy range in approximately 4.5% of patients; approximately a fourth of these patients, or 1% overall, will contract leukemia (*41–43*). Data from Hiroshima indicate that the frequency of chromosomal mutation increased substantially in lymphocytes in residents exposed to ionizing radiation. In addition, a 20% increase in mutation frequency was observed in workers involved in the cleanup at Chernobyl (*44*). Studies in mice report that with a 1-Gy exposure, there is an increase in mutation frequency in spermatogonia, indicating that germ cells also are sensitive to ionizing radiation (*45*).

### *Tissue Effects: Noncarcinogenic Alterations*

In most tissues, relatively large radiation doses are required to induce overt tissue injury or organ failure. Al-

though there are notable exceptions (e.g. bone marrow), single doses of >10 Gy are generally required to induce significant tissue dysfunction. However, after exposure to doses of 1–10 Gy, measurable effects can be detected in many tissues, including persistent and transient alterations in protein expression, growth factor activity, and normal cell and tissue function (*46*). Although the significance of such changes with respect to normal tissue radioresponse after moderate doses has not been determined specifically, similar tissue changes have been observed in a number of other pathological conditions. Thus it is likely that such changes can contribute to radiation response. Our knowledge in this area, however, is incomplete, and further studies, particularly long-term studies, are needed to evaluate the health impact of such tissue effects of radiation.

Tissue damage is the result of damage to stem cells, parenchymal cells, stromal cells, and endothelial cells as well as to the signaling processes through which these cell types communicate with each other and with the extracellular matrix (*46–48*). Over the past decade, molecular biological approaches have been employed to define subcellular and biochemical events occurring after irradiation. Much of this work has relied on *in vitro* model systems in which cells are considered as autonomous units, responding to damage as independent entities. However, tissues are highly integrated systems in which cell–cell interactions play major functional roles under physiological and pathological conditions. Thus the response of individual cells in a culture dish can be misleading when determining what occurs *in situ.* Moreover, the history of cells and their microenvironment directly affects how they respond to stimuli. Not only do irradiated cells modify the tissue microenvironment, but the irradiated microenvironment also influences subsequent cell/tissue responses. Application of the technique of laser capture microdissection (LCM) (*49*), which allows for the *in situ* analysis of specific cell populations within normal and tumor tissue, should provide relevant information in this research area. Currently, critical deficiencies exist in our understanding of how irradiated cells and the microenvironment interact and function.

In certain tissues, stem and precursor cells are critical targets for radiation. They can undergo rapid apoptosis after exposure and are particularly sensitive to moderate radiation doses. For example, in both rats and mice in the hippocampal region of the brain, which is associated with learning and memory, radiation doses as low as 0.5 Gy induce significant apoptosis in neural precursor cell populations, with a very steep dose response between 0–2 Gy (*50*). Subsequent to the induction of apoptosis, there is a significant reduction in neurogenesis (*51–55*). Given that the decrease in neurogenesis detected after other types of injury results in cognitive impairment (*56, 57*), one can speculate that radiation-induced changes in these cells might have similar effects. It is well established that exposure of normal brain to radiation during higher-dose treatment for cancer can result in cognitive impairment, but as yet the pathogenesis of these changes has not been fully elucidated. In the brain, as in other tissues, critical questions include how microenvironmental factors influence the outcome after irradiation, and how radiation affects differentiation and mitogenesis. Understanding these relationships is critical in developing strategies to ameliorate the consequences of radiation exposure of tissues.

In the absence of overt tissue damage, persistent radiation effects may contribute to evolving pathology or response to subsequent trauma, disease or the aging process. For example, radiation has been shown to produce chronic oxidative stress (see below) (*21, 58*), and there are a number of degenerative conditions as well as aging that have been associated with decreased antioxidant status and increased oxidative stress. In addition, persistent changes in growth factor activity after irradiation may initiate a cascade of events resulting in delayed injury in susceptible tissues or individuals (*46*). Given the potential significance of the interaction of low-dose radiation with various forms of tissue pathology as well as trauma or stress on the tissue, considerable research is required to define the potential risks and to understand the mechanisms responsible.

To address the many factors involved in moderate-dose, noncarcinogenic effects in tissues, it will be necessary to employ existing experimental models and to develop new models. The use of mice genetically modified in their expression of potentially critical molecules (e.g. TGFB and SOD) in various pathways relevant to specific disease end points would facilitate investigation of the role of these molecules in response to radiation. Co-culture models, in which cells from different types of tissue and/or cells plus matrix are grown together, can be used to delineate functional and molecular analyses of tissue radiation response, which depends upon individual cell response, cell–cell and cell–matrix interactions, and microenvironmental factors.

### *Assessing Gene Expression and Encoded or Modified Proteins*

In addition to DNA damage, ionizing radiation induces a complex pattern of gene expression that depends on cell type (*26, 59–61*). Specific patterns of radiation-induced gene expression can now be analyzed using microarray gene chip technology. This technology is being applied to irradiated cell culture models as well as to *in vivo* experimental systems. Signatures of radiation-induced gene expression may ultimately aid in identifying genetic determinants responsible for the variations in radiation sensitivity within a population, defining molecular targets for radioprotective strategies, and serving as biomarkers for human radiation exposure. Radiation-induced gene expression can also be evaluated using real-time polymerase chain reaction assays (*62*).

Radiation-responsive proteins, which may be easier to detect than radiation-induced gene expression, have considerable potential as biodosimeters. Such proteins may be the

result of gene expression or possibly a protein directly altered by radiation. Tissue-specific protein biomarkers detected in peripheral blood have been described for an *in vivo* murine system (*23, 27, 63–66*), which suggests the possibility of providing diagnostic information of organ-specific radiation injury. Radiation-induced gene and protein expression are active areas of research that will contribute to both the fundamental and applied levels of normal tissue radiobiology.

*Oxidative Stress and Tissue Fibrosis*

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are formed and degraded by all aerobic organisms. In normal cells, ROS are believed to play an important role in intracellular signaling and redox regulation. ROS/RNS generation and removal are in balance in the presence of effective antioxidant defenses (antioxidants and antioxidant enzymes). Any increase in the ratio of ROS/RNS generation to antioxidant defenses can create cellular stress. A sufficient degree of stress can initiate mitochondrial changes that in turn can lead to a cascade of irreversible damage. Indicators of oxidative stress have been detected in *in vitro* models after irradiation as well as in the kidney and central nervous system after irradiation of rodents (*22, 55, 58, 67*).

Fibrosis, a debilitating late response occurring in a number of critical normal tissues, is an example of radiation-induced injury that may involve oxidative stress. Radiation-induced fibrosis has been viewed as a chronic, progressive, untreatable injury. However, this view is being challenged by a new paradigm of fibrosis as a wound-healing response involving complex and dynamic interactions among several cell types and the extracellular matrix. This suggests the possibility of developing therapies that inhibit or reverse the fibrotic process induced by radiation exposure. A growing body of evidence suggests that chronic oxidative stress is an important factor in the etiology and development of fibrosis. Antioxidants, particularly SOD (superoxide dismutase), have proven to be effective for inhibiting and reversing fibrosis in preclinical models (*68–70*), an observation that supports the contention that it may be possible to intervene in the chronic-progressive process. Recent development of novel SOD mimetics offers the promise of improved clinical therapies for ROS-mediated injury (*71*).

