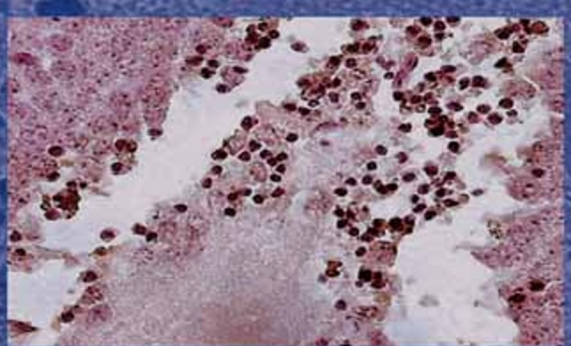
MEDICAL RADIOLOGY

Radiation Oncology

L. W. Brady
H.-P. Heilmann
M. Molls · C. Nieder

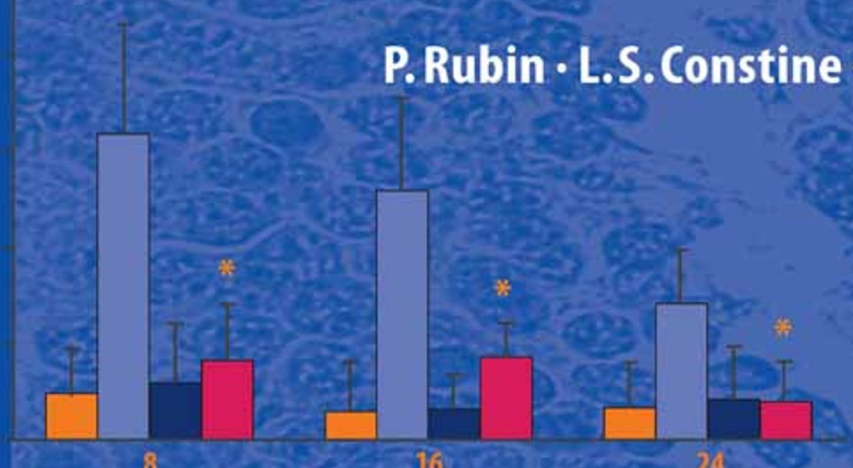
CURED II ▪ LENT

Cancer Survivorship Research and Education



Late Effects on Normal Tissues

P. Rubin · L. S. Constine · L. B. Marks · P. Okunieff
Editors



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P. Rubin · L.S. Constine
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Cancer Survivorship Research and Education

Late Effects on Normal Tissues

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With 39 Figures in 62 Separate Illustrations, 42 in Color and 25 Tables

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Dedications

Dedication to Joel Tepper for CURED II meeting

Joel E. Tepper: Dedicated scholar, teacher, physician and leader

Joel was born in Brooklyn, soon after WWII, making him one of the earliest baby boomers. He grew up in Massachusetts and attended college at the Massachusetts Institute of Technology, where he studied electrical engineering. He went on to medical school at Washington University in St. Louis, graduating in 1972. Joel did his medical internship at Presbyterian-St. Luke's in Chicago.

He continued his training as a resident and fellow in Radiation Medicine at the Massachusetts General Hospital. There, he had the good fortune to train under many giants in our field, including Herman Suit and C.C. Wang. There, he started what would prove to be his very prolific career as an author, penning many manuscripts, including several dealing with the initial world-wide experience with proton beam radiation therapy. Following training, Joel served as the Chief of Radiation Therapy at the Malcolm Grow Air Force Medical Center. Joel then spent several years as a senior investigator at the National Cancer Institute, and had the good fortune to work with Eli Glatsein, Tim Kinsella, Allen Lichter, Steve Rosenberg, Jim Mitchell, Liz Travis, and many other talented investigators. There, he gained experience with intra-operative radiation therapy techniques, gastrointestinal cancers and soft tissue sarcomas.

Joel returned to MGH in 1981. During the subsequent six years, he further developed his expertise in several areas including intra-operative radiation therapy, soft tissue sarcoma, and most notably gastrointestinal malignancies. He rapidly became one the nation's experts in the care of patients with gastrointestinal malignancies, and in the use of combined modality therapy. He is at present Principal Investigator of the UNC GI SPORE grant, a prestigious NCI translational research award that spans many departments within the medical school.

In 1987, Joel moved to the University of North Carolina at Chapel Hill to become Professor and Chairman of the Department of Radiation Oncology. During the subsequent 20 years at UNC, Joel has proved to be a dedicated scholar, physician teacher, and leader.

As a scholar, Joel has authored over 150 publications on a wide array of topics, and has conducted numerous clinical trials. Through these efforts, Joel has helped to define the optimal treatment for most GI malignancies. His broad expertise is evident by his work as the founding editor of Seminars in Radiation Oncology, and his authorship of several books. His expertise extends well beyond radiation oncology, as he has helped to lead efforts to better de ine the roles of surgery, chemotherapy and radiation in GI malignancies. He is a true oncologist.

As a physician teacher, Joel has strived to provide outstanding care for many patients-both through his direct interactions with patients, and through his educational and scholarly activities. He has lectured extensively throughout the world, helping to spread knowledge to others. He has helped to organize numerous educa-

tion conferences, such as being co-Director for three years of the ASCO/AACR Vail Conference on Clinical Trial Methodology, and has mentored countless residents, students and young faculty. Through these efforts, Joel has helped to train a generation of physicians and leaders in our field.

Joel's most impressive accomplishments lie in the realm of leadership where he has applied his skills in a broad array of local, national and international capacities. At UNC, in addition to serving as Chairman of the Department of Radiation Oncology, he served as an Associate Director of the Lineberger Cancer Center, and has held many leadership/administrative positions within the UNC Health System.

Nationally and internationally, Joel has served the greater oncology community on multiple levels. He served on countless ASTRO, ASCO, CALBG, AACR committees. Most notably, he is the chair of the NCI GI-Intergroup (GI Steering Committee), is on the ASCO Board of Directors, and has served as ASTRO President from 2002 – 2003, and subsequently as ASTRO Chairman of the Board. Through these many efforts, he has been a strong advocate for the fields of oncology, radiation oncology, and, most importantly, patients with cancer. His outstanding service to our field was recognized by his nomination to the first class of ASTRO Fellows in November, 2006.

Joel and his wife Laurie are active in the local Durham Chapel Hill Jewish community- serving on various committees to help the community at large. Joel is fortunate to have two daughters and three grandchildren.

Perhaps one of the most humbling aspects of reviewing Joel's outstanding career is the recognition that he has done all of this despite serious personal challenges. It is with great admiration and respect that the CURED II meeting, and this publication, is dedicated to the career and accomplishments of Dr. Joel E. Tepper.

Lawrence B. Marks

Foreword

The evolution of treatment programs for cancer has been predicated on the safe intensi ication of radiation therapy, chemotherapy, and biologic adjuvants. The emphasis on combined integrative multimodal programs of management have led to markedly increased survivorship, which now exceeds 64% overall, and is much higher for selected malignancies, such as 87% for breast cancer and 80% for all childhood cancers combined.

Combined integrative multimodal programs of management are more aggressive in character and often miss the potential risk of normal tissue reactions or tolerance. This past emphasis has led to increased efforts to prevent or avoid normal tissue damage during combined modality treatment and to manage and rehabilitate affected patients. This requires understanding of tissue tolerances to treatment and dealing effectively with not only early but also late effects on normal tissues. Concomitant with this requirement is the need to follow the patients carefully for years after completion of treatment. Late effects may not occur early but may occur long after the cessation of treatment. This balance requires understanding of the maximum potential of benefit versus decreasing the potential for toxicity from the treatment.

The second volume on late effects in normal tissues by Rubin et al. is an important statement with regards to early and late effects from radiation therapy, as well as from combined therapy. It contributes signi icantly to the basic understanding of the problem, the need for long-term follow-up, and the criteria by which one would identify and treat the early and late effects of treatment. The volume represents a significant contribution in this field of endeavor.

Philadelphia — Luther W. Brady
Hamburg — Hans-Peter Heilmann
Munich — Michael Molls
Bodø — Carsten Nieder

Introduction

Multimodal treatment is the hallmark for the rising success of cancer cure rates. The more aggressive the treatment delivery in terms of dose, time and volume for radiation and chemotherapy, the more adverse effects in normal tissues can be anticipated. The major paradigm shift is to be aware of the new focus of cancer survivorship; that is to say, the promise of prolonging life means that quality of life needs to be maintained. The life worth saving must be worth living. The issues that need to be addressed can be stated succinctly. The new definition of a cancer survivor is not 5 years cancer free survival, but begins at Day 0, before treatment is initiated.

Anatomy is foremost for the radiation oncologist to focus upon with our new sophisticated techniques of intensity modulated radiation treatment (IMRT) and image-guided radiation treatment (IGRT). The ability to concentrate and increase the tumor dose is at the price of exposing surrounding normal tissues to significant radiation doses. Although such normal tissue radiation doses are well within tolerance, any radiation dose to a normal tissue is above threshold. Restated, radiation (and chemotherapy) use up the mitotic potential of a normal tissue, accelerate senescence, and cause mutations and genome instability that in time may be expressed as a second malignant tumor (SMT). Collateral unintended damage is inevitable in surrounding neighboring sites as rotational techniques focus on the tumor volume. Our highly refined computerized technology-driven radiation delivery systems concentrate high tumor doses on target; however, the anatomic sites in the immediate neighboring environment are also irradiated. Thus, the gross, clinical, and planning tumor volumes (GTV, CTV, PTV) expressed as a series of repetitive contours need to be replaced by the in situ normal tissues contents. Our physics, not our biology, has created the illusion of a selective effect in our ability to ablate cancers by heightening doses beyond 50–60 Gy to smaller tumor volumes. However, there is a parallel need to concentrate on the normal tissue volumes (NTV) being simultaneously exposed (Rubin CURED I). These fall into three categories and each deserves to be carefully assessed. They are expressed in terms of TNM cancer nomenclature:

NT are normal tissues inside the Tumor volumes GTV, CTV, PTV.

NN are normal tissues in Neighboring sites in the same transverse axial planes outside the CTV.

NM are normal tissues in more reMote systemic sites and receive scattered radiation from leakage to the whole body.

NTV: The normal tissue sites within the GTV, CTV, PTV that receive doses approaching or exceeding tolerance doses, i.e., 50–60 Gy

NNV: The neighboring normal tissue volumes surrounding the cancer that receive below tolerance doses that are well above the threshold, i.e., >10 Gy.

NMV: The more remote systemic tissues that receive minimal doses that reside in creating genomic instability and mutate chromosomal DNA, i.e., >1 Gy

Biology of the biocontinuum is essential to comprehend the complexity of molecular, subcellular mitochondrial components, nuclear DNA and cytoplasmic RNA and protein messengers triggered by all modalities, i.e., radiation, chemotherapy, and surgery. Each mode releases a microarray of molecular events resulting in a

perpetual cascade of cytokines and chemokines that are the paracrine, autocrine, and endocrine messages amongst cells in normal tissues. The multimodal treatment causes normal tissues to respond both to subtolerance high doses and to above threshold lower doses beyond the range of target tumor volumes. Embedded in normal tissues that constitute organs are a large variety of cells that fall into five major categories: the parenchymal stem cell, the endothelial cell or vascular component, the fibroblast (cyte), the macrophage, lymphocyte, in the interstitium and then unique mature functioning parenchymal cells, critical to its structure and physiology. Microstructure as to tissue organization is as essential as the anatomy's macrostructure to complete the picture of events triggered by cancer treatments.

Biocontinuum refers to the sequence of ongoing events once a normal tissue or organ is perturbed by radiation chemotherapy. Rubin and Casarett's paradigm of the continuing effect in normal tissue(s) over time is built on a number of key premises:

Radiation doses that exceed the tolerance will be expressed differently over time depending on the tissue's cell kinetics, i.e., fast, slow, or no cell renewal.

There is no latent period histologically in that cellular changes always precede clinical manifestations.

There is the persistence of the memory of the radiation and compensatory mechanisms of cell regeneration or cell repopulation determining the severity of the ultimate injury expressed clinically.

There is an acceleration of the aging process expressed by the slope of cellular depletion, tissue atrophy, or the organ's senescence that is altered by radiation (dose, time, volume factors).

Another injurious event by any form of trauma (surgery) or drugs (chemotherapy) can be additive or synergistic and alter the aging slope in time uncovering radiation residual damage, i.e., recall reaction.

Co-morbidities such as infection, hypertension, diabetes, and obesity can contribute to the slope of the senescing tissues and accelerate the late effect earlier.

Common Toxicity Criteria (CTC) have been applied to assess the quality of survival as applied to cancer patient long-term survivorship. In the 1950s, the concept of late effects was considered unique to radiation. However, over the past two decades the Common Toxicity Criteria scales initially applied to chemotherapy's acute adverse events (v1.0). Since late changes due to drugs were not recognized until years later, v2.0 incorporated radiation acute toxicity, and, more recently, in v3.0 incorporated radiation's late toxicity, by recognizing that these criteria were equally applicable to chemotherapy. It is with the creation of the Office of Cancer Survivorship at NCI that long-term effects of chemotherapy were recognized as indistinguishable from late effects. It is with the publication of IOM/NRC "Cancer Survivor to Cancer Patient, Lost in Transition" that late effects are being listed and are applicable to all cancer treatment modalities.

There is a price to pay for being a cancer survivor, but one needs to be a long-term cure to have a late effect.