*Bystander Effects*

The bystander effect is the induction of biological effects in cells not directly hit by radiation. It has been demonstrated by three different techniques: medium transfer (*72*), a low fluence of $\alpha$ particles (*73–75*), and single-cell-diameter microbeams (*76*). It has been observed using a number of biological end points including cell lethality (*72*), formation of micronuclei (*73, 76*), mutation (*75*), oncogenic transformation (*77, 78*), sister chromatid exchange (*74*), and gene expression (*73, 77*). Two mechanisms have been hypothesized: transmission of a signal through cell-to-cell gap junctions (*73*) and release of a signal into the extracellular space (*24, 72*). The bystander effect appears to predominate at very low doses of radiation. A single nuclear traversal by a high-LET particle such as an $\alpha$ particle or, for low-LET $\gamma$ rays, doses as low as 0.01 Gy can induce bystander responses (*74, 79*). In general, the majority of effects described are detrimental to the affected cells. This suggests that at low doses of radiation, bystander effects may amplify the biological effectiveness of a given radiation dose by increasing the number of cells injured beyond those directly exposed to radiation (*79*).

To date, the information on radiation-induced bystander effects comes almost exclusively from *in vitro* tissue culture experiments. It is not clear what types of bystander effects might be observed in three-dimensional tissues or intact organisms, or how important these effects might be in the dose range of 1–10 Gy. However, a second related epigenetic phenomenon associated with *in vivo* and *in vitro* exposure to radiation has also been described: the induction of clastogenic factors which can be found in plasma from irradiated humans (*80–83*). Culturing normal human peripheral blood lymphocytes in medium containing plasma from irradiated individuals can result in significantly more chromosomal aberrations than in those cultured with plasma from nonirradiated individuals (*84*). Clastogenic factors have been described after a range of doses of radiation and include such diverse exposures as radiotherapy patients (*82, 83*), Japanese A-bomb survivors (*84*), salvage personnel at Chernobyl (*80*), and children exposed at Chernobyl (*81*). These factors appear to be extremely persistent in irradiated individuals, with clastogenic activity observed $>30$ years after the initial exposure (*84*).

*The Adaptive Response*

The adaptive response to radiation is the ability of a very low dose of radiation to induce cellular changes that alter the level of subsequent radiation-induced or other damage. If low doses of radiation predictably induce a protective response in cells exposed to subsequent low doses of radiation or to spontaneous damage, this could have a substantial impact on estimates of adverse health risk from low-dose radiation. This phenomenon has been observed in model systems from cell culture up to whole animals (*78, 85–88*). However, adaptive responses do not seem to be induced at the moderate dose levels of interest to this report.

*Mechanisms of Susceptibility to Carcinogenesis and Tissue Injury*

In a nuclear accident or intentional exposure, the vast majority of an irradiated population will likely receive a dose of $<1.5$ Gy and will not develop any acute radiation symptoms. Cured cancer patients are likely to survive for many years. Although the risks are low, survivors in all

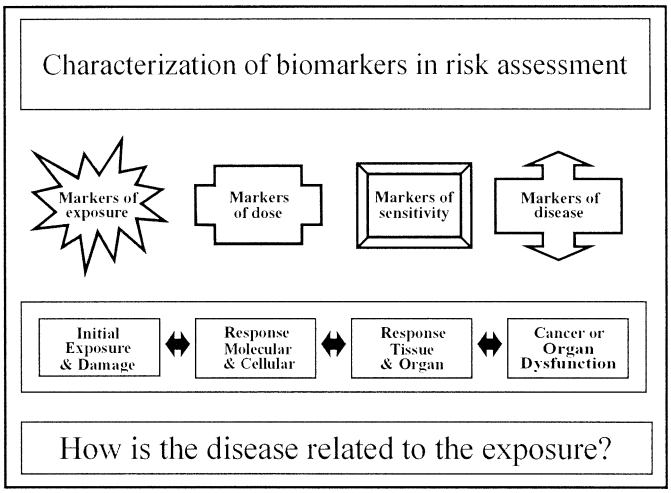


**FIG. 1.** The relationship between exposure and effect. The molecular, cellular and tissue responses vary among individuals; appropriate biomarkers can then be useful in determining an individual's risk and therefore possible therapeutic intervention. Figure provided by A. L. Brooks.

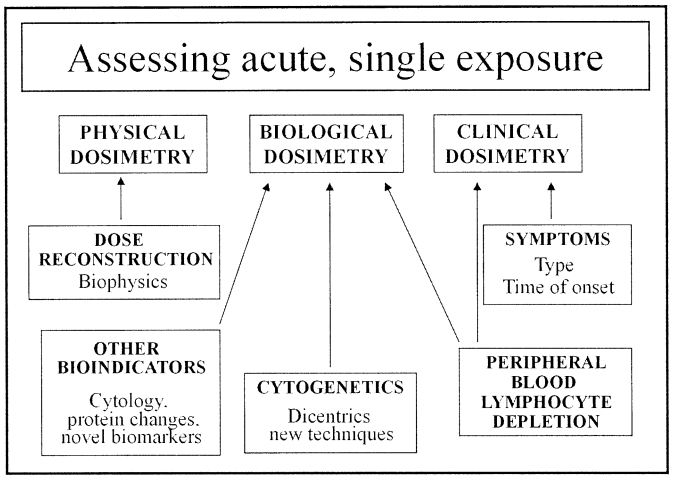


**FIG. 2.** Biodosimetry for clinical use: current state of the science. Figure provided by P. Voisin, Institut de Radioprotection et Sûreté Nucléaire (IRSN), Fontenay aux Roses, Cedex, France.

radiation exposure groups will be at some increased risk for development of a radiation-induced malignancy. Ionizing radiation is an established mutagenic and carcinogenic agent, albeit a weak one (*89*); however, the underlying mechanisms responsible remain to be fully determined. Radiation is known to induce chronic inflammation, genomic instability, and expression of genes involved in anti-apoptosis. As the pathways involved in radiation-induced oncogenesis are elucidated and the mechanisms of noncarcinogenic late tissue damage are defined, treatments to prevent secondary malignancy or injury could be conceived. Furthermore, because individuals vary in their susceptibility to such complications, research is needed to develop biological markers and assays that can determine individual risk.

In regard to noncarcinogenic tissue damage, animal models and human studies suggest that individual subjects have naturally differing expression of cytokines that have significant effects on the expression of radiation toxicity (*25, 90–98*). Clarification of these mechanisms and development of suitable biomarkers would provide important information for determining long-term risk and potential preventative treatment.

## BIODOSIMETRY AND BIOMARKERS

### *Summary of Critical Information*

In accidental or intentional exposure, life-threatening injuries must be treated first, followed by appropriate decontamination procedures for exposed individuals. Biodosimetry combined with physical dosimetry then becomes a priority because individuals may respond differently to the same dose. The underlying concepts of biodosimetry and biomarkers are summarized in Fig. 1, which relates the concept of exposure to ultimate biological effect (i.e. a disease or illness).

### *Biomarkers*

At exposures of 1–10 Gy, there are currently a number of useful biomarkers that have the sensitivity to quantify exposure expeditiously. Medical response for radiation accidents involves the use of multiple parameters of physical dose, biological dosimetry, and clinical diagnostics, as illustrated in Fig. 2.

Because a biomarker is an indicator of biological processes, the time at which it should be sampled depends on the type of exposure and the end point as well as on the type of tissue to be sampled. Table 3 indicates how current biomarkers would be used in situations of external exposure Table 4 provides similar information for internal exposure.

The selection of the proper biomarker of radiation exposure depends on the exposure scenario and on the tissues available for sampling. The ''gold standard'' for external exposure has been dicentric chromosomal aberrations scored in peripheral blood lymphocytes (*17, 40, 99–101*). Blood sampling should be performed 1 day after exposure to ensure adequate circulation of blood to obtain a representative sample (*99*). However, other markers of exposure in lymphocytes are available (*102*), including PCC (*30, 37, 103*), changes in the expression of well-defined genes (*26, 63*), and the number and characterization of lymphocytes (*19, 20, 104, 105*).

For the moderate radiation doses considered in this report, the frequency of dicentrics per cell would be very high and thus would not require scoring many cells to estimate the level of the radiation exposure (*30*). Lymphocytes from peripheral blood would be available for cytogenetic biodosimetry analysis for several days after exposure to doses up to 4 Gy. However, blood lymphocytes are very sensitive to cell killing so that after higher doses this cell population

**TABLE 3**
**Biomarkers of External Exposure**

| Exposure type | Biological samples | Test and sampling time |
|---|---|---|
| Acute whole-body | Blood lymphocytes | Blood count and molecular and cellular changes in tissue at early time after exposure |
| | Buccal mucosa cells | |
| | Tooth enamel | ESR (electron spin resonance)—any time after exposure |
| Chronic whole-body | Blood lymphocytes | Chromosomal changes—any time after exposure. |
| | Tooth enamel | ESR—any time after exposure |
| Acute partial-body | Blood lymphocytes | Molecular and cellular changes—early time after exposure |
| | Target organ | Functional assay, possibly tissue biopsy |

is depleted as a function of both dose and time after exposure (*104, 105*). In the 1.5–7-Gy dose range, dose estimates can also be obtained from measurement of lymphocyte depletion kinetics from peripheral blood cell counts in this early time (1–7 days) after exposure (*104, 106*). In the dose region where lymphocytes are depleted, biomarkers in other tissues need to be considered and developed further.

Another tissue that is readily available and easily sampled and that provides a source of epithelial cells is the buccal epithelium (*107*). It is possible to sample viable cells, score micronuclei, and obtain RNA and DNA samples. Additional research is needed on other potential biomarkers that can be employed using this cell type, such as PCC and FISH. Detection of changes in electron spin resonance (ESR), being studied in the teeth of rats, provides a very sensitive indicator of dose into the 20-mGy range (*108*).

Fallout could result in nonuniform exposure from internally or externally deposited radioactive materials. In a nuclear accident or bioterrorism event, internal deposition of radioactive isotopes may occur, despite efforts to prevent or minimize ingestion and inhalation. Long-lived ingested isotopes will cause less acute lethality even after high doses because of their protracted exposures, but they could still cause late tissue damage and an increased risk of cancer. Biokinetic models can be used to determine the dose from internally deposited radioactive materials using input data based on whole-body and target-organ counting and measurements of samples of blood, urine and feces and to determine if intervention is needed (*109*). Most biomarkers of tissue damage have limited usefulness for internally deposited radioactive materials since the tissue in which the radiation is concentrated is not usually available for evaluation. This is especially true for $\alpha$-particle-emitting radionuclides, where the range in the tissue is only a few tens of micrometers.

## RADIATION PROTECTORS AND TREATMENT OF RADIATION EXPOSURE

### *Summary of Critical Information*

The treatment of individuals exposed to whole-body radiation will depend on clinical status, ensuing clinical response, and estimates of exposure level. For patients with indications of potentially lethal levels of exposure, standard supportive care regimens developed for patients undergoing total-body irradiation in preparation for bone marrow transplantation should be employed. This includes the use of antibiotics and antiemetics, perhaps supplemented by the use of chelators for specific isotopes to which the individual may have been exposed (*110, 111*). However, current therapeutics are limited, and effective prophylaxis and treatment of radiation injuries will require novel strategies to prevent hematopoietic, GI, pulmonary, renal and cutaneous syndromes and their associated long- and short-term effects.

**TABLE 4**
**Biomarkers of Internal Exposure**

| Exposure type | Biological samples | Sampling time |
|---|---|---|
| $\beta$-particle/$\gamma$-ray emitters | Partial- (including target organ) and whole-body counting | Early time and multiple counts postexposure |
| | Body fluids (blood, urine, saliva, etc.), expired air, nasal swipes, and fecal samples | Multiple counts postexposure |
| | Cells or tissue from target organ | Any time postexposure |
| $\alpha$-particle emitters | Body fluids (blood, urine, saliva, etc.), expired air, nasal swipes, and fecal samples | Early time and multiple counts postexposure |
| | Cells or tissue from target organ | Any time postexposure |

Historically, considerable scientific effort has been put into the development of chemical radioprotectors with antioxidant properties that might be taken by individuals prior to entry into a radioactively contaminated site. Current limitations of such radioprotectors are that the radioprotective effects are not long-lasting, toxicity is associated with their use at cytoprotective doses, and they are most effective when administered prior to exposure to radiation (*112*). Growth factors and cytokines have also been investigated for their ability to prevent radiation-induced damage and to accelerate recovery of tissue stem cells and their precursors after radiation exposure. The most promising are the hematopoietic growth factors such as G-CSF (*113*), GM-CSF (*114–118*), SCF (*119*), IL11 (*120, 121*), MGDF (*122*), Flt-3 ligand (*123, 124*), IL7 (*125, 126*), and new epithelial cell-specific growth factors, such as keratinocyte growth factor, KGF (*127–132*). As our ability to treat the acute radiation syndromes improves, late damage to other organ systems will become evident and will need to be addressed. This is also relevant to cancer treatment with radiation alone and/or combined with chemotherapeutic or biological agents as well as to other types of radiation exposures. Recently, strategies have been developed to reverse certain long-term radiation-induced physiological imbalances in tissues, with some success. Although the mechanisms by which this can be achieved are not fully known, the role of free radicals and redox state in mutagenesis, carcinogenesis and normal tissue injury after radiation exposure is a highly promising area of research that needs to be explored.

### *Chemical Radiation Protectors*

In the past, development of drugs for use in radioprotection focused on chemicals possessing antioxidant properties. At present the phosphorothioate, amifostine (Ethyol®), is the only radioprotector drug that has been approved by the FDA and is applicable for decreasing the incidence of moderate to severe xerostomia (dry mouth) in patients undergoing radiation therapy for the treatment of head and neck cancer (*112, 133*). This agent is the most studied of the radioprotector drugs developed by the Antiradiation Drug Development Program of the U.S. Army Medical Research and Development Command (*134*). However, toxicity may limit its general applicability in that it often requires co-administration with an antiemetic agent. Clearly, there is a need for the development of additional agents that can prevent and ameliorate radiation injury.