Rochester — Philip Rubin
Rochester — Louis S. Constine
Durham — Lawrence B. Marks
Rochester — Paul Okunieff

Contents

CONCURED:

Defining the Leading Edge in Research of Adverse Effects of Treatment for Adult-Onset Cancers

Lois B. Travis

CONTENTS

1.1 Introduction

The number of cancer survivors in the United States has tripled since 1971, and is growing by 2% each year [1]. The burgeoning number of patients reflects improvements in early detection, supportive care, and therapy. In 2003, there were an estimated 10.5 million cancer survivors, representing 3.5% of the US population [2]. Among all cancer patients, the 5-year relative survival rate is now almost 66% [2]. Given the increasing survival after a cancer diagnosis, identification and quanti ication of the late effects of cancer and its therapy have become critical. Further, this growing and heterogeneous population provides important opportunities for clinical and epidemiologic research into cancer biology, long-term treatment effects, and prevention. In November 2004, the National Cancer Institute sponsored a workshop, whose major objective was to provide perspective on the research agenda, design considerations, and infrastructure needed to understand the underlying genetic mechanisms of late neoplastic effects in cancer survivors and thus, to facilitate the development of evidence-based long-term management and intervention strategies. Participants represented a group of experts in the fields of epidemiology, statistics, molecular genetics, clinical genetics, pharmacogenomics, informatics, radiation biology, medical oncology, pediatric oncology, and radiation oncology, and the advocacy community. Workshop proceedings were published as a Commentary in [3]. Although the focus of the workshop was second primary cancers, given the high associated mortality [4–6], participants emphasized that most of the infrastructural and design approaches that support research in this area also provide a sound basis for the study of other important physiologic late effects and psychosocial concerns in cancer survivors [7].

1.2 Research Infrastructure for Studies of Cancer Survivorship

Recommendations of the NCI-sponsored workshop are reproduced in Table 1.1. First, there was a high level of enthusiasm for the establishment of a multi-center cancer survivor cohort derived from large institutions [3]. The advantages of such a cancer survivorship coalition, as now realized by the Consortium for Cancer Research and Education

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Table 1.1. Workshop recommendations for future research: genetic susceptibility and second primary cancers (modified from [3])

Develop research infrastructure for studies of cancer survivorship	**Promote the development of new technology, bioinformatics, and biomarkers**
• Institute a systematic, national approach to develop research infrastructure for studies of genetic modifiers of late effects of cancer treatment, including second malignancies • Provide for rigorous ascertainment of multiple primary cancers with clinical annotation, detailed treatment data, and biospecimen collection • Establish multicenter cohorts of cancer survivors, with recruitment of trans-disciplinary research teams dedicated to research the late effects of therapy • Expand the capacity of National Cancer Institute cooperative groups to ascertain and study long-term outcomes in clinical trial populations, in support of survivorship research	• Identify new technologies for the analysis of germline and somatic genetic alterations to determine their contributions to second cancer risk • Reduce the amount of tissue and DNA needed for various assays, with standardization of protocols for whole genome amplification • Develop molecular profiles of tumors that incorporate analyses of etiologic pathways and therapeutic targets related to second cancers and other late outcomes
Create a coordinated system for biospecimen collection	**Support the development of new epidemiologic methods**
• Standardize biospecimen collection, laboratory procedures, and documentation for blood and other DNA sources, normal tissue from target organs, and tumor tissue • Develop a centralized biospecimen repository or a tracking system ("virtual repository") to permit sample retrieval from multiple storage centers. Institute mechanisms for scientific review of specimen use and administrative procedures for specimen control • Support methodologic research to enhance the quality and lower the cost of biospecimen collection, processing, storage, and distribution	• Develop efficient epidemiologic study designs to investigate the role of genetic susceptibility to multiple primary cancers, including genetic modifiers of risk associated with treatment effects or other etiologic factors • Develop optimal approaches for selection of controls for case-control studies in which both treatment and genetic susceptibility play important roles • Include a biospecimen component in all study designs
	Develop evidence-based clinical practice guidelines
	• Implement pilot studies of interventions to prevent second cancers within genetically defined, high-risk groups of patients • Integrate smoking cessation programs into research designs • Support research to provide evidence-based follow-up care for cancer survivors

(CONCURED), are numerous. In institutions which comprise CONCURED, the status of cancer patients is usually updated routinely through hospital-based tumor registries. Detailed information with regard to cancer diagnosis and exposure data (radiation therapy and chemotherapy) is available and of high quality. Even though a number of cancer centers have independently initiated their own cancer survivorship programs, as reviewed in Travis et al. [3], NCI workshop participants foresaw that only a coalition of centers would have sufficient statistical power to serve as a platform for additional research into late effects, in particular gene-environment interactions. CONCURED represents the first step in the realization of the vision published in 2006 [3]. The success of this collaborative approach is already reflected in the investigation by Klem et al. [8] examining features of breast cancer after Hodgkin lymphoma. In this study, a total of 264 patients with second primary breast cancer was assembled from numerous, collaborating institutions across the US. In contrast, in the largest previous study to date, an international effort among population-based cancer registries, was required to assemble 105 women who developed breast cancer after being treated for HL at age 30 or less [9].

As pointed out by participants at the 2004 NCI-sponsored workshop, CONCURED can also serve as the source of patient subcohorts that are selected based on eligibility criteria that enrich populations for those at high risk of late effects. Such criteria include specific cancer treatments previously demonstrated to carry a high risk of second cancers (e.g., high-dose, extended-field radiation therapy for Hodgkin lymphoma [10]; long-term, high cumulative doses of alkylating agents [11, 12]; a specific clinical phenotype at the time of the first cancer diagnosis that might elevate the risk of treatment-related cancer (e.g., nevoid basal cell carcinoma syndrome); the presence of field effects in normal tissues that represent an increased risk; or specific genetic traits). CONCURED might serve as a study base to examine the potential risk of second cancers and other late effects associated with newer treatment

modalities such as intensity-modulated radiotherapy (IMRT) [13], with the prospective identification and follow-up of patients. Importantly, CONCURED should also have sufficient statistical power to examine the under-studied area of the influence of race and ethnicity in the susceptibility to late effects of cancer treatment, as reviewed at the May 2007 meeting [14].

1.3 A Platform for Studies of Gene–Environment Interactions

Two of the five major recommendations put forth by participants in the 2004 NCI-sponsored workshop [3] were directed to the creation of a coordinated, multi-center system for biospecimen collection and the promotion of the development of new technology, bioinformatics, and biomarkers, respectively (Table 1.1). In this regard, it was noted that an infrastructure based on large cancer centers also provided the advantage that biologic specimens (peripheral blood and tumor and normal tissue from target organ) could be prospectively collected from cancer patients at presentation. This is in contrast to other cohort sources, such as population-based cancer registries, in which available specimens must be requested from constituent hospitals and laboratories, who typically store only archived paraffin-embedded tissues. The limitation of single cancer center studies to date has been the relatively small sample size; it was recognized by participants in the 2004 NCI-sponsored workshop [3] that only a multi-center effort would have sufficient statistical power to address the role of gene-environment interactions in the late effects of treatment. Thus, the vision outlined by NCI workshop participants included the establishment of programs at multiple centers using common infrastructure, common data collection instruments, and common state-of-the-art biospecimen collection, processing, storage, and distribution systems, in support of hypothesis-generating and hypothesis-testing research, with a goal of the identification of those genetic characteristics that might make cancer survivors especially susceptible to treatment-related second cancers [3]. This type of comprehensive approach to biobanking is another goal of CONCURED, and at the recent meeting, several proposals which address the issue of gene–environment interactions in the role of late effects were presented [15, 16].

1.4 Development of Epidemiologic Methods and Predictive Models

In the NCI-sponsored workshop, the use of a large survivorship network to serve as the study base for the development of new epidemiologic methods was discussed (Table 1.1) [3]. The application of traditional cohort and nested case-control designs to studies of treatment-related second primary cancers was recently reviewed [17], with both designs applicable to the study of other late effects. However, whereas standard methods have proven highly effective in defining dose–response relations between treatment and adverse outcomes, new analytic paradigms are needed to explore gene–environment and gene–gene interactions [18]. For case-control studies, these include counter-matching on therapy in studies where both treatment and genetic susceptibility may play important roles [18]. In general, new types of hypothesis-generating models and customized research methods are needed to more efficiently study the various determinants of second cancers and other late effects.

There is an increasing emphasis on the development of predictive models for the occurrence of the adverse sequelae of treatment. Travis et al. [19] recently published estimates of the cumulative absolute risk of breast cancer 10, 20, and 30 years after treatment for HL according to various dose categories of mantle radiotherapy and the administration of alkylating agents. As emphasized in an accompanying editorial by Longo [20], these types of risk estimates, which are easily communicated to patients, would be optimal for all adverse outcomes of cancer treatment. Already in CONCURED, proposals are being considered to predict the risk of second cancers comparing photon megavoltage IMRT versus conformal proton treatment [21].

1.5 Evidence-Based Clinical Practice Guidelines

The implications for research opportunities in large cohorts of cancer survivors also include the provision of definitive recommendations for evidence-based care and cost-effective strategies for patient follow-up [3] (Table 1.1). Pilot studies of interven-

tions to prevent or ameliorate the risk of late effects can also be undertaken. Thus, as part of the CONCURED effort, Ng et al. [22] present a progress report of the effectiveness of breast MRI screening in female survivors of HL. Moreover, a new proposal to examine the feasibility of using chest CT to screen for lung cancer in survivors of HL who smoke was discussed by Ng, given the multiplicative interaction reported between either chest radiotherapy or alkylating agent treatment for HL and tobacco use and subsequent lung cancer risk [23, 24].

At the CONCURED meeting, a proposal to screen for second cancers of the gastrointestinal tract in survivors of HL who received pelvic and abdominal irradiation was also evaluated [25]. Based on additional analyses of the results of an international study by Hodgson et al. [26], a cumulative risk of 20% for colorectal cancer in HL patients over 50 years of age was predicted. Thus, the rationale for such a feasibility study seems well-founded.

Whether long-term cardiovascular complications in survivors of HL can be ameliorated by exercise may also be explored in other CONCURED research endeavors [27]. Already, a greater survival has been shown in physically active breast cancer survivors with high vegetable-fruit intake regardless of obesity [28]. Thus, the application of interventions such as lifestyle modifications in cancer survivors holds great promise, and deserves further study.

1.6 A Trans-disciplinary Approach

The successful establishment of effective trans-disciplinary cancer survivorship programs requires the attention of dedicated clinical and research teams [3]. For example, in the Living Well After Cancer Program (www.pennhealth.com), a multidisciplinary team of clinicians (including medical oncologists, radiation oncologists, clinical oncology nurse practitioners, nutritionists, cardiologists, cancer rehabilitation specialists, psychiatrists, and psychologists) and researchers (including those with expertise in genomics, cancer biology, biostatistics, epidemiology, and behavioral science) integrate the clinical and research arms of the program. An institutional review board-approved clinical research protocol follows data on symptoms, follow-up, and quality of life; provides feedback to health care providers regarding these problems in individual patients; and manages the recruitment of patients into studies. Participants at the 2004 NCI-sponsored workshop [3] emphasized the importance of dedicated survivorship staff and the need to budget for the costs of screening, etc. in developing these resources.

Comment

In 2005, the Institute of Medicine and the National Research Council of the National Academies issued a report, *From Cancer Patient to Cancer Survivor: Lost in Transition* [29]. Indeed, one chapter of this report was devoted to survivorship research, and emphasized the need to study large number of patients who survive their cancers for many years. The report pointed out that sample sizes should also be large enough to include individuals who will manifest unusual late sequelae. As did participants at the 2004 NCI-sponsored Workshop [3], the report stated that one mechanism to accrue large numbers of cancer survivors who represent the diversity of the US is to conduct multi-institutional collaborative research [29]. Thus, CONCURED represents in part the realization of the research component of the IOM report. Until the results of research undertaken in large survivorship programs are available, scienti ic progress over the last few decades has made it possible nonetheless to identify selected treatment regimens which are associated with exceptionally high risks of late effects. Although individual susceptibility factors remain largely unknown, groups of exposed patients can still be selected for close monitoring. Whenever effective screening methods (e.g., mammographic examination) are available, these should be included in patient follow-up. Preventive strategies (e.g., smoking cessation, avoidance of ultraviolet light) may also diminish the risk of selected late effects, and cancer survivors should be encouraged to adopt practices consistent with a healthy lifestyle. Even though cancer treatment represents a double-edged sword, it should be kept in mind that many treatments have been accompanied by sizable improvements in patient survival. Thus, the benefits associated with many cancer treatments greatly exceed the risk of developing adverse effects. Further, it should always be kept in mind that the late adverse effects of cancer and its treatment may not necessarily be attributable to prior therapy, but also re lect the effect of shared etiologic factors, environmental exposures, host characteristics, pa-

tient co-morbidities, underlying hepatic and renal function, lifestyle factors and combinations of influences, including gene–environment and gene–gene interactions [30]. Research undertaken in large well-constructed cohorts of survivors, such as CONCURED, should be able to clarify the roles of these various influences on the risk of late effects, identify genetically susceptible populations, and also provide the basis for evidence-based prevention and intervention efforts.

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Bioimaging In Vivo to Discern the Evolution of Late Effects Temporally and Spatially

2

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CONTENTS

Summary

- Imaging is a powerful tool for measuring regional radiation-induced normal tissue changes, independent of radiation volumes.
- Several functional imaging tools (e.g., single photon emission computed tomography [SPECT], positron emission tomography [PET], and magnetic resonance imaging [MRI]), have been used to monitor radiation-induced injury in a variety of different tissues, including the lung, heart, brain, liver, and salivary glands.
- The degree/extent of changes in regional imaging may be associated with changes in global organ function (e.g., clinical symptoms) at various time points.

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2.1 Introduction

The utility of radiotherapy (RT) in cancer management is based on the premise that treatment will cause greater damage to tumor than to surrounding normal tissue. Thus, the risks of normal tissue toxicity largely determine the dose and volume used clinically.

Normal tissue injury encompasses a wide range of effects with varying clinical impact, depending on treatment site and organ of interest. Further, the acceptability of RT-induced morbidities changes over time as treatment techniques evolve. If we are successful in our goals to cure more patients of their cancers, late normal tissue effects will become of greater concern. It is critical that improvements in therapies maximize both cure and quality of life (QOL).