Other agents are under development in the laboratory (*1*). Nitroxides, represented by the compound tempol, scavenge free radicals formed by ionizing radiation (*21, 135, 136*). Both aminothiols (amifostine) and nitroxides have been found to be effective in protecting against radiation toxicity to cells and tissues and appear to reduce mutagenesis and carcinogenesis in rodents (*137, 138*). It is unknown whether these agents will have similar anticarcinogenic effects in humans.

Amifostine, even at low noncytoprotective doses, is effective in protecting against radiation-induced mutagenesis and carcinogenesis in rodents (*137, 139*). Because the dose of amifostine in mice needed to protect against radiation-induced mutagenesis is about one-twentieth that required to protect against cell killing, it may be possible to develop both oral and topical forms of drug administration for use in an antimutational and/or anticarcinogenesis application. The lower drug dose needed for this use is likely to exhibit less toxicity.

An important limitation of the current radioprotectors is the requirement that they be administered intravenously (*140*). Although this may be achieved under controlled clinical conditions, such as with radiotherapy patients, this limits its applicability under emergency conditions in the field. There is ongoing research into the administration of radioprotectors by a subcutaneous route.

Another potential radioprotector that currently is being studied is the antioxidant enzyme superoxide dismutase (SOD). This may be considered a biological agent in that SOD has been modulated by gene therapy (*69, 141*). Chemical radioprotector treatments may act by inducing SOD, as noted below. Both superoxide and hydroxyl radicals generated by ionizing radiation are rapidly destroyed by SOD with the generation of hydrogen peroxide, which is converted by intracellular catalase to oxygen and water (*136*). Overexpression of intracellular manganese superoxide dismutase (MnSOD, SOD2) has been demonstrated to be radioprotective in rodents (*69*). The gene therapy approach has been demonstrated to be effective in preclinical testing (*69, 141*), and clinical trials are planned for further evaluation using radiation doses $>10$ Gy. Antioxidants must be administered prior to radiation exposure to be effective protectors, because the half-life of radiation-induced free radicals is so short that free radical damage is essentially complete by $10^{-3}$ s (*136*).

Although antioxidants generally work best if given around the time of irradiation, recent observations may change this concept: Thiol-containing drugs such as *N*-acetylcysteine, oltipraz, captopril and amifostine, as well as cytokines such as KGF (keratinocyte growth factor), TNF (tumor necrosis factor), and IL1 (interleukin 1) can induce production of MnSOD, and it may be worth examining these compounds in postirradiation settings (*142*). For example, amifostine can increase MnSOD 24 h after administration; resistance to 2 Gy is similar at this time whether the amifostine is present or has been removed (*142*). Although the prolonged radioprotective effects could be advantageous for postexposure treatment in an environmental radiation exposure, this might not necessarily be suitable for radiotherapy, where treatments are given daily, and persistent radioprotectors could reduce tumor response. The potential of any radioprotective agent for cancer treatment will require attention to dose, schedule and mechanisms of protection and avoidance of tumor protection.

Additional classes of radioprotectors under development

in the laboratory include a group of agents called ''neutraceuticals'', which includes plant flavonoids such as orientin and genistein (*143*)[4] and vitamin E analogs (*144–146*).

*Biological Agents*

The use of biological agents to limit damage after radiation exposure draws heavily on clinical and preclinical experience with hematopoietic cytokines and other growth factors (*147*). In contradistinction to chemical agents that protect all or most tissues, growth factors target specific cell populations, and their use is best considered in the context of specific radiation-induced syndromes.

*Treatment of the Hematopoietic Syndrome*

Strategies to counter this syndrome come from the field of bone marrow transplantation. Options include the use of cytokines that expand specified stem and progenitor cell populations *in vivo* and *in vitro*, as well as the use of stem cell transplants. Numerous cytokines have been demonstrated to prevent radiation-induced hematopoietic deficiency in animal models. There is sufficient clinical experience using these agents in the treatment of chemotherapy-induced myelosuppression to be able to assess their probable utility in a setting of acute whole-body exposure to moderate radiation doses. The primary goal in such situations is to eliminate the obligate periods of neutropenia and thrombocytopenia (low white blood cell and platelet counts). Most preclinical and clinical experience has been obtained with G-CSF and GM-CSF (see above), which shorten the duration of neutropenia and time to recovery of neutrophils in myelosuppressed patients subsequent to chemotherapy or myeloablative (marrow ablative) conditioning prior to stem cell transplant and are approved for use in these indications by the FDA. These benefits translate into fewer days on antibiotics, less risk of infection, and significantly less morbidity. G-CSF and GM-CSF have been safely administered to hundreds of thousands of patients.

The Centers for Disease Control and Prevention (CDC) and the Armed Forces Radiobiology Research Institute (AFRRI) are working with experts in the field of growth factors to develop guidelines for the use of these agents in radiological terrorism settings.[5] The FDA has provided assistance in consideration of submission of an IND (investigational new drug) and discussion of requirements for licensure of growth factors for treatment of radiation-induced injury under the Animal Rule (see Glossary, Appendix 4). According to the requirements for licensure under the Animal Rule, definitive studies are needed in acutely irradiated animals to demonstrate the impact on clinical end points such as mortality, infection, febrile neutropenia and, potentially, incidence of malignancies. If such studies support licensure, it would potentially remove the requirements for study of such growth factors for radiation injury under IND. Early discussion with the FDA to define acceptable end points is recommended.[6]

Numerous other cytokines have been tested in preclinical models, but few have entered into common clinical use. They may, however, be of value as radioprotectors within the framework under consideration in this report. The following agents may be effective if given either before or after irradiation. Stem cell factor (SCF) acts on both primitive and mature progenitor cells and is best given before exposure (*119*). It is approved for clinical use in Europe but not in the U.S. Preclinical studies have shown that recombinant SCF can protect against lethal irradiation, elicit multilineage hematopoietic responses and increases in bone marrow cellularity, and increase the number of circulating peripheral blood progenitor cells (PBPCs) in a dose-dependent manner. Both preclinical and early clinical studies using recombinant methionyl human SCF plus recombinant methionyl human granulocyte colony-stimulating factor (Filgrastim®) have demonstrated increased PBPC mobilization compared to the use of either factor alone (*148*).

Thrombocytopenia has been more difficult to combat than neutropenia, but is perhaps less of an immediate problem after chemotherapy or radiation exposure. Currently there is only one cytokine, IL11 (Neumega®, oprelvekin), that is FDA-approved for reducing chemotherapy-induced thrombocytopenia (*149–151*). Unfortunately, IL11 has proven at times to be only modestly efficacious clinically and has elicited significant toxicities (*152*). Thrombopoietin (TPO) (*153, 154*) and megakaryocyte growth and development factor (MGDF) (*155*) continue in both preclinical and clinical trials, but both require further investigation. Clinical development of TPO and MGDF was limited by the development in the patient of neutralizing antibodies to the treatment. Cytokines for reconstituting the immune system, such as IL7 and Flt-3 ligand, are under development and may prove of value in treatment after radiation exposure (*156, 157*).

Moderate-dose radiation exposure of the magnitude associated with neutropenia and thrombocytopenia will lead to the development of subacute anemia approximately 3 months after the exposure (*158*). This condition can be treated with blood transfusion in emergency settings but can be addressed more effectively over the long term with cytokines including erythropoietin (Epogen®, Procrit®) (*159*) and novel erythropoiesis stimulating protein (NESP, darbepoetin) (*160*).

*Stem Cells and Immune Function*

Cytokine-based therapy of radiation injury has fewer logistical problems and is less technically demanding than stem cell transfer using either auto- or allotransplants, al-

[4] M. R. Landauer, T. M. Seed, V. Srinivasan and A. Shapiro and C. Takimoto, Phytoestrogenic isoflavone compositions, their preparation and use thereof for protection and treatment of radiation injury, U.S. and International Patent Application Filed June 12, 2001.

[5] J. Waselenko *et al.*, in preparation.

[6] Personal communication, Dr. Amy Rosenberg.

though the latter may be advantageous under specific conditions. The approaches are not mutually exclusive. For example, banking of autologous cells may be desirable prior to entry of personnel into contaminated areas. Cytokine-mobilized peripheral blood and umbilical cord blood are the most readily available sources of stem and progenitor cells for autologous or allogeneic transplantation (*161, 162*). Cord blood is rather low in cell numbers for transplantation into adults, but methods to expand hematopoietic stem and progenitor cells *in vitro* using combinations of cytokines and cell selection technologies may make this a valuable resource in the future. Because of the paucity of compatible HLA-matched stem cell donors and the length of time needed to find them, allogeneic stem cell transplants will have a very limited application for accidental and intentional exposures.

Despite the probable utility of the therapies mentioned above, there is a need for novel strategies to counter the defects in immune function and increased mortality associated with the hematopoietic syndrome. One new approach uses the steroid 5-androstenediol (androst-5-ene-3 beta,17 beta-diol, AED) (*163, 164*). In rodents, AED stimulated myelopoiesis, ameliorated neutropenia and thrombocytopenia, and enhanced resistance to infection after exposure to ionizing radiation. Further preclinical research is needed using large animals to confirm efficacy and to define the best setting for evaluating this drug in humans.

The effects of moderate-dose radiation on the fate of memory T and B cells mediating protection against infectious disease after irradiation require further study. Initial studies of allospecific memory responses *in vivo* after irradiation with 3–5.5 Gy show an abrogated memory response (*165, 166*). Should this be the case in humans, irradiated individuals could lose their ability to fight conventional infections and those from bioterrorist weapons. Further information on this effect should be accumulated from studies conducted in animals and from patients undergoing total-body irradiation.

### *Other Organ Systems*

As our ability to treat the hematopoietic syndrome improves, damage to other organ systems will become evident and will need to be addressed. This is very relevant to clinical cancer treatment with radiation and with combined modality therapy with radiation plus chemotherapeutic or biological agents.

### *Treatment of Gastrointestinal (GI) Syndrome*

Whole-body radiation doses in the range of 2–6 Gy are sufficient to produce severe leukopenia and predisposition to death from infection. Moderately higher doses (7–12 Gy) cause a more acute death attributed to the GI syndrome. Crypt cell death and possibly endothelial cell death in the submucosal vessels occur in the higher end of this range and above (*47, 48*). The crypts and villi of the small bowel are affected, with damage appearing histopathologically within a few days after irradiation. Thus deaths that occur in less than 10 days after exposure are usually attributed to the GI syndrome.

Loss of the integrity of the mucosal surface predisposes to sepsis, fluid and electrolyte loss, and malabsorption. Supportive measures that include the use of antibiotics and fluid administration are important (*167, 168*). A unique feature of the GI tract is the option for use of oral and nonabsorbable therapies, in addition to intravenous therapies. Altering subclinical effects of GI syndrome in the lower-dose range is likely to reduce lethality from bone marrow syndrome, even at doses less than 7 Gy. Nonabsorbed orally administered antibiotics are of proven benefit in immunosuppressed patients.

Some hematopoietic growth factors appear to protect against GI syndrome as well, although the mechanism is unclear. Agents that specifically protect epithelial surfaces need to be explored in more detail and new agents developed. Keratinocyte growth factor (KGF) is the only epithelial-specific growth factor currently available (*128, 130, 131*). It mediates proliferation, differentiation and homeostasis in a wide variety of epithelial cells, including type II pneumocytes (*129, 132*), keratinocytes (*96*), hepatocytes (*169*), gastrointestinal epithelial cells (*128, 130, 131*), and uroepithelial cells (*170*). In preclinical models, KGF has been shown to prevent oral and lower GI tract mucositis (*127–131*), hemorrhagic cystitis (*171*), pulmonary injury (*132*) and alopecia (*172*), and it can be effective if given before or after irradiation (*128*). Recombinant human KGF is currently in clinical trials for mucositis.

### *Kidney and Lung*

Chronic renal failure is a late complication of exposure to radiation in the myeloablative dose range (*173*). There is a need for better understanding of this syndrome. Radiation-induced chronic renal failure can evolve to end-stage renal disease requiring chronic dialysis or renal transplantation and result in a shortened life span. There is growing evidence that the renin-angiotensin system is important in the expression of renal radiation injury (*174*). Progression of established radiation nephropathy in rats was delayed by continuous treatment with captopril, an angiotensin-converting enzyme (ACE) inhibitor, or an angiotensin II Type-1 (AT1) receptor antagonist (AII blocker, e.g. losartan) (*175*). There is extensive clinical experience with these agents for cardiovascular disease, and they are well tolerated.

In the rat, these interventions are particularly important between 3 and 10 weeks after irradiation, which supports the concept that there are specific and sequential events in the pathogenesis of kidney failure (*175*). The underlying mechanisms require investigation to enhance our understanding of their optimal use in this context. Nonetheless,

these agents are promising and are already available for clinical use.

In addition to protecting against radiation nephropathy, both ACE inhibitors and AII blockers have been found to protect rats against radiation-induced pneumonitis and fibrosis (*176*). There are biological reasons to suggest that they might also protect the central nervous system. Keratinocyte growth factor (KGF) stimulates the differentiation of Type II pneumocytes into Type I pneumocytes that are responsible for gas exchange in the lung (*132*). Currently, no clinical data are available on postirradiation use of these drugs to ameliorate radiation-induced pneumonitis, and they should be investigated in this regard.

*Radiation Fibrosis*

The concept that late effects can be ameliorated by treatments given some time after irradiation has been supported by the finding that pentoxifylline with tocopherol can reverse fibrosis in human patients (*144*). The mechanisms of these effects are not understood, as pentoxifylline has multiple effects on cytokine production, red cell deformability, and cell cycle progression. Cu/Zn and MnSOD have similar effects in ameliorating radiation-induced fibrosis in pigs and in patients (*70, 71*), and they also reduce the incidence of radiation-induced cystitis (*177*), suggesting that some aspect of oxidative stress is involved (see above). Studies with ACE inhibitors, pentoxifylline and SOD have provided clear evidence that late consequences of irradiation can be reversed, even if treatment is initiated some time after exposure (*178*). Studies on the underlying mechanisms are urgently required so that the pathways that are involved can be specifically targeted and new drugs can be developed.

*New Approaches for Developing Drugs to Protect Normal Tissues: High-Throughput Screening*

High-throughput screening (HTS) has been used for a number of years by academia and the pharmaceutical industry as a tool for drug discovery. HTS can also be applied to the identification of novel radioprotectors and protectors against normal tissue injury from a variety of stresses. For this to occur there are three basic requirements:

1. Agents to test (combinatorial libraries composed of synthetic small molecules and/or libraries of natural products).
2. Assay systems amenable to automation.
3. Appropriate normal tissue targets.