Normal tissue injury can be expressed in several ways (Table 2.1), and the reported frequency of "injury" depends on the endpoint chosen.

The current review addresses functional imaging as a means to assess regional RT-induced normal tissue injury. Functional imaging allows for the early detection and quantification of subclinical regional injury, and is a powerful tool in this area of study.

Table 2.1. Example of different endpoints that can be used to study RT-induced injury, organized on the basis of objective vs. subjective and regional vs. global assessments

	Objective	Subjective
Regional	Imaging (e.g., CT-defined increases in tissue density)	Pain from local ulceration
Global	Creatinine clearance, pulmonary function tests	Shortness of breath, fatigue

A variety of imaging modalities have been used, including PET, SPECT, CT, and MRI. We herein review organ sites with the most available data on functional imaging and clinical endpoints: lung, heart, liver, brain, and salivary glands.

2.2 Lung Injury

Symptomatic pulmonary injury following RT for cancers in and around the thorax is common, occurring in up to a third of patients. The early phase of RT-induced lung injury (radiation pneumonitis) usually presents within 6 months of RT, and is commonly characterized by cough and dyspnea [1]. Late fibrotic injury usually evolves and becomes clinically manifested ≥6 months post-RT, and is characterized by progressive dyspnea, radiologic findings, and possible mortality [1, 2]. For patients treated for lung cancer, ~5%–35% will develop symptomatic lung injury, and 50%–100% will develop radiologic evidence of lung injury (the majority of which are asymptomatic) [3–8]. Similarly, for patients treated for breast cancer, 0%–34% may develop symptomatic lung injury, and 0%–63% of patients may develop radiologic changes [9–14].

Review of RT-induced lung injury by several investigators using various non-invasive imaging techniques has been previously described in detail [15, 16]. In brief, nuclear medicine imaging provides a sensitive means to assess regional lung function. Investigators from the Netherlands Cancer Institute (NKI), Princess Margaret Hospital (PMH), and Duke University have related changes in regional perfusion/ventilation [via single photon emission computed tomography (SPECT)] and/or tissue density [via computed tomography (CT)] to the 3D radiation dose map. There is a clear association between regional dose and changes in regional perfusion/ventilation/density. Further, there appears to be an association, albeit weak, between the integrated response (e.g., the sum of changes in regional perfusion) and changes in whole lung function [7, 17, 18].

Recent reports have focused on radiation-induced lung injury in the context of stereotactic radiosurgery. In a study of 31 patients receiving stereotactic radiosurgery for primary or metastatic lung lesions, Aoki et al. noted asymptomatic increases in CT density 2–6 months post-RT, and later fibrotic reactions at 6–15 months post-RT [19]. While all 31 patients developed radiographic changes, no patients developed severe symptoms (e.g., Grade ≥2 or requiring steroids). When follow-up CTs were compared to the dose distribution on the treatment planning CT, investigators observed that the minimal dose for the development of CT-defined changes in lung tissue ranged from 16 to 36 Gy. Figure 2.1 illustrates the pre- and post-RT change in CT for a patient treated at Duke with radiosurgery for synchronous pulmonary lesions.

Contrast-enhanced magnetic resonance imaging (MRI) may also be used to describe perfusion characteristics of various phases of RT-induced lung injury [20]. Several studies from Japan suggest MRI can detect RT-induced lung injury in animal models. In the clinical setting, Yankelevitz et al. and Ogasawara et al. used MRI to study perfusion characteristics of

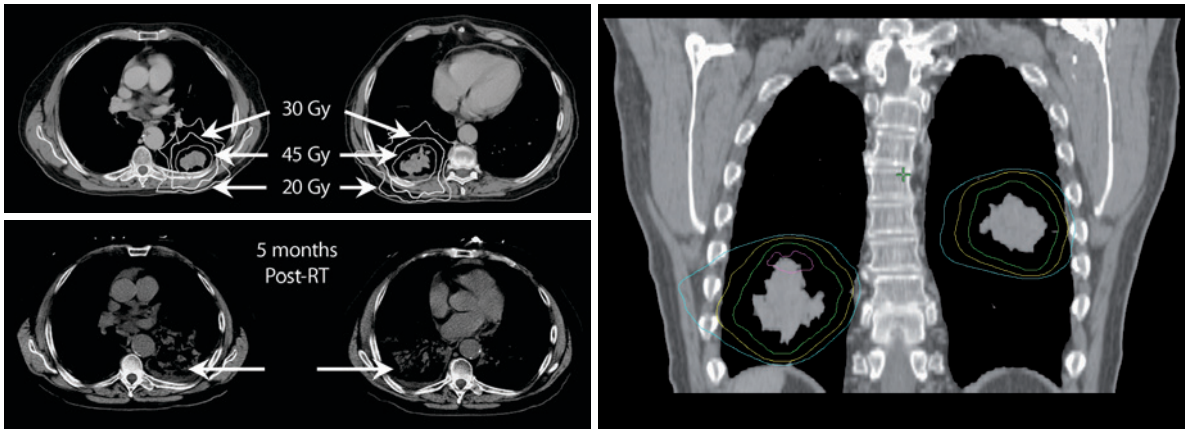

Fig. 2.1. Patient with synchronous bilateral lung lesions treated with radiosurgery at Duke University. Follow-up CTs at 5 months post-RT showed marked increases in regional tissue density in the symptomatic patient

Table 2.2. Frequency of radiographic and symptomatic changes following thoracic irradiation

Reference	No. of cases	Disease site	Radiographic follow-up	Radiologic endpoint	Rate	Clinical endpoint	Rate
CT/radiographs							
[129]	54	Lung, breast, Hodgkin's disease	6 Months	↑ Lung density	36/54 (67%)	RP	10/54 (19%)
[12]	33	Breast	9 Months	↑ Lung density	24/33 (73%)	Cough/ dyspnea	13/33 (39%)
[130]	37	Breast	0.7–10 Years	↑ Radiopacity	16/37 (43%)	–	0/37 (0%)
[131]	75	Hodgkin's disease	3–10 Years	↑ Radiopacity	12/75 (16%)	–	0/45 (0%)
[6]	184	Lung, breast, lymphoma	24 Months	↑ Lung density	162/259 (63%)	Dyspnea	34/175 (19%)
SPECT/scintigraphies							
[131]	75	Hodgkin's disease	3–10 Years	↓ Perfusion	29/45 (64%)	–	0/45 (0%)
[137]	25	Lymphoma	18 Months	Dose-dependent reductions in perfusion/ ventilation and partial recovery	25/25 (100%)	RP	4/25 (16%)
[135]	110	Breast, lymphoma	48 months	Dose-dependent reductions in perfusion/ ventilation and partial recovery	110/110 (100%)	–	–
[136]	106	Lung	3 Months	Dose-effect relation for perfusion and CT density	25/25 (100%)	–	–
[6]	184	Lung, breast, lymphoma	24 Months	↓ Perfusion	168/230 (81%)	Dyspnea	34/175 (19%)
[132]	79	Lung, lymphoma, breast, other thoracic tumors	~65 Months	Progressive dose-dependent reductions in regional perfusion	79/79 (100%)	–	–
[134]	20	Breast	1 Year	↓ Lung clearance	10/10 (100%)	Mild RP	2/20 (10%)
MRI							
[21]	10	Lung	3.5 Years	↑ Signal intensity on T1 and T2 weighted images	10/10 (100%)		
[20]	9	Lung	0.5–7 Months	Asymmetric enhancement on dynamic perfusion MR	9/9 (100%)	Acute RP/ RT ibrosis	
[133]	40	Lung, esophagus	None (pre RT image)	↑ Vascular resistance on velocity-encoded cine MR in patients with RP	–	RP	9/40 (23%)
PET							
[138]	73	Lung	38 Months	↑ FDG uptake	55/73 (75%)		
[24]	36	Esophagus	1–3 Months	Linear relation between radiation dose and normalized FDG uptake	36/36 (100%)	–	–
[23]	101	Esophagus	3–12 Weeks	Linear relation between radiation dose and normalized FDG uptake	–	≥Grade 2 CTC symptoms	66/101 (66%)

RP, radiation pneumonitis; FDG, fluorodeoxyglucose
Some data estimated from published reports

RT-induced lung injury (Table 2.2) [20, 21]. In a recent study by Muryama et al., velocity-encoded cine (VEC) MRI was used to investigate whether pulmonary arterial flow as a function of time could be used to predict radiation pneumonitis (RP) [22].

A recent study from M.D. Anderson noted dose dependent changes in regional FDG-PET activity in 101 patients assessed 3–12 weeks post-RT for esophageal cancer [23]. Further, the severity of these regional in lammatory changes appeared to be significantly correlated to the probability of symptoms.

Data from several studies regarding radiographic changes in the lung following thoracic RT, seen on SPECT, CT, MRI, and PET, are summarized in Table 2.2. Figure 2.2 illustrates some of these imaging findings and correlation to dose.

2.3 Heart Injury

RT to the thorax may induce both early and late cardiac effects if portions of the heart are included in the radiation field. Patients with breast cancer

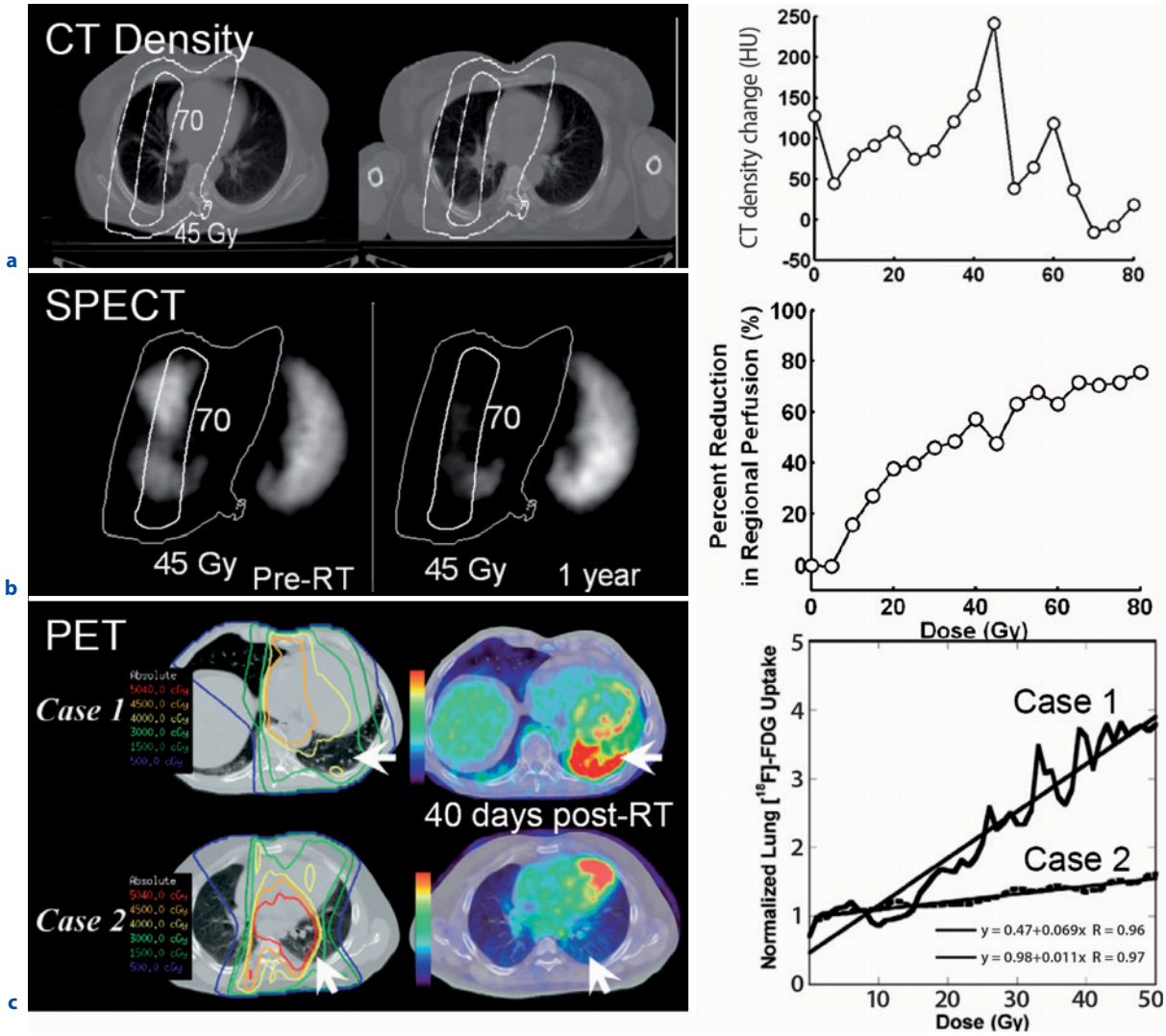

Fig. 2.2a–c. Pre- and post-RT lung CT (Panel **a**, from Duke), SPECT perfusion scans (Panel **b**, from Duke), and FTG-PET images (Panel **c** from MDAH, [24]). For each image pair, the associated isodose lines are shown. On the right side are dose response curves for changes in regional density, perfusion, and metabolic activity, respectively

and Hodgkin's disease are particularly at risk for developing late myocardial damage, due to their longevity and possibly also due to the frequent use of anthracycline-containing chemotherapy. In general, one has to wait at least 10 years post-treatment to see these effects manifest clinically [25]. The use of radiologic methods may allow for the early detection of treatment-associated dysfunction. Recent studies have investigated the incidence of cardiac effects on patients receiving RT for lung and esophageal cancer. There are some preliminary data available on newer imaging technologies such as cardiac MRI and PET to assess RT-induced cardiac injury in patients with thoracic cancers. The vast majority, however, of available data regarding the imaging of RT-induced heart injury use SPECT myocardial perfusion imaging in patients with breast cancer and Hodgkin's disease. An in-depth discussion of RT-induced cardiovascular injury in lymphoma patients is discussed elsewhere in this book (see Chap. 10).