A number of libraries are currently available and more are being developed; assays amenable for high-throughput analysis can be developed based on a compound's ability to alter the function of a specific protein or modify a biological process. The most difficult task will be determining the specific protein or process to target in the HTS approach. This will require an increased understanding of the cellular and molecular events that regulate the radioresponse of normal tissues. However, based on the current understanding of the radiobiology of normal tissue, possible targets for use in HTS include apoptosis, cell cycle progression, DNA repair, oxidative stress, TGFB-mediated gene transcription, and activity of various other cytokines. The discovery of compounds that inhibit these events not only may lead to the identification of radioprotectors but also may provide insight into the mechanisms regulating the radioresponse of normal tissues.

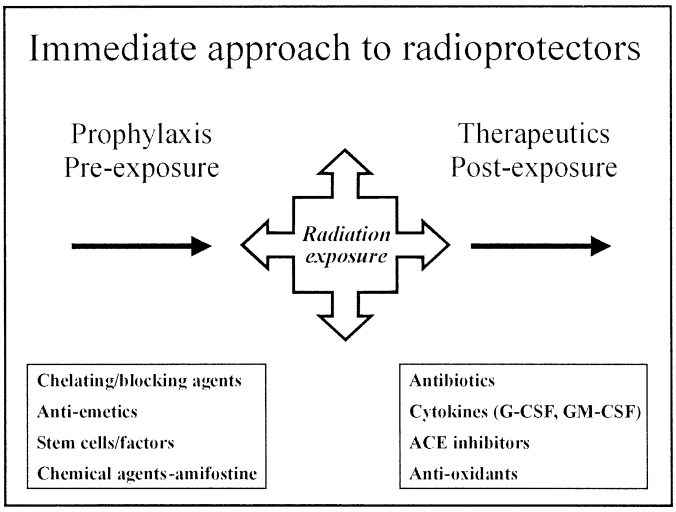


**FIG. 3.** Pre- and postexposure measures for reducing radiation injury, available now or within about 1 year.

*Approaches to Radioprotection*

Timelines for the development of effective new therapies cannot be stated with certainty. To help conceptualize the state of the science, approaches were arbitrarily divided into three categories:

1. *Immediate* approach indicates drugs and biological agents that have been used in patients. Analogues of these drugs would require further development over a longer time frame (illustrated in Fig. 3, showing agents that are given either before exposure for prophylaxis or after radiation exposure as therapy to ameliorate damage).
2. *Medium-term* approaches, estimated to be ready for clinical use in about 1–3 years, indicate approaches that are already under development in the laboratory but require additional research.
3. *Long-term* approaches are those in earlier stages of development in the laboratory that may lead to new treatments in several years.

*Agents for Radioprotection: Summary of Critical Information*

There is a pressing need to develop better agents using both empirical and mechanistic approaches. Interdisciplinary strategies and coordination will be essential in achiev-

ing the scientific and population-based goals. The underlying general principles for development include:

1. Basic research into biological mechanisms ranging from molecular biology through whole-animal studies.
2. Establishment of appropriate animal models and research facilities to study normal tissue injury and radiation protectors in long-term experiments.
3. High-throughput screening and evaluation of molecular targets of radiation injury in normal tissues.
4. Ongoing interaction and dialogue between scientists, industry and regulatory agencies;
5. Adequate supply and distribution of effective drugs; orally administered compounds are highly desirable.
6. For clinical radiation therapy, the assessment of whether a given radioprotector affects tumor radioresponse.

*Available Radioprotective Agents*

For the individual categories, the target tissue and pertinent research questions are included.

## PROPHYLACTIC ADMINISTRATION (PRE-EXPOSURE)

### *Immediate*

#### Amifostine and other aminothiols

*Target tissue:* bone marrow, GI tract, salivary gland (approved by FDA), lung, kidney, liver, spermatogonia, hair follicles (amifostine is not effective in central nervous system).

*Research needs:* explore and develop additional agents including those with potential for oral or topical delivery; protection of renal function; protection of lung function; protection of central nervous system function (with newer agents). *For radiation therapy*, determine whether new agents protect tumors.

#### KGF (keratinocyte growth factor)

*Target tissue:* epithelial tissue, hair follicles.

*Research needs:* schedule and dose; determine effect on GI immunity and bacterial infection. *For radiation therapy*, determine whether new agents protect tumors.

#### Antiemetics

*Target tissue:* GI tract- and central nervous system-related nausea.

*Research:* none.

#### Stem cell banking

*Target tissue:* bone marrow.

*Research needs:* means of *in vitro* expansion of hematopoietic stem cells; potential use of umbilical cord blood.

### *Medium-term*

#### Nitroxides

*Target tissue:* whole body.

*Research needs:* time/dose/efficacy, toxicity, pharmacokinetics; determine mechanism of effect; explore role in post-treatment protection and anticarcinogenesis. *For radiation therapy*, determine whether new agents protect tumors.

#### MnSOD

*Target tissue:* mitochondria (therefore, potentially all tissues).

*Research needs:* schedule and dose; *in vivo* studies of different organs; duration and magnitude of effect; induction of MnSOD by reducing and other agents, delivery (gene therapy)—can it reach target? *For radiation therapy*, determine whether new agents protect tumors.

#### AED (5-androstenediol)

*Target tissue:* bone marrow, immune cells.

*Research needs:* effects on bone marrow biology. *For radiation therapy*, determine whether new agents protect tumors

#### SCF (stem cell factor)

*Target tissue:* bone marrow.

*Research needs:* combination with other growth factors or radioprotectors; toxicity. *For radiation therapy*, determine whether new agents protect tumors.

#### Antioxidants

#### (vitamin E, selenium, *N*-acetyl cysteine, captopril, mesna, oltipraz)

*Target tissue:* whole body or specific tissues.

*Research needs:* determine localization, tissue-specific protection, long-term effects. *For radiation therapy*, determine whether new agents protect tumors.

### *Long-term*

#### Prostaglandin/COX2 inhibitors

*Target tissue:* whole body, CNS.

*Research needs:* efficacy studies.

## THERAPEUTIC ADMINISTRATION (POSTEXPOSURE)

### *Immediate*

#### ACE inhibitors (other receptor blockers)

*Target tissue:* kidney, lung, possibly CNS.

*Research needs:* animal studies; mechanisms; clinical trials for radiation therapy.

**Growth factors (G-CSF, GM-CSF, KGF, EPO)**

*Target tissue:* bone marrow, whole body.
*Research needs:* time of delivery postexposure. *For radiation therapy*, determine whether new agents protect tumors

**Chelating and isotope-competing agents**

**(Prussian blue,[7] DTPA, EDTA, potassium iodide, penicillamine, alginates)**

*Target tissue:* thyroid, bone marrow.
*Research needs:* determine isotope specificity.

**Pentoxifylline/Vitamin E/SOD**

*Target process:* fibrosis.
*Research needs:* mechanism, schedules; further clinical trials.

**Antiemetics**

*Target tissue:* GI, CNS.
*Research needs:* none.