2.3.1 SPECT

SPECT scans provide a noninvasive assessment of myocardial perfusion and function (changes in wall motion and left ventricular ejection fraction). Scans taken in early years following RT may be able to assess early the subclinical damage. The incidence of perfusion defects appears to be related to the volume of left ventricle irradiated and largely persists up to 6 years after RT in patients irradiated for breast cancer [26–28]. Perfusion defects have been associated with episodes of chest pain, and wall motion abnormalities [26, 28–30], but their clinical implications may not be well understood. Further, relatively large perfusion defects may cause reductions in ejection fraction [26, 31]. Data from several studies relating radiographic changes in the heart, as seen on SPECT, in patients treated for breast cancer and Hodgkin's disease, and preliminary data from studies in esophageal and lung cancer are shown in Table 2.3. Unlike the data for breast cancer, the results as assessed by SPECT for esophageal and lung cancer are limited and somewhat mixed. It may be more dif ìcult to draw conclusions about the incidence of RT-induced cardiac injury in this group of patients as many may have pre-existing heart disease and associated related risks. Additional follow-up may be needed.

There is some concern that the abnormalities detected on SPECT may be due to attenuation artifacts related to RT-induced scarring of the breast/chest-wall; i.e., RT causes pericardial scarring that may lead to an "arti ìcial" defect in the anterior myocardium. Additional study is underway to assess for this confounding issue.

2.3.2 MRI

Nuclear medicine imaging provides both qualitative and quantitative information about regional and global cardiac function [32] and has been suggested to be a sensitive means to assess myocardial injury in patients with coronary artery disease [33, 34]. MRI provides assessments of myocardial wall thickness and, with delayed hyper-enhancement, allows direct visualization of myocardial injury/ ìbrosis, and is more sensitive in assessing subendocardial injury. Both MRI and the nuclear medicine techniques provide information regarding wall motion and ejection fraction, but MRI has better spatial resolution and thus may be more accurate [35–37]. Conversely, quantification of myocardial perfusion is better developed with SPECT than with MRI [36, 37]. While SPECT images only the left ventricle, MRI affords the possibility to assess global cardiac function.

At this time, MRI has only been applied to the study of RT-induced cardiac disease for a small number of patients with lung cancer. In preliminary abstracts from MD Anderson and Duke University, no apparent changes on cardiac MRI have been observed in the small patient numbers evaluable [38, 139]

2.3.3 Cardiac PET

There is increased interest in the use of cardiac positron emission tomography (PET) to provide a map of regional myocardial perfusion. PET has been suggested as having improved resolution and accuracy as compared to SPECT. In addition, PET may require shorter exam times than SPECT, but is similarly limited to imaging only the left ventricle [39]. A case report noted increased FDG uptake within cardiac regions receiving ≥25 Gy approximately 4 years earlier [40]. The patient was asymptomatic and had a normal ECG.

Table 2.3. Summary of studies using myocardial perfusion scintigraphy/SPECT to assess for RT-induced cardiac injury in patients with thoracic malignancies. (Adapted with permission from [128])

Reference[a]	Years of RT	No. of cases	Median radiographic follow-up	Subgroup	Perfusion defects	
Breast cancer – retrospective						
[41]	1971–1976	37	18.4 Years	Left-sided photons or electrons	25%	(5/20)
			19 Years	Right-sided photons or electrons	0%	(0/17)
[42]	1978–1983	90	13 Years	Left-sided RT	12%	(4/34)
				Right-sided or no RT	4%	(2/56)
[43]	1982–1990	16	7.9 Years	Left-sided electrons	44%	(4/9)
				No RT	57%	(4/7)
[44]	1987–1993	17	8.4 Years	Left-sided photons	0%	(0/17)
[30]	1987–1995	36	6.7 Years	Left-sided photons	71%	(17/24)
			8.3 Years	Right-sided photons	17%	(2/12)
Breast cancer – prospective						
[45]	1993–1994	12	1.1 Year	Left-sided photons	100%	(4/4)
				Left-sided electrons	25%	(2/8)
[26][b]	1998–2001	114	0.5 Year	Left-sided photons	27%	(21/77)
			1 Year	Left-sided photons	29%	(16/55)
			1.5 Years	Left-sided photons	38%	(13/34)
			2 Years	Left-sided photons	42%	(11/26)
[28][b]	1998–2006	44	3 Years	Left-sided photons	38%	(3/8)
			4 Years	Left-sided photons	58%	(7/12)
			5 Years	Left-sided photons	67%	(4/6)
			6 Years	Left-sided photons	67%	(2/3)
Other disease sites						
[46]	1967–1985	16	9.3 (2.5–21.5) Years	Lymphoma	0%	(0/16)
[47]		26	15 (4–20) Years	Lymphoma	61%	(14/23)
[48]	1978–1988	31	7 (3–11) Years	Lymphoma	84%	(21/25)
[49]	1964–1992	112	11.2 (1.0–31.5) Years	Lymphoma	7%	(7/100)
[50]		49	75 (28–208) Months	Lymphoma	78%	(32/41)
[51]	1964–1994	294	6.5 (4.0–8.4) Years	Lymphoma	12%	(32/274)
[52]	2005–2006	51	3 Months	Esophageal cancer	54%	(14/26)
				No RT	16%	(4/25)
[38]		13	2 Months, 6 months	Lung cancer	–	–[a]
[139]	2006–2007	12	3, 6, 12, and 18 months	Lung cancer, Mesothelioma	50%	(6/12)

[a] At least one patient with a new perfusion abnormality. Limited data in available abstract
[b] Some patient overlap, incidence of new perfusion defects listed
Some data estimated from published reports

2.4 Liver Injury

Previous radiation therapy techniques limited the utility of radiation to the liver, due to the liver's low whole organ tolerance. Data from studies dating back to the 1960s indicated that doses to the whole liver up to 30–35 Gy using standard fractionation resulted in a 5% risk of radiation-induced liver disease (RILD), while smaller volumes of the liver could tolerate higher doses [53–56]. With the advent of 3D conformal therapy and more recently IMRT, it has become increasingly important to assess the impact RT-induced liver injury. Radiologic changes are often evident on irradiated livers prior to, or even in the absence of, clinical symptoms [54, 57]. Such imaging changes have been reported 6 months to 6 years post-RT [53, 54, 58] (Table 2.4).

2.4.1 CT Perfusion Studies

Investigators at the University of Michigan have used CT to detect RT-induced liver injury. CTs obtained 2–3 months post-RT revealed low attenuation within irradiated areas in of 74% of 31 patients studied [66]. Using CT-based perfusion imaging, Cao et al. assessed the relationship between local radiation dose and changes in regional portal vein perfusion, similar to perfusion imaging studies of the lung and heart post-RT from other institutions noted above [58]. In 10 patients with unresectable primary or metastatic hepatic tumors, reductions in regional portal vein perfusion ~1.5 and 3 weeks into treatment (i.e., during RT) were related to changes in perfusion at 1 month post-RT. Conceivably, one may be able to alter therapy based on normal tissue changes noted early-on during a proposed course of therapy, thereby further individualizing therapy.

Munden et al. noted new CT liver abnormalities in 40% (8/20) of patients ≈ 8 (range 5–11) weeks post-IMRT for mesothelioma [59]. The abnormalities were in the liver periphery, corresponding to the regions receiving > 45 Gy. All patients with CT-defined abnormalities were asymptomatic and had normal liver function tests. For those patients with limited additional follow-up, the majority of the abnormalities resolved; however, additional data with longer follow-up is warranted.

2.4.2 MRI

MRI also provides a non-invasive method of imaging the RT-induced liver disease. On conventional MR images, irradiated liver tissues show T1-weighted hypointensity and T2-weighted hyperintensity, potentially due to increased water content

Table 2.4. Imaging assessment of RT-induced liver disease

Reference	No. of cases	Disease	RT technique median dose (range)	Follow-up	Radiologic changes	Clinical RT-hepatitis
CT						
[66]	31	Primary or metastatic hepatic tumors	High-dose conformal RT 59 Gy (48–73 Gy)	8- to 12 -Week intervals	74%	6%
[59]	20	Mesothelioma	IMRT in 25 fractions to 45–50 Gy	16 Weeks Range (3–116)	40%	0%
[58]	10	Primary or metastatic hepatic tumors	67.5 Gy (48–78 Gy)	1.5, 3 Weeks during RT; 1 month post-RT	↓Regional perfusion	–
MRI						
[60]	10	Hodgkin's disease	–	2, 4, 6, 12 Weeks	30%	0%

[a] For post-contrast T_1-weighted images
Some data estimated from published reports

[60, 61]. However, severe hepatic fibrosis can cause hypointensity on T2-weighted images that has been observed in irradiated patients with Budd-Chiari syndrome [62].

Special MR imaging techniques may provide a more precise differential diagnosis of radiation-induced hepatic injury. In gadolinium-enhanced dynamic studies, the irradiated liver parenchyma shows early hyperintensity that becomes more prominent and persists at the end of the dynamic studies [61]. Superparamagnetic iron oxide-enhanced (SPIO) MR imaging may also be a sensitive modality for early and late radiation-induced liver injury [62–65].

2.5 Brain Injury

Symptomatic brain injury from radiotherapy is common and likely underestimated due to limited lifespan of the majority of treated patients and the subtlety of findings [67–71]. Neurocognitive alterations can range from subtle cognitive dysfunction, such as mild short-term memory loss 1–6 months after treatment, to global irreversible/progressive neuropsychological deficits such as personality change and an overt decrease in IQ > 6 months post RT [72–75]. The situation is further complicated by neurotoxic effects of the tumor as well as effects of surgery and/or chemotherapy. The changes in normal brain following therapy for brain tumors is often complex; however there is an ever-increasing volume of data using imaging studies to better differentiate changes in normal brain tissue post-RT from recurrent disease. There is, however, limited data on the implications of these radiologic findings and changes in the neurocognitive function of patients receiving RT (Table 2.5).

Animal studies reveal that cranial irradiation leads to deficits in learning and memory, which may be comparable to human reports of cognitive dysfunction following RT [76]. In these animal reports, there is anatomic variability in radiosensitivity, as well as dose related deficits of learning and memory [77–81].

Post-RT, conventional CT and MRI can reveal morphologic abnormalities in the brain. However, these are non-specific and may reflect RT-induced normal tissue changes/inflammation/necrosis, tumor-related changes, or surgery-induced changes (e.g., enhancement along the resection margin) [82]. Functional brain imaging (e.g., SPECT and PET) may be able to distinguish between recurrent tumor and normal tissue changes such as radiation necrosis [82–85]. The majority of studies have evaluated the response of tumor to radiotherapy rather than the effect on normal tissue.

2.5.1 SPECT

SPECT can illustrate changes in cranial blood flow following RT. A Japanese study by Araki et al. utilized Xenon 133 SPECT to evaluate changes in mean cerebral blood flow of non-tumor bearing areas in 40 patients as compared with 40 normal volunteers [86]. Mean blood flow increased during therapy in some patients, compared to normal controls. At 3 months post-RT, significant reductions in blood flow were seen in three patients.

Harila-Saari et al. studied 25 patients with acute lymphoblastic leukemia (ALL) treated with either intrathecal (IT) chemotherapy or RT. SPECT perfusion defects were noted in 11/25 (44%), eight of whom received chemotherapy alone and three who received cranial RT [87]. The degree of SPECT abnormality has not been associated with neuropsychologic changes post-RT.

2.5.2 PET

There are limited data regarding the effects of RT on glucose metabolism in the brain via FDG-PET and clinical symptoms, and the available data is contradictory. Kahkonen et al. evaluated 40 long term survivors of ALL, half of whom received methotrexate and cranial RT [88]. No major differences were found in regional glucose metabolism in various defined cortical and subcortical anatomical areas for irradiated vs. non-irradiated groups. Pre-RT imaging was not available in these patients. Munley et al. retrospectively evaluated eight patients with both pre- and post-RT FDG-PET imaging [89]. There were no changes in regional metabolic activity in areas of brain receiving doses up to 50 Gy. Above 50 Gy, the effects varied, one decreasing and others increasing to varying degrees. Both of these studies were very small.

Table 2.5. Radiographic changes to the brain after irradiation

Reference	No. of cases	Radiographic follow-up	Post-RT radiographic response/outcome
SPECT			
[86][a]	40	3 Months	• ↑ Mean blood flow during RT • ↓ In blood flow in three RT patients post-RT
[87]	25	Varied (9–13 months)[b]	• Defects in 44% (11/25) – eight chemo alone, three RT alone • Impairment in neuropsychological functioning in 19/22 (86%) • No significant difference in intelligence testing between normal vs. abnormal SPECT
Diffusion weighted (DW)-MRI			
[110]	18	1–3 Month intervals starting 4 months post-RT	• ADC ratios and means significantly ↓ for recurrent vs. non-recurrent lesions
[111]	17	–	• Marked hypointensity [67%, (8/12)], in lesions due to RT-injury vs. recurrent tumor • Maximal ADC values significantly ↓ for recurrent vs. non-recurrent lesions
[1]HMRSI			
[97]	9	0.5–10.5 Years	• Widespread chemical changes in white matter after RT
[112]	18	Mean 4.6 years (range 3.0–9.6 years)	• ↓ NAA in RT-induced temporal lobe changes • Cr levels relatively more stable than Cho or NAA levels • Cho levels may be increased, normal, or reduced
[113] (MRS and DW-MRI)	55	2-Month intervals (1.5–12 months) 3–4 months intervals (12–36 months)	• Cho/ NAA, Cho/Cr, and ADC ratios and means significantly ↓ in regions of RT injury vs. recurrent tumor • MRS with DW MRI correctly classified 96.4% of subjects as recurrent disease or RT injury (100% correct for RT injury group)
[114] (I-IMT SPECT [1]HMRS)	25	9.7 Months	• I-IMT SPECT significantly ↓ for recurrent disease vs. treatment related changes • SPECT yielded more favorable results in differentiating recurrent tumor vs. post-RT changes
[115]	100	2 Years	• Oscillations in Cho/NAA and Cho/Cr ratios seen in 8 month cycles • Maxima in Cho/NAA and Cho/Cr ratios seen 2 months after RT
PET			
[91]	11	3 Weeks; 6 months	• ↓ FDG uptake, correlating with neuropsychological deficits

[a] Data extracted from abstract

[b] Radiographic follow-up not distinguished between patients with and without RT. Follow-up time measured from end of systemic therapy or RT

[1] HMRSI, proton MR spectroscopic imaging; ADC, apparent diffusion coefficient; NAA, N-acetyl-aspartate; DW, diffusion-weighted; Cr, creatine; Cho, choline; I-IMT, 123-iodine-α-methyl tyrosine

Some data estimated from published reports

Mineura et al. reported on seven patients studied with ^{15}O PET before and after RT for gliomas [90]. At 1 month post-RT, there were increases in PET-de ined regional blood flow in the contralateral grey matter felt to be normal tissue by CT. At longer time points, there were significant decreases in blood flow from pre treatment compared with later studies.