***Medium-term***

**Pentoxifylline**

*Target process:* fibrosis.
*Research needs:* derivatives; mechanism. *For radiation therapy*, determine whether new agents protect tumors

**Amifostine (anticarcinogenic effects)**

*Target process:* mutagenesis, carcinogenesis (given within 3 h of exposure).
*Research needed:* mechanism; human model system; possibly future clinical trials.

**Tempol and other nitroxides**

*Target tissue, process:* whole body, fibrosis.
*Research needed:* analogues; efficacy, *in vivo* studies. *For radiation therapy*, determine whether new agents protect tumors.

**Stem cell transplants**

**(bone marrow, umbilical cord blood, peripheral blood, liver, CNS)**

*Target tissue:* bone marrow, CNS, liver.
*Research needs:* define stem cell populations, schedules/cell numbers required.

[7] Prussian blue (request for New Drug Application from FDA): http://www.fda.gov/cder/drug/infopage/prussian_blue/q&a.htm.

***Long-term***

**MGDF, IL11**

*Target tissue:* bone marrow.
*Research needs:* isolate, identify. *For radiation therapy*, determine whether new agents protect tumors.

**Flt3 ligand, IL7**

*Target tissue:* bone marrow, thymus/lymphocytes.
*Research needs:* effects on immunity. *For radiation therapy*, determine whether new agents protect tumors.

**Agent combinations**

*Target tissue/process:* all.
*Research needs:* define appropriate combinations, efficacy, schedules/doses.

**Prostaglandin/COX2 inhibitors**

*Target tissue/process:* inflammation, all tissues.
*Research needs:* efficacy, mechanism. *For radiation therapy*, determine whether new agents protect tumors.

## DETAILED RECOMMENDATIONS

1. *Research* is needed in the following areas to increase understanding of the fundamental effect of ionizing radiation on human biological systems.
   a. Determine genetic and epigenetic mechanisms that govern individual susceptibility to radiation, including those involved in cell death, the bystander effect, cancer induction, organ-specific damage, and the fibrotic response.
   b. Develop and characterize genetic, chromosomal, gene expression, and protein biomarkers of exposure in the range of 1–10 Gy.
   c. Define the effects of ionizing radiation on function of tissue stem cells (proliferation, differentiation and migration). Both acute and long-term animal studies are essential to determine the consequences of radiation-induced stem cell dysfunction.
   d. Define the effects of ionizing radiation on function of parenchymal cells of tissues and organs that develop chronic radiation injuries (e.g. proliferation, apoptosis, cytokine response and production).
   e. Conduct long-term animal studies to determine the consequences of radiation-induced parenchymal cell dysfunction, as well as stromal and endothelial cell populations.
   f. Continue long-term organ and animal toxicity studies of ionizing radiation alone and in combination with radioprotector drugs and biological agents.
   g. Conduct epidemiological studies of late normal tis-

**TABLE 5**
**Key Recommendations from Meeting Report. Modifying Normal Tissue Damage Postirradiation. Report of a Workshop Sponsored by the Radiation Research Program, National Cancer Institute, Bethesda, Maryland, September 6–8, 2000 (*179*)**

| | |
|---|---|
| Long-term support | Late effects develop months to years after therapy; long-term preclinical and clinical studies will be necessary to track the process. |
| Multidisciplinary approach | Radiation biologists, physcists and oncologists will need the assistance of pathologists, physiologists and geneticists, as well as experts in functional imaging, would healing, burn injury, molecular biology, and medical oncology. |
| LENT/SOMA scoring system | An effective scoring system is essential for assessing late effects in patients, and for comparing treatments. Objective scoring systems must replace subjective systems as they are developed and validated. The system should be computerized and refined for ease of use. |
| Tissue sharing; tissue bank | A repository of irradiated and unirradiated normal tissue could be useful resources for research. |
| Mechanistic studies | It will be necessary to identify potential targets for interventions and how they will be most useful in a clinical setting. |
| Dose modification factors | Radiation dose–response studies in clinically relevant dose ranges and treatment schedules will identify potential treatments and assist in choosing therapies for clinical trials. |
| Study models | Models to study late effects and their mechanisms must be chosen carefully and, in some cases, new models should be developed. |

sue toxicity in people exposed to radiation in cancer treatment and in accidental or intentional exposure.
   h. Identify molecular targets for intervention in ionizing radiation-induced injury.
   i. Investigate the role of oxidative stress in the cellular and tissue response to ionizing radiation and the role of antioxidants for prevention and treatment of injury.
2. *Technologies* will be required for investigations of ionizing radiation-induced injury.
   a. Develop systems for analysis of gene and protein expression of normal tissues (normal tissue ''chips'').
   b. Develop high-throughput assays based on molecular targets to identify novel protectors of normal tissue injury.
   c. Develop detection technology for rapid analysis of molecular biomarkers of radiation exposure for large numbers of samples. Automate sample preparation and analysis for cytogenetic bioassays.
3. *Treatment strategies*
   a. Develop treatment strategies for use before and after exposure based on optimizing current approaches and on newly discovered molecular, cellular and tissue targets.
   b. Facilitate cooperation and collaboration among industry, government agencies and the academic communities for the development, testing and production of new agents.
   c. Coordinate drug development strategies with the FDA, optimizing preclinical development, including using the Animal Rule, which allows adding an indication to the label of a drug when human trials are not ethical or feasible.
4. *Ensuring sufficient expertise*
   a. Increase the pool of researchers with expertise in normal tissue and animal radiation biology. There is a very serious shortage of such individuals.
   b. Increase the pool of experts in health physics, radiation protection and dosimetry.
   c. Support long-term animal studies in radiation toxicology and effective protection strategies.
   d. Recruit individuals with expertise in cellular biology, molecular biology, physiology and wound healing to the normal-tissue radiobiology field.
   e. Include training in long-term late effects of ionizing radiation, chemotherapy and biotherapy in the education of oncologists.
   f. Support national capabilities for medical radiological response.

## APPENDIX 1

### Summary of RRP-NCI Workshop on Modifying Normal Tissue Damage Postirradiation (*179*)

The Radiation Research Program of NCI held a workshop in September 2000 entitled ''Modifying Normal Tissue Damage Postirradiation.'' The group focused on the higher doses encountered in radiation therapy, but the underlying mechanistic studies are relevant to the current moderate-dose workshop. The workshop brought together experts in radiation oncology and radiation biology with those outside the radiation field, including physiology, functional imaging, inflammation, wound healing, and molecular biology, to identify research opportunities that could lead to development of treatments to prevent or reverse late effects. Late effects develop in the months to years after treatment and include such problems as fibrosis, radionecrosis, stricture, fracture and ulceration. The risk depends on the dose and schedule of irradiation, chemotherapeutic agents, the tissue or organ, the volume irradiated, the time after irradiation, precipitating factors such as surgery or dental extraction, and predisposing factors in the patient, such as genetic susceptibility and comorbid conditions. Late effects were thought to be inevitable and irreversible, but we are now looking at the development of late effects as a process similar to wound healing or inflammation, involving a series of steps that might be redirected toward more satisfactory healing. There are a number of studies that suggest this is possible. Key recommendations of the workshop are included in Table 5.