Early data from a prospective Duke study of 11 patients utilizing FDG and O–15 PET revealed reductions in FDG uptake in regions of the brain receiving >40 Gy in comparison to pre-treatment scans [91]. These FDG changes were found additionally to correlate with changes on neuropsychologic testing. Additional study, however, is needed to confirm and more precisely characterize these findings.

2.5.3 MRI

In animal models, changes in brain tissue on MRI have been found to be dependent on dose and are progressive with time [92]. Diffusion-weighted MRI has been used to characterize and differentiate morphologic features including edema, necrosis, and tumor tissue. This approach is based on differences in apparent diffusion coefficient (ADC), resulting from changes in the balance between intracellular and extracellular water and changes in structure of the two compartments, with these conditions [93].

Price et al. studied four patients who received RT for low grade gliomas with MR perfusion imaging. There appeared to be RT-induced decreases in relative cerebral blood volume (rCBV) and blood flow (rCBF) within 3 months of RT in regions receiving >32 Gy [94]. In children with brain tumors, radiotherapy combined with or without chemotherapy often leads to the development of late neurocognitive sequelae. Using high-resolution MRI, Liu et al. measured the thickness of the cerebral cortex in medulloblastoma patients [95]. Cortical thickness maps showed relatively thinner cortex in multiple brain regions that were age and gender related. They reported that the areas of cortex undergoing development are more sensitive to the effects of treatment of medulloblastoma.

2.5.4 Magnetic Resonance Spectroscopy

Magnetic resonance spectroscopy (MRS) can measure aspects of brain metabolism in vivo by showing the spatial distribution and ratios of compounds that are present within the neurons, or participate in membrane or energy metabolism, e.g., N-acetyl aspartate (NAA), choline (Ch), and creatine (Cr) [96, 97]. Signals from multiple metabolites can be noninvasively measured within a single measurement period. In normal brain tissue, the largest signal arises for NAA, which serves as a marker of neuronal density and neuron functionality [98, 99]. Several studies have demonstrated decreases in NAA levels in normal brain after conventionally fractionated radiotherapy, which may occur even with low dose RT (<6 Gy) and recover within months after completing RT.

Kaminaga and Shirai serially studied 20 patients with MRS pre-RT, and ≈ 9 days and 15 months post treatment. They noted decreased NAA and increased choline at the longer follow-up time [104]. Choline was found in cellular membranes at high levels, which investigators suggested led to rapid membrane turnover or disruption. In a similar study from Zeng et al., MRS and diffusion-weighted imaging were performed 6 weeks post-RT, and serially every 2 months for the first year, then at 3- to 4-month intervals over 2 and 3 years [105]. Investigators found significantly lower levels of CH/NAA and Ch/Cr ratios ($p < 0.01$) in 55 patients assessed for radiation injury vs recurrent disease. These two variables reportedly could differentiate recurrent disease vs. normal tissue toxicity in 85.5% of the 55 subjects. With the addition of diffusion weighted imaging data [and the apparent diffusion coefficient (ADC)], authors reported a higher accuracy when differentiating radiation-induced injury vs. recurrent gliomas. Plotkin et al. compared the ability of I-IMT SPECT and H^1MRSI to differentiate recurrent tumor vs. radiation changes in 25 patients previously treated with RT for glioma. Using a 1.62 cutoff for I-IMT SPECT uptake, SPECT yielded a higher sensitivity, specificity, and accuracy as compared to H^1MRSI.

2.5.5 Functional Magnetic Resonance Imaging

Another modality being utilized increasingly to study brain plasticity in diseased patients is functional magnetic resonance imaging (fMRI) [106]. Blood-oxygen-level-dependent (BOLD)-based fMRI is based upon changes in brain oxygen content, which occur with changes in neuronal activity [107]. These changes can be induced by a number of stimuli, most commonly visual but inclusive of cognitive tasks [108]. One small study of 16 survivors of childhood cancers and 27 healthy subjects, demonstrated feasibility of using this technique to investigate brain function in survivors of childhood cancer, and found that the BOLD signal for both survivors and healthy subjects was qualitatively similar in timing and shape [109]. However, computer-aided analyses did detect significant quantitative differences in the BOLD signal of the survivors vs. healthy subjects.

2.6 Parotid Gland Injury

The parotid glands may be injured by RT during therapy for head and neck tumors, and can impact speech, chewing, and swallowing [117]. The incidence of clinical parotid dysfunction appears to be most related to the RT dose delivered, the percent of parotid volume irradiated, and the pre-RT parotid function [118].

2.6.1 SPECT and PET

^{11}C-methionine PET activity in the parotid glands is reduced following RT for head and neck cancer [119]. As has been done for the lung and heart, changes in planar salivary gland scintigraphy (SGS) and SPECT can be related to the RT dose in order to define the dose-response relationship for parotid dysfunction [118, 120]. It appears that doses as low as 10–15 Gy can result in a >50% loss of salivary gland function, measured by comparing the pre- and post-RT salivary excretion fraction (SEF) seen on SPECT. In a study from Medical University of Lübeck (Germany), significant alterations in radiotracer uptake in irradiated salivary glands of rabbits demonstrated that functional impairment could be assessed by scintigraphy as early as 24 h post-irradiation [117].

2.6.2 MRI

MRI has been used to evaluate salivary gland diseases, due to its excellent soft tissue contrast and the visualization of characteristic changes resulting from RT [121, 122]. A reduction in MRI-defined apparent diffusion coefficients (ADC) has been noted in patients with RT-induced dysfunction as assessed by scintigraphy [122].

Data from several studies relating radiographic changes in the parotid gland using PET, SPECT, and MRI, are shown in Table 2.6.

2.7 Conclusions

Functional imaging can be used quantitatively to detect RT-induced normal tissue injury in a variety of organs. In general, these imaging abnormalities manifest soon after (or even during) RT. Hence, it may be a powerful tool for the early detection of normal tissue injury and for the study of potential mitigators of such injury in humans. Radiologically defined normal tissue injury may be related to short/long-term clinically meaningful injury (e.g., global organ function), but further study is needed to better quantify this association (Table 2.7). Additional work is needed to develop methods and standards to quantitatively score radiologic injury.

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Portions of this document were adapted from [15].

Table 2.6. Radiographic changes in the parotid glands after irradiation of the head and neck

Reference	No. of cases	Radiographic follow-up months post-RT Median (range)	Type of radiographic study	Radiographic response/outcome
[119]	8	6 Months (minimum)	^{11}C-methionine PET	Metabolic clearance of ^{11}C-methionine in the parotid and submandibular glands decreased with increasing RT dose
[123] [a]	12	21 Months (8–54)	^{11}C-methionine PET	Dose-response analysis revealed a sigmoid relationship with a threshold dose of 16 Gy, and mean TD_{50} of 30 Gy
[124]	9	1.5 and 6 months	Magnetic resonance sialography (MRS)	Comparison of pre- and post-RT images revealed RT-induced decreases in visibility of the parotid and submandibular ducts, at 1.5 months, but subsequent improvement at 6 months
[121]	52	Within 24 months post-RT	MRI	RT-induced volume reduction of parotid
[122]	21	1 Month	MRI and salivary gland scintigraphy (SGS)	• Mean apparent diffusion coefficient (ADC) of dysfunctional parotids decreased by 23% on diffusion-weighted imaging post-RT • No significant change of ADCs of functional parotids
[125]	39	1 and 4 months	Salivary gland scintigraphy (SGS)	The mean loss of SEF in the spared parotid was 67% and 19% in 1 and 4 months post-RT, respectively. Normal excretion function was regained in 75% of the spared parotids
[126]	96	1.5 and 12 months	Salivary gland scintigraphy (SGS)	Reduction in salivary excretion fractions (SEF) from 44.7% to 18.7% at 6 weeks and to 32.4% at 12 months post-RT
[127]	16	1 and 9 months	Salivary gland scintigraphy (SGS)	Maximal excretion ratio dropped from 53.5% to 10.7%, and 23.3% 1 and 9 months post-IMRT, respectively
[120]	21	1 Month	Salivary gland scintigraphy (SGS) plus SPECT	Linear correlation between RT-induced changes in SEF on SGS-SPECT and RT dose
[118]	16	7 Months (6–10)	Salivary gland scintigraphy (SGS) plus SPECT	Median reduction in salivary excretion fractions (SEF) of 100% (range 17%–100%) observed 7 months post-RT

[a] Includes four of the same patients as the other Buus study [123]
SEF, salivary excretion fraction
Some data estimated from published reports

Table 2.7. Sample attempts to relate changes in regional radiologic studies to changes in global organ function

Organ	Regional radiologic assay	Degree of regional injury associated with reported global injury?
Lung	CT, SPECT PET	Yes; PFTs (Duke, NKI), symptoms (MDAH)
Heart	SPECT, MRI, PET	Unclear; EF (Duke, NKI, MDAH)
Liver	CT perfusion, MRI	Unclear, hepatitis (University of Michigan; University Tsukuba, Japan)
Brain	MRI, PET	Yes; neuropsychological deficits (Duke)
Parotid	PET, SPECT, MRI	Mixed; SEF (University of Leuven, Beligium; Toyko Medical and Dental University, Japan)

PFT, pulmonary function test; EF, ejection fraction; SEF, salivary excretion fraction.

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Association Between Single Nucleotide Polymorphisms and Susceptibility for the Development of Adverse Effects Resulting from Radiation Therapy

3

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CONTENTS

3.1 Summary

A small, but significant number of radiotherapy patients develop adverse responses to treatment, manifested as either normal tissue/organ damage or the development of a radiation-induced cancer. The ability to predict which patients are at greatest risk for radiation toxicity would be of great benefit in optimizing treatment decisions. One promising approach for the development of a predictive assay is through the use of genetic information. The main source of genetic variation among individuals is single nucleotide polymorphisms. Much of the early work to identify single nucleotide polymorphisms (SNPs) associated with the development of radiation injury focused on candidate genes. These studies have provided results indicative of a genetic basis for radiosensitivity, but it is clear that this approach is too limited in its scope to identify the SNPs that could serve as the basis for a predictive assay with clinical applicability. However, with completion of HapMap II and the development of high density SNP microarrays, it is now feasible to conduct genome wide studies which promise to lead to the identification of SNPs that will serve as the basis of an assay with suf ìcient sensitivity and specificity to be useful in the routine screening of cancer patients to identify those individuals at greatest risk for the development of adverse effects resulting from radiotherapy.

3.2 Introduction

The widespread recognition that modern radiation therapy can provide a sustainable cure for many people diagnosed with cancer, or at least delay disease progression, has led to its acceptance as a standard treatment option. However, as is true with all forms of cancer therapy, some patients experience morbidity resulting from their treatment. Although there are well-documented dosimetric explanations or underlying medical conditions responsible for the injury experienced by some patients who received radiotherapy, this explanation is not appropriate for many people. Often, the adverse response is simply ascribed to unknown individual variations. Important evidence in support of genetic factors being responsible for the differences in radiosensitivity between patients was obtained through an examination of radiation-induced telangiectasia in breast cancer patients [1]. It was observed in this study

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that the variation in the progression rate to the development of telangiectasia was relatively large for the same radiation treatment. A determination was reached that 80%–90% of the disparity was due to deterministic effects related to the existence of possibly genetic differences between individuals, whereas only 10%–20% of the variation could be explained through stochastic events arising from the random nature of radiation-induced cell killing and random variations in dosimetry and dose delivery. In addition to normal tissue injury, it must be recognized that radiation is a carcinogen. As a result, radiotherapy may increase the risk for the development of a new cancer. Extensive epidemiologic evidence has been obtained consistent with the conclusion that radiotherapy may cause many of the second malignancies observed in long-term cancer survivors for whom radiation successfully controlled their initial tumor [2–12].

3.3 Predictive Assays

The development of an assay capable of predicting which radiotherapy patients are most likely to manifest adverse radiation effects represents a long sought after goal [13]. Despite modest success, the effort to achieve this aim continues since an assay capable of predicting clinical radiosensitivity would allow customization of radiotherapy protocols. It has been estimated that a signi ıcant improvement in the therapeutic index could be achieved using this approach [14, 15]. These efforts are also re ỉective of the new era of "personalized" medicine [16–18], which recognizes the increasing importance of cancer survivorship for the roughly 10 million Americans who have survived a cancer diagnosis [19]. For these individuals, there is an increased focus on the quality of life 5, 10 or 20 years following treatment. The goal for this field of research is therefore to develop a robust, speci ỉc assay for cancer patients who are eligible for radiotherapy to enable individual dose adjustment based upon the response of each patient to this test [14, 15, 20, 21]. It is suggested that the area of research utilizing assays to predict the response of radiotherapy patients based upon genetic pro ỉles be termed "radiogenomics". Hence, radiogenomics is a new manifestation of the developing field of personalized medicine, which uses detailed information about a patient's genotype in order to select a medication, therapy or preventative measure that is particularly suited speci ỉcally to that patient.

There have been numerous efforts to identify predictors of clinical radiosensitivity. However, none of these assays has been implemented in the routine practice of radiotherapy as these efforts have failed to yield biomarkers that could serve as the basis for an assay which would possess the level of level of sensitivity and specificity necessary for a clinically useful predictive test [22]. In recent years, attention has focused upon the identification of genetic factors as the basis for an assay to predict which patients are at increased risk for complications secondary to radiation treatment. With the recognition that large and well-characterized patient populations are essential for the performance of these genetic studies, several broad international efforts have begun whose aim is the creation of biorepositories and databanks of radiotherapy patients. Major biorepositories have been established under the auspices of the GenePARE (genetic predictors of adverse radiotherapy effects) project [22], GENEPI [23] which was initiated by ESTRO and RadGenomics [24] which is comprised of Japanese patients.

Although the emphasis for much of the research performed has focused upon susceptibility to tissue and organ damage from radiation, there is increasing recognition that the development of second malignancies, particularly in children, is of great concern. Due to the success of radiotherapy and other forms of cancer treatment, many young people are being cured of their cancers only to develop a new radiation-induced cancer some years later. It would therefore also be advantageous if a predictive assay could be advanced that would help to identify which of these patients are most likely to develop a second malignancy from the radiation used to treat their first cancer.

Hence, the overall goal of this area of research is to identify those individuals from the general patient population who are most likely to suffer pronounced radiation-induced normal tissue damage and/or a radiation-induced malignancy. Although these radiosensitive patients may be better suited to a surgical treatment approach, paradoxically, these people could alternatively represent a subset of patients who are optimal candidates for radiotherapy, given that their cancers presumably harbor the identical sequence alterations associated with normal tissue toxicity. This highlights the potential

for radiotherapy dose modification as radiosensitive tumors theoretically could require lower total treatment doses than their genetically non-variant counterparts. Conversely, for the vast majority of patients who do not possess genetic variants associated with radiosensitivity, it may be possible to dose escalate and potentially achieve a larger number of cancer cures.

It should also be noted that through this research, genetic markers may be identified that are associated with radioresistance. For these patients, it may be possible, and even necessary if the possession of the SNP confers radioresistance to their cancer, to treat them with a greater dose of radiation.

3.4 Candidate Gene Studies

The sequence of the genetic material between all people is roughly 99.9% identical. However, approximately once every 1,000 nucleotides, a person may have a alternate nucleotide in the DNA sequence, which is referred to as a single nucleotide polymorphism (SNP). Many of the estimated 10 million SNPs that are thought to be present in the human genome can lead to a substitution of one amino acid for a different one in a protein, or could occur in an important functional region of a gene that can cause a person to be more likely to develop a certain disease, affect drug metabolism, or possibly render that individual more susceptible to the development of complications resulting from a radiation treatment [25].

A great deal of work has been performed in recent years in an effort to identify the candidate genes and SNPs in these genes that are associated with clinical radiosensitivity. This work is summarized in Table 3.1. Although candidate gene studies have provided critical evidence supportive of a genetic basis for clinical radiosensitivity, this approach has reached an impasse in terms of its ability to provide findings that will translate into a useful predictive assay for the following reasons: (1) Although a number of studies have detected correlations between possession of a minor SNP allele with an increased incidence of either radiation injury or second malignancy, the results of early studies have not been routinely validated in subsequent work (Table 3.1); (2) There is relative ignorance of the full spectrum of genes and proteins that are associated with the development of radiation injury and/or radiation-induced cancers; (3) Even if all of the important genes that encode the essential protein products associated with radiation toxicity were included in candidate gene studies, it is not certain whether any of these genes would possess SNPs that would both alter protein function and be present at a high enough frequency in the population to be of importance; (4) Critical SNPs associated with radiosensitivity may not be located within genes, but in regulatory portions of the DNA.

3.5 Genome Wide SNP Association Studies

It has become now clear that candidate gene studies are far too limited in scope to enable identification of SNPs to meet two criteria for useful biomarkers to form the basis of a predictive assay. These two essential characteristics are that the SNP must be present in at least a few per cent of the overall population and that possession of the SNP confers a significant elevation in the relative risk for the development of radiation toxicity. Therefore, it is now recognized that only through the performance of genome wide association studies will it be possible to identify SNPs that could form the basis of a predictive assay. This approach has just become feasible within the past few years due to two important scienti ic advances that have provided the ability to screen the entire human complement of genetic material to identify SNPs associated with clinical radiation responses. The first is the HapMap Project that has identi ied a substantial portion of the SNPs present in the human genome [26]. The second critical advance is the development of high density SNP microarrays which has enabled genotyping for less than $0.001 per SNP [27, 28]. Hence, it is likely that the path leading to the identi ication of SNPs that will form the basis of a predictive assy for clinical radiosensitivity will involve the performance of genome wide association studies. This approach has achieved a great deal of success in other areas of biomedical research which is reflected in the marked increase in the number of genome wide association studies being reported in which SNPs associated with a series of diseases and treatment reactions have been discovered [29].

Table 3.1. Candidate gene studies

Reference	First author	Irradiated site	Gene(s) screened	Number of subjects [a]	Result
[24]	Suga	Breast	999 SNPs in 137 candidate genes	399	Association between haplotype GGTT in CD44 with an increased incidence of early skin reactions. Association between haplotypes CG in MAD2L2, GTTG in PTTG1, TCC and CCG in RAD9A and GCT in LIG3 with a reduced risk for early skin reactions
[30]	Hall	Prostate	ATM	17	[b] Association between "significant mutations" with proctitis and cystitis
[31]	Duell	[c] Self reported	XRCC1	1286	No association between the codon 399 SNP with an increased incidence of breast cancer
[32]	Severin	[d] Multiple sites	RAD21	19	Association between the nucleotide 1440 SNP with adverse radiation effects
[33]	Iannuzzi	Breast	ATM	46	Association between "significant" SNPs with subcutaneous fibrosis and telangiectasia
[34]	Offit	Hodgkin's disease	ATM	64	No association between protein truncation mutations with the development of breast cancer
[35]	Andreassen	Breast	[e] TGFB1, SOD2, XRCC3, XRCC1, APEX	41	Association between the SNPs in TGFB1 codon 10 and nucleotide -509, SOD2 codon 16, XRCC3 codon 241 and XRCC1 codon 399, with an increased risk for subcutaneous fibrosis
[36]	Angele	Breast	ATM	566	Association between the codon 1853 SNP with an increased risk for adverse effects. Association between the SNPs at nucleotides IVS22-77 and IVS48 + 238 with a decreased risk for adverse radiation responses
[37]	Bremer	Breast	ATM	1100	No association between protein truncation mutations with either acute or late radiation effects
[38]	Moullan	Breast	XRCC1	566	Association between codons 194 and 399 SNPs with adverse radiation effects
[39]	Quarmby	Breast	TGFB1	103	Association between the nucleotide -509 and 869 SNPs with subcutaneous ibrosis
[40]	Andreassen	Breast	TGFB1, SOD2, XRCC1, XRCC3, APEX and ATM	52	Association between the TGFB1 codon 10 and nucleotide -509 SNPs with an increased risk of altered breast appearance
[41]	DeRuyck	Cervix / endometrium	XRCC1, XRCC3 and OGG1	62	Association between the XRCC3 nucleotide IVS5-14 SNP with an increased risk of late radiation effects and the XRCC1 codon 194 SNP with a reduced incidence of late effects
[42]	Millikan	[c] Self reported	XRCC3, NBS1, XRCC2 and BRCH2	4333	Association between possession of the minor allele for 2-4 SNPs in the screened genes with an increased risk for breast cancer and number of lifetime mammograms
[43]	Andreassen	Breast	ATM	41	Association between the codon 1853 SNP with subcutaneous fibrosis

Table 3.1. Continued

Reference	First author	Irradiated site	Gene(s) screened	Number of subjects [a]	Result
[44]	Andreassen	Breast	TGFB1, XRCC1, XRCC3, SOD2 and ATM	120	No association for any of the screened SNPs with risk for subcutaneous fibrosis
[45]	Cesaretti	Prostate	ATM	37	Association between missense SNPs (cause substitution of the encoded amino acid) with rectal bleeding and erectile dysfunction
[46]	Damaraju	Prostate	[f] Multiple genes		Association between the LIG4 codon 568 and ERCC2 codon 711 SNPs and the CYP2D6*4 splicing defect, with bladder and rectal toxicity
[47]	DeRuck	Cervix / endometrium	TGFB1	218	Association between the SNPs at nucleotides -1.552delAGG, -509 and in codon 10 were associated with development of late radiotherapy effects
[48]	Cesaretti	Prostate	ATM	108	Association between multiple SNPs with proctitis when the radiation dose to rectal tissue was quantified
[49]	Edvardsen	Breast	ATM	462	Association between the rs1801516 SNP with a reduced frequency of telangiectasia and the rs1800058 SNP with a reduced risk for pleural thickening and lung ìbrosis
[50]	Giotopulos	Breast	TGFB1 and XRCC1	167	Association between the TGFB1 -509 SNP with an increased risk of ìbrosis and an association between the XRCC1 codon 399 SNP with an increased risk of telangiectasia
[51]	Ho	Breast	ATM	131	Association between the codon 1853 SNP with the development of fibrosis and telangiectasia
[52]	Meyer	Prostate	ATM	721	No association between the codon 1054 SNP with either urinary morbidity or erectile dysfunction
[53]	Peters	Prostate	TGFB1	141	Association between SNPs at either nucleotide -509, codon 10 or in codon 25 with a decline in erectile function. Association between the SNP at nucleotide -509 with an increased risk of late rectal bleeding
[54]	Edvardsen	Breast	GSTM1, GSTP1, and GSTT1	542	Association between the GSTP1 codon 105 SNP with pleural thickening

[a] Includes cases and controls.
[b] Use of the term "association" indicates that a statistically significant association was reported (generally based upon use of a *p*-value of 0.05). It should be noted that most studies did not correct the *p*-value for multiple testing.
[c] Self-reported occupational and medical irradiations.
[d] Breast, tonsillar fossa, cervix, anus, vagina, testis, thymoma, and lymphoma.
[e] When multiple genes and SNPs were screened, a note was indicated only for significant associations that were detected.
[f] BRCA1, BRCA2, ESR1, XRCC1, XRCC2, XRCC3, NBN, RAD51, RAD52, LIG4, ATM, BCL2, TGFB1, MSH6, ERCC2, XPF, NR3C1, CYP1A1, CYP2C9, CYP2C19, CYP3A5, CYP2D6, CYP11B2, and CYP17A1.

Identification of the genetic factors associated with clinical radiosensitivity will have important and direct implications upon patient care as this will provide a basis to predict which patients diagnosed with cancer are at greatest risk for radiation toxicity resulting from radiotherapy. Upon detection of one or several SNPs associated with normal tissue injury or a heightened risk for a second malignancy in a particular patient, either a surgical approach to treatment can be considered, or recommendations may be made that can reduce the risk of morbidity resulting from radiotherapy. An added bene ìt of genetic testing is that once a potentially radiosensitive population is identi ìed, then the vast majority of cancer patients who prove negative for possession of the SNPs associated with susceptibility to the harmful effects of radiation can consider radiotherapy with less concern regarding complications. In fact, it is possible that traditional treatment doses have been limited by the subset of radiosensitive patients as radiation oncologists generally treat to normal tissue tolerance, the dose at which complications that cause signi ìcant morbidity begin to appear in the patient population. It may therefore be feasible as a result of genetic testing to increase the standard treatment dose and possibly achieve more cancer cures among the population of people who do not harbor the genetic alterations associated with adverse radiotherapy responses.

3.6 Conclusion

The performance of genome wide studies to identify SNPs associated with a susceptibility for the development of either radiation injury or a second malignancy from radiotherapy will be of great value as this will permit the creation of a predictive assay that will help patients and their doctors to decide upon an optimal treatment plan for each individual.

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Prospective Second-Cancer Risk Estimation for Contemporary Radiotherapeutic Protocols

4

David J. Brenner and Igor Shuryak

CONTENTS

4.1 Introduction: Radiotherapy-Related Second-Cancer Risks

The ability to predict radiation-induced cancer risks associated with modern radiation therapy protocols should allow the risks of second cancers to be included, and potentially minimized, in radiation therapy treatment plan optimization. This consideration is of increasing importance in light of the increasing number of younger patients undergoing radiation therapy, and with increasing survival times. As screening programs lead to earlier treatment and at younger ages, and as improvements in radiotherapy result in longer survival times, the issue of radiation-induced second cancers is becoming increasingly important [1, 2]. The 5-year relative survival rate for prostate cancer in the US has increased from about 67% in the mid 1970s to about 98% in the 1990s [3], while the mean age at diagnosis decreased from 72 to 69 [4]. The corresponding 10-year relative survival rates are now 76% for both prostate and breast.

Of particular importance in this regard are radiation-induced second cancers in childhood radiotherapy survivors [5–7], who: (a) are probably inherently more sensitive to radiation-induced carcinogenesis than adults, and (b) hopefully have more years of life remaining.

An example of the magnitude of the risks of concern can be seen from the results of a retrospective tumor-registry-based study [8] which compared second cancers in prostate cancer survivors who had radiotherapy, vs. those who had surgery: Here, the risks of developing a radiation-associated second malignancy after prostate cancer radiotherapy were estimated as 1 in 290 (all years), 1 in 125 for 5+ year survivors, and 1 in 70 for 10+ year survivors. As expected, second-cancer risks are much higher in long-term survivors of pediatric radiotherapy, approaching 25% at 30 years [6].