## APPENDIX 2

### Workshop: Molecular and Cellular Biology of Moderate-Dose Radiation and Potential Mechanisms of Radiation Protection, Bethesda, MD, December 17–18, 2001

| **December 17** | **Presenter** |
|---|---|
| *Introduction and welcome* | Norman Coleman, James Deye, William F. Blakely, Bruce Wachholz |
| *Genetic effects* | *Moderator:* Julian Preston |
| Chromosomal damage | Joel S. Bedford |
| Mutation and carcinogenesis | Howard L. Liber |
| Oxidative stress | Michael E. C. Robbins, David Gius |
| Gene expression | Gayle E. Woloschak, Sally A. Amundson |
| Protein expression | Alexandra C. Miller, David Boothman |
| *Epigenetic effects* | *Moderators:* Noelle Metting, Richard Pelroy |
| Bystander effect | William F. Morgan, Eric Hall |
| Cellular/tissue effects | Mary Helen Barcellos-Hoff, John Fike |
| Biological dosimetry | William F. Blakely, Antone L. Brooks |
| *Accidental medical exposure response* | *Moderators:* W. F. Blakely, Robert C. Ricks |
| Assessment, Diagnosis, and Clinical Care | W. F. Blakely, Ronald E. Goans |
| *Radiation protectors* | *Moderators:* William H. McBride, Helen Stone |
| Radiation protector—amifostine | David J. Grdina |
| Radiation protector—nitroxides | James B. Mitchell |
| Radiation protector—SOD | Joel Greenberger |
| Radiation protector—Angiotensin II inhibitors | John E. Moulder |
| Radiation protector—growth factors and cytokines | Paul Okunieff, Thomas M. Seed, Thomas MacVittie |
| Use of stem cells and marrow transplantation | Ian McNiece, Michael Bishop |
| High-throughput screens | Philip Tofilon |

**December 18—Breakout groups**

I. Detection and Biology (Chair, Julian Preston; Co-Chair, John Fike, NCI, Rosemary Wong)

II. Protection (Chair, William McBride; Co-Chair, David Grdina; NCI, Helen Stone)

Breakout reports—presentation of draft report/recommendations and group discussion

## APPENDIX 3

### Workshop Participants and Attendees

**Participants**

Sally A. Amundson, NIH, NCI
Col. Edward Baldwin, DOD, USAF
Mary Helen Barcellos-Hoff, Lawrence Berkeley Laboratory
Joel S. Bedford, Colorado State University
Michael Bishop, NIH, NCI
William F. Blakely, DOD, AFRRI
David Boothman, Case Western Reserve University
David Brizel, Duke University
Antone Brooks, Washington State University, Tri-Cities
C. Norman Coleman, NIH, NCI
Curtis E. Cummings, DOD, AFRRI
Nancy Daly, ASTRO
John Fike, University of California, San Francisco
Amato Giaccia, Stanford University
David Gius, NIH, NCI
Ronald Goans, Oak Ridge Associated Universities
Mary Beth Grace, DOD, AFRRI
David Grdina, University of Chicago
Joel Greenberger, University of Pittsburgh
Eric Hall, Columbia University
Alan Huston, DOD, USN
John M. Jacocks, DOD, AFRRI
David G. Jarrett, DOD, USAMRIID
K. Sree Kumar, DOD, AFRRI
Michael R. Landauer, DOD, AFRRI
Robert Leedham, FDA
Howard Liber, Massachusetts General Hospital
Richard S. Lofts, DOD, AFRRI
Min Lu, FDA
Thomas MacVittie, University of Maryland
Kali Mather, DOD, USAF
William McBride, University of California, Los Angeles
Ian McNiece, University of Colorado
Noelle Metting, DOE
Alexandra C. Miller, DOD, AFRRI
James Mitchell, NIH, NCI
William Morgan, University of Maryland
John Moulder, Medical College of Wisconsin
Ruth Neta, DOE
Paul Okunieff, University of Rochester
Richard Pelroy, NIH, NCI
Pataje G. S. Prasanna, DOD, AFRRI
Julian Preston, EPA
Robert C. Ricks, Oak Ridge National Laboratory
Michael E. C. Robbins, Wake Forest University
Sara Rockwell, Radiation Research Society
Amy Rosenberg, FDA
Walter Schimmerling, NASA
Thomas Seed, DOD, AFRRI
Venkataraman Srinivasan, DOD, AFRRI
Helen Stone, NIH, NCI
Donald L. Thompson, FDA
Horace Tsu, DOD, AFRRI
Bruce Wachholz, NIH, NCI
Joseph Weiss, DOE
Mark Whitnall, DOD, AFRRI
Gail Woloschak, Argonne National Laboratory
Robert Yaes, FDA

**Observers**

Richard Cumberlin, NIH, NCI
James Deye, NIH, NCI
Albert Fornace, NIH, NCI
Peter Inskip, NIH, NCI
Francis J. Mahoney, NIH, NCI
Steven Simon, NIH, NCI
Paul Strudler, NIH

## APPENDIX 4

### Glossary of Abbreviations

*Agencies and organizations*

| | |
|---|---|
| AFRRI | Armed Forces Radiobiology Research Institute |
| ASTRO | American Society for Therapeutic Radiation Oncology |
| CDC | Centers for Disease Control and Prevention |
| DHHS | Department of Health and Human Services |
| DOD | Department of Defense |
| DOE | Department of Energy |
| EPA | Environmental Protection Agency |

| | |
|---|---|
| FDA | Food and Drug Administration |
| NASA | National Aeronautics and Space Administration |
| NCI | National Cancer Institute |
| NIH | National Institutes of Health |
| NCRP | National Council on Radiation Protection and Measurements |
| REAC/TS | Radiation Emergency Assistance Center/Training Site |
| RRP | Radiation Research Program, NCI |
| USAMRIID | U.S. Army Medical Research Institute of Infectious Diseases |

*Scientific terminology*

| | |
|---|---|
| AII | Angiotensin II |
| ACE | Angiotensin-converting enzyme |
| AED | 5-Androstenediol |
| AT1 | Angiotensin II type 1 |
| CSF | Colony-stimulating factor; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte/macrophage colony-stimulating factor |
| CNS | Central nervous system |
| COX | Cyclo-oxygenase |
| ESR | Electron spin resonance |
| FISH (mFISH) | Fluorescence *in situ* hybridization (mFISH, multiplex FISH) |
| GI | Gastrointestinal |
| Gy | Gray, unit of absorbed dose of radiation |
| IL | Interleukin (different interleukins have different numbers, e.g. IL1, IL11) |
| IMRT | Intensity-modulated radiation therapy |
| *In vitro* | In glass (or in the laboratory, but not in animals) |
| *In vivo* | In animal models |
| IND | Investigational new drug (FDA) |
| KGF | Keratinocyte growth factor |
| LCM | Laser capture microdissection |
| $LD_{50}$ | Lethal dose for 50% of people or animals exposed |
| MGDF | Megakarocyte growth and development factor |
| MV | Megavolt (unit of energy) |
| NDA | New Drug Application (FDA) |
| PBPC | Peripheral blood progenitor cells |
| PCC | Premature chromosome condensation |
| RNA | Ribonucleic acid |
| RNS | Reactive nitrogen species |
| ROS | Reactive oxygen species |
| SCF | Stem cell factor |
| SKY | Spectral karyotyping system |
| SOD | Superoxide dismutase |
| Sv | Sievert, unit of equivalent or effective dose used in radiation protection |
| TGFB | Transforming growth factor β |

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