Using retrospective techniques, many such studies of second cancer risks after radiation therapy have been reported [2, 8–19]. However, radiotherapy treatment techniques are constantly changing, particularly in terms of escalating treatment dose [20–22], altered dose fractionation/protraction [23–26], and, as discussed in the next section, differing normal-tissue dose distributions, such as from intensity-modulated radiation therapy (IMRT) [27, 28]. Consequently, results from second-cancer studies which are typically the results of treatments that took place several decades ago, cannot generally be directly applied to modern-day protocols. This is-

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sue can be addressed, as is described in this chapter, by developing models that prospectively predict, through use of organ doses or dose distributions, second cancers associated with current radiation therapeutic treatments. Such models also provide insight into the basic mechanisms of radiation carcinogenesis [29] and, as we argue, are an essential first step towards systematic reduction of long-term radiotherapy-induced second-cancer risks.

4.2 The Potential Significance of Altered Normal-Tissue Dose Distributions: Intensity-Modulated Radiation Therapy and Second-Cancer Risks

There are two potential reasons why the change from 3D conformal radiotherapy (3D-CRT) to IMRT might result in a change in radiation-induced second malignancies risks [27, 30–35]. First, compared to 3D-CRT IMRT involves smaller volumes of normal tissues receiving higher doses, but larger volumes of tissues receiving smaller doses. Clearly the significance of this in terms of radiation-induced malignancies will depend will depend on the shape of the dose-risk relationship. Second, delivery of a specified dose to the isocenter from a modulated field requires the accelerator to be energized for longer, and so more monitor units are needed, typically increases being factors of about 3, but with a range of from about 2–8 [35–37], compared with delivering the same prescribed dose from an unmodulated field. It follows that patient dose due to leakage radiation may be increased, although its spatial distribution and magnitude will depend on many interrelated factors, including the head shielding design, design and operation of the multileaf collimator, beam energy, and the details of the IMRT modulation.

To date, there have only been fairly crude estimates of second cancer risks after IMRT compared with 3D-CRT. Followill et al. [34] estimated a fatal cancer risk after pelvic IMRT of 1.0%, compared with 0.4%–0.6% for the corresponding 3D-CRT treatment. Hall and Wuu's cancer mortality estimates [27] were 1.8% (IMRT) vs. 1.0% (3D-CRT), and the fatal cancer risk estimates by Kry et al. [35] were 2.9%–3.7% (IMRT) vs. 1.7% (3D-CRT). So each of these estimates concluded that IMRT would roughly double the second-cancer risk, compared with 3D conformal radiotherapy.

These estimates are, however, all very crude [38]. In particular they are based on linear extrapolations of low dose (≤ 2 Gy) cancer risks that were generated for radiation-protection purposes from A-bomb survivors, to high, fractionated, radiotherapeutic doses. There has been a considerable literature suggesting that this is not a reasonable approach [11, 39], though to date alternatives have not been available. Here we describe some new approaches towards understanding and predicting dose-effect relations for radiation-induced cancer at radiotherapeutic doses.

4.3 Mechanisms of Radiation-Induced Cancer at Radiotherapeutic Doses

4.3.1 The Standard Model

Radiation therapy can deliver very high doses of radiation to regions in organs that are in or close to the target volume [40]. In earlier approaches to high-dose risk estimation, radiation-induced carcinogenesis at high doses was assumed to be governed primarily by two competing cellular processes [41], "initiation" and "inactivation". Initiation is the production of changes that make a stem cell premalignant; examples are chromosomal translocations, such as the Philadelphia chromosome [42], or other cytogenetic abnormalities such as inversions, small-scale mutations, deletions, duplications, or aneuploidy [43–45]. Inactivation prevents a stem cell from having viable progeny, examples being mitotic death or apoptosis.

The assumption that radiation carcinogenesis is primarily governed by initiation and inactivation has generally been quantified using the standard linear–quadratic–exponential (LQE) equation [41]; for reviews, see [29, 46, 47]. The LQE equation describes the excess relative cancer risk (ERR) after a single acute dose of radiation (D) as

$$\mathrm{ERR} = (aD + bD^2)\exp(-\alpha D - \beta D^2) \qquad (4.1)$$

where a and b are linear and quadratic coefficients for initiation, and α and β are linear and quadratic

coefficients for inactivation. The LQE equation uses the classic linear-quadratic (LQ) form both for radiation-induced initiation ($aD + bD^2$) and for radiation-induced inactivation $\exp(-\alpha D - \beta D^2)$.

For small and intermediate radiation doses, Equation 4.1 predicts that ERR is an increasing function of dose, as is seen epidemiologically [11, 12, 48, 49]. At high doses, however, the exponential cellular inactivation term, $\exp(-\alpha D - \beta D^2)$, in this LQE equation leads to very small predicted ERRs; that is, essentially all radiation-initiated premalignant stem cells would be inactivated by the radiation. As shown in Figure 4.1 [29], this prediction of the LQE equation is inconsistent with recent estimates of radiation-induced solid cancer risks, in that a rapid decrease in the ERR at high doses is not observed.

4.3.2 A More Realistic Model

Consequently, the standard LQE initiation–inactivation model has been extended [29] to include a third mechanism, in addition to initiation and inactivation, of radiation-induced carcinogenesis at high doses. Speci ically, symmetric stem-cell proliferation (i.e., a stem cell dividing into two daughter stem cells) occurs in response to radiation-induced cell killing [55–58], and replenishes the number of stem cells in that organ. Because repopulating cells can only travel very small distances, at least for solid organs, they will have been near the treatment field at the time of irradiation, so will have received signi icant doses, and so will contain a significant fraction of stem cells with pre-malignant damage. Symmetric proliferation, which takes place both during and after radiation therapy, and will thus increase the high-dose cancer risk, as any proliferating stem cell that has pre-malignant damage can pass that damage on to its progeny.

In fact there is a great deal of quantitative biology in the literature about repopulation kinetics [55–58], which can be reasonably grafted on to the standard initiation/inactivation model, resulting in a quantitative initiation/inactivation/proliferation model, as discussed in the next section.

Figure 4.2 schematizes the three mechanisms which appear to dominate radiation-induced carcinogenesis at radiotherapeutic doses. The standard model incorporates only the first two mechanisms, namely initiation and inactivation.

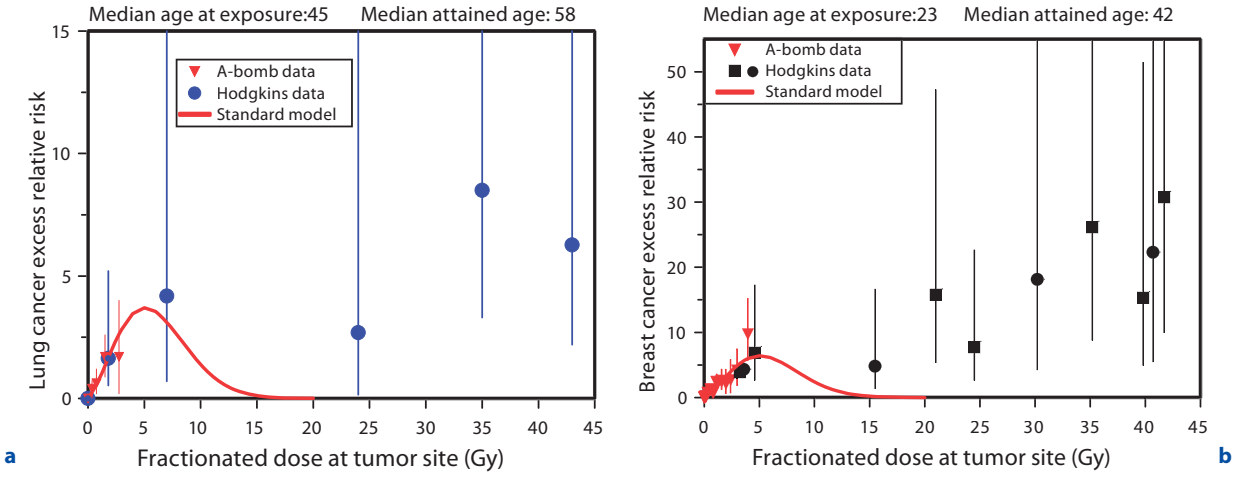

Fig. 4.1a,b. Excess relative risks for radiation-induced lung cancer (**a**) and breast cancer (**b**). The lower-dose data points from A-bomb survivors [50, 51], and the data points at high doses are from studies of lung cancer [52] and breast cancer [53, 54] after radiotherapy of Hodgkin's disease patients. The *solid curves* in each panel represent fits to the A-bomb data using the standard "initiation + killing" LQE model [41], which involves a balance solely between induction of pre-malignant cells and cell killing, without considering cellular repopulation. It is clear that the predictions of this standard LQE model are inconsistent with the high-dose data

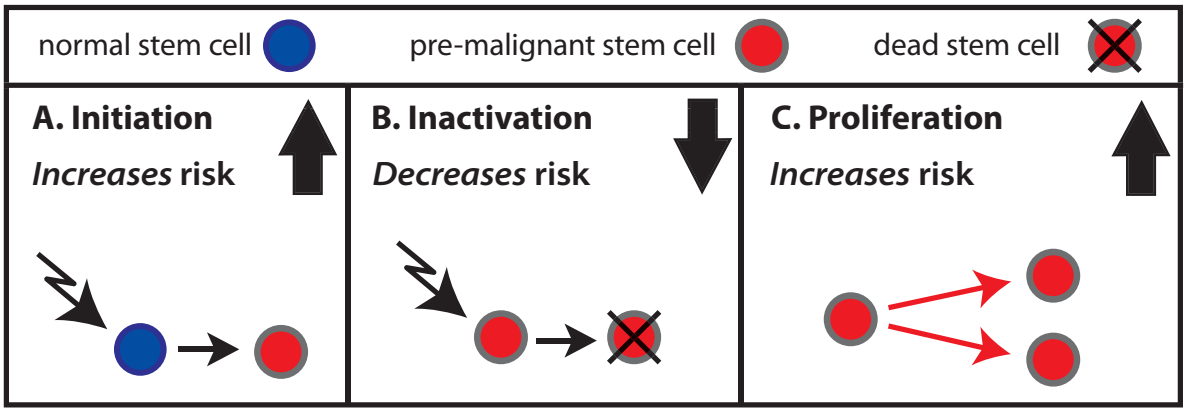

Fig. 4.2. The three dominant processes affecting the probability of radiation-induced cancer at radiotherapeutic doses. The standard model incorporates only the first two of these mechanisms

4.4 An Application: Prospective Estimation of Radiotherapy-Induced Second-Cancer Risks

The stem cell initiation/inactivation/proliferation model [29, 59, 60] outlined here provides a practical approach [61] for predicting organ-specific high-dose cancer risks based on: (a) cancer risk data from A-bomb survivors (who were exposed to lower doses), (b) the demographic variables (age, time since exposure, gender, ethnicity) of the population/individual of interest, and (c) an organ-specific parameter describing radiation-induced cellular repopulation, which has previously been estimated both for breast and lung [29]. First, ERRs are directly estimated for single radiation exposures at moderate doses, based on cancer incidence data among A-bomb survivors [50, 62]. Second, a well established methodology described by Land and colleagues [63] (and almost identically in the recent BEIR-VII report [64]) is used to adjust the dose-dependent ERRs from the A-bomb survivors to apply to the demographics (age, time since exposure, gender, ethnicity) of the individual or group under study. These two steps are implemented through publicly available on-line software (Interactive RadioEpidemiological Program, IREP version 5.3 [65]). Finally, these moderate-dose ERR estimates for single exposures are adjusted to fractionated high-dose radiation exposure, using the initiation/inactivation/proliferation model [29] outlined above.

This augmented cancer risk model is able to well describe demographics-specific epidemiological data for radiotherapy-induced carcinogenesis [29, 59]; examples are shown in Figure 4.3.

The approach can, in principle, generate organ-specific ERR estimates for any given radiotherapeutic dose and fractionation scheme, for any given set of demographics (in particular age at exposure, and time post exposure). Essentially all that is needed are dose-volume histogram (DVH) data for the organ or organs of interest. In this "dosimetric + risk-modeling" method, each incremental small volume in the DVH, ΔV_j, is associated with a total dose $D_j = j\Delta D$. Given the associated $ERR(D_j)$, estimated as described above, the overall predicted ERR is the volume-average of these local ERRs, i.e., $ERR = (1/V) \sum_j ERR(D_j)\, \Delta V_j$, where V is the organ volume. An example is given in Figure 4.4, based on results reported by Koh et al. [61].

4.5 Future Directions

Understanding and quantifying second cancer risks is, we believe, the first step towards being able to reduce them, through hardware and software optimization – conceptually in the same way as classic early and late sequelae have been reduced by advances in hardware and by treatment planning. Having said

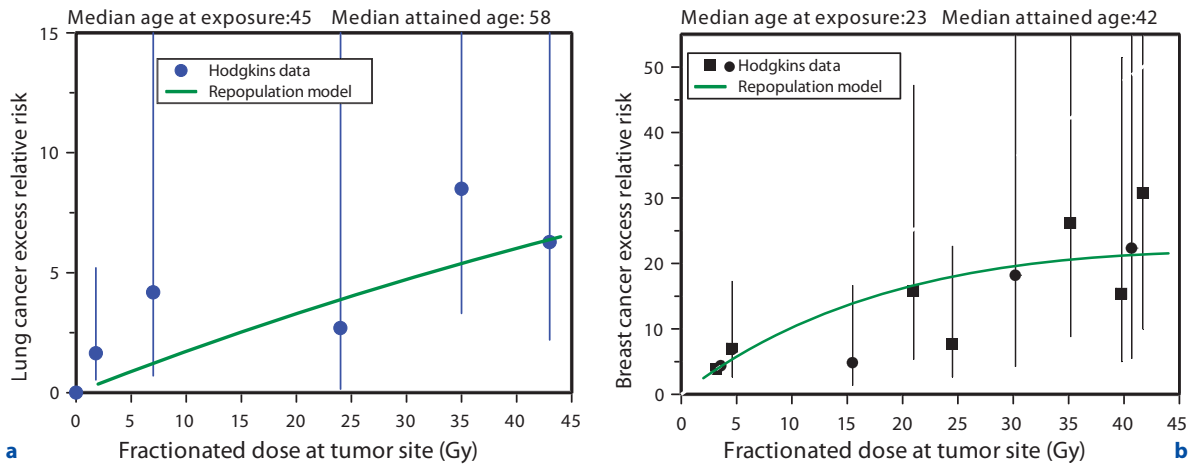

Fig. 4.3a,b. Measured and predicted excess relative risks for lung cancer (**a**) and breast cancer (**b**) induced by high doses of fractionated ionizing radiation. The data points are from studies of second cancers after radiotherapy of Hodgkin's disease patients, as in Figure 4.1, and the curves are estimates using the methodology [29] outlined here

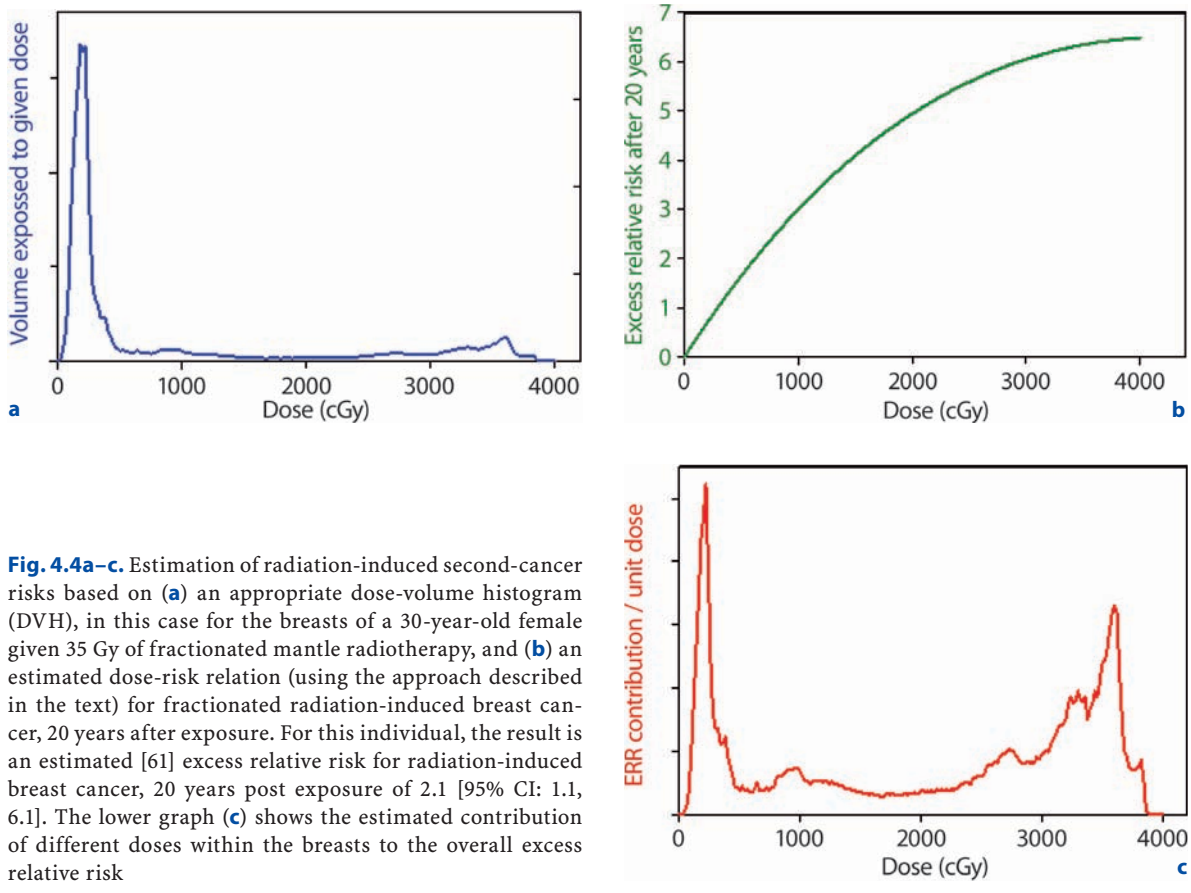

Fig. 4.4a–c. Estimation of radiation-induced second-cancer risks based on (**a**) an appropriate dose-volume histogram (DVH), in this case for the breasts of a 30-year-old female given 35 Gy of fractionated mantle radiotherapy, and (**b**) an estimated dose-risk relation (using the approach described in the text) for fractionated radiation-induced breast cancer, 20 years after exposure. For this individual, the result is an estimated [61] excess relative risk for radiation-induced breast cancer, 20 years post exposure of 2.1 [95% CI: 1.1, 6.1]. The lower graph (**c**) shows the estimated contribution of different doses within the breasts to the overall excess relative risk

this, it is crucial to ensure that any changes in treatment technique designed to decrease second-cancer risks do not impact negatively on primary tumor control.

We are a long way from being able to estimate radiation-induced cancer risks *ab initio*, i.e., solely based on biologically-based models. The approach described here, which appears to be reasonably promising, is to use cancer risks originally estimated in A-bomb survivors, modify them for the demographic cohort or individual of interest, and then extrapolate these risks to higher doses using the quantitative biological models described here. Finally, combining the results using organ-specific dose volume histograms allows realistic prospective estimates of radiotherapy-related second cancer risks.

Of course, there remain considerable uncertainties in these modeling approaches. For example, it remains unclear to what extent radiation-induced second cancer risks depend on the primary cancer, over and above the different dose distributions. A recent study of CNS tumors in survivors of childhood cancers concluded that "after adjustment for radiation dose, neither original cancer diagnosis nor chemotherapy was associated with risk" [5], but the question is still open and important.

Donaldson and Boyer, commenting on IMRT, suggested [66] that "*the impact of multi-field, low-dose radiation exposure, and higher total body doses from leakage radiation associated with longer "beam-on" times and leaf transmission, carry risks of radiation carcinogenesis that cannot be accurately addressed*". In this regard, we have reported here some advances towards quantitative prospective estimation of radiotherapy-induced second-cancer risks.

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Bioengineering in the Repair of Irradiated Normal Tissue by Bone Marrow Derived Stem Cell Populations

5

Joel S. Greenberger

CONTENTS

5.1 Introduction

5.1.1 The New Paradigm for Understanding Ionizing Irradiation Tissue Damage

While the basic principles of radiation chemistry and radiation molecular biology have not changed, there has been significant modi ication in our understanding of cellular interactions involved in irradiation tissue damage [1–5]. Ionizing irradiation induces radiation chemical changes in cells, principally targeting oxygen and water molecules which results in formation of superoxide, hydroxyl, and other free radical moieties, within fractions of a second after radiation exposure [2]. Formation of nitric oxide, and its combination with superoxide leads to formation of peroxynitrite, a potent pro-oxidant [6, 7], which in combination with the other radical oxygen species leads to significant lipid peroxidation as well as direct binding to nuclear DNA that results in single and double strand breaks [8–11]. In the last decade, it has become clear that DNA strand breaks are repaired rapidly, certainly within 15 min after irradiation of cells in culture [4, 5], and that this repair involves a complex interaction of multiple rapid response genes, which initiate by site specific phosphorylation of the ataxia telangiectasia protein (ATM) on a specific phosphorylation site [12]. Concatenation of multiple proteins at the site of DNA strand break leads to induction of pathways for homologous recombination or homologous end joining in the case of double strand break repair [11, 12]. Deletion or inactivation or one or more components of the complex protein concatenation at DNA strand break sites leads to a reduced kinetics of DNA repair, radiosensitivity of cells in culture, and increased irradiation damage to tissues or organs in vivo [12].

While our understanding of the initial events involved in DNA strand breaks and repair, remain basically the same, there has been new appreciation for the distal steps in the pathway of ionizing irradiation response that follow rapid repair of DNA strand breaks.

Nuclear to cytoplasmic, and specifically mitochondrial communication of signals from DNA strand breaks has become the focus of intense investigation in basic radiation biology [4, 5, 13–17]. Translocation of multiple proteins from the nucleus to the mitochondria has been described following ir-

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radiation of cells in culture, including movement of p53, activation of p21, and the stress activated protein (SAP) kinases including BAX [4, 5, 18–20]. Localization of these nuclear proteins to the mitochondria after irradiation has been associated with profound biochemical and physiological changes in the mitochondrial membrane. Increased calcium transport across the mitochondrial membrane, is associated with activation of mitochondrial (neuronal) nitric oxide synthase, resulting in mitochondrial production of nitric oxide [6, 19]. Mitochondrial membrane changes associated with SAP kinase concentration include increased production of superoxide which in combination with nitric oxide leads to peroxynitrite formation and lipid peroxidation [6–9, 15]. A potentially critical element in the mitochondrial changes that are associated with irradiation induced apoptosis (programmed cell death) is the step involving interaction of Cytochrome C with cardiolipin [14]. Recent evidence has indicated that Cytochrome C, a natural component of the electron transport cascade which normally functions to stabilize mitochondrial generation of ATP during respiratory metabolism, has been shown to be associated 70% in binding to the mitochondrial phospholipid cardiolipin [14]. Only around 30% of cytochrome c is free within the inter-cisternae space between the outer and inner mitochondrial membranes [14]. Lipid peroxidation associated with ionizing irradiation induced changes in the mitochondria results in release of cardiolipin from Cytochrome C [14]. Furthermore, changes in Cytochrome C induced by ROS convert Cytochrome C into a peroxidase which can further denigrate cardiolipin and other mitochondrial lipids [14, 15]. Shunting of one of the breakdown products of cardiolipin, phosphatidyl-serine from the mitochondrial to the cell membrane is associated with annexin 5 expression, leading to one of the signals for apoptosis and/or signal for phagocytosis by inflammatory cells in the microenvironment [14, 15]. Cytochrome C leakage from the mitochondria, follows the release of Cytochrome C from cardiolipin, and represents a "point of no return" [14] in the apoptotic pathway since free Cytochrome C in the cytoplasm has been associated with activation of caspase-3, leading to PARP (poly-ADP-ribosylpolymerase) and the formation of DNA strand breaks in the nucleus that are observed in the apoptosis [4, 5].

Thus, the "new paradigm" for irradiation induced cellular injury involves initial DNA damage which is rapidly repaired, but results in a signal through the cytoplasm to the mitochondria which results in lipid peroxidative changes in the mitochondrial membrane [4, 5, 14], and release of Cytochrome C that then induces a secondary damage effect called apoptosis. There follows a delayed series of DNA strand breaks which are detected in the apotag or Olive-tail assay in vitro or identi ication of apoptotic bodies in vivo [4, 5, 21]. Studies from the 1960s and 1970s with alkaline sucrose gradient analysis of DNA fragmentation of the nucleus, following increasing doses of irradiation, may actually have been measuring those strand breaks induced by secondary apoptosis, rather than the initial DNA strand breaks [1].

5.1.2 General Concepts of Bioengineering for Tissue Repair

There is great enthusiasm for combining biological materials with chemically synthesized scaffolds in tissue repair. Synthetic polymer, and micro-porous materials have been utilized in maxillofacial surgery and in the therapy of non-union of bone fractures [74–81]. Combining tissue culture grown bone marrow stromal cells (progenitors of osteoblasts and chondrocytes) with scaffolding has led to novel approaches for experimental bone reconstruction [78, 81]. Biological engineering has also incorporated techniques utilizing keratinocytes, or bone marrow stromal cells, genetically engineered to express increased levels of humoral factors, including colony stimulating factors, and cytokines, involved in tissue repair [82]. Such approaches have been used to modify skin grafts for burn therapy, and also in the repair of vascular grafts including coronary microvasculature. Transplantation of embryonic stem cells, tissue derived stem cells, or bone marrow stem cells into stereotactically defined regions of the brain has been utilized in experimental approaches to treat Parkinson's Disease, stroke, and damaged tissues following brain or spinal cord injury [79, 80]. Biologically engineered cells secreting neurotrophic growth factors have also been applied in these approaches [79].

In all the above examples, the role of the tissue microenvironment into which complex organic and in-organic repair modules are inserted, has been described as of critical importance.

Ionizing irradiation damaged tissue poses a unique challenge for the use of stem cell therapies in tissue repair. Unlike burn, or traumatic tissue injury, ionizing irradiation damaged tissue may show

only microscopic histopathology [2]. In the case of the oral cavity mucosa, esophagus, and lung, microvascular edema, inflammatory cell in ìltrates, and then delayed apoptotic body formation may be the only indications of early irradiation damage. Circulatory elaboration of cytokines may rapidly cause systemic symptoms, but the sites of major irradiation injury may reveal damage days to weeks after injury [2]. Furthermore, the kinetics of induction of the late effects (fibrosis, vascular telangiectasias) may vary dependent upon dose, fraction size, and volume irradiated as well as sources of co-morbidity including prior surgery.

While ionizing irradiation damage poses unique challenges to tissue engineering, there are common factors which link many forms of tissue injury and can guide strategies for using stem cell transplantation in tissue repair. A common challenge in the development of stem cell therapies for bioengineering of irradiated tissue repair, is focused on the pathophysiology of the irradiated microenvironment. Irradiated tissues demonstrate many common associations with premature aging, including graying of hair, loss of elasticity of skin, and delayed healing of wounding [3]. At the biochemical level, reduction in antioxidant pools is associated with increased cumulative injury from radical oxygen species production [83–86]. Transplanting unirradiated stem cell populations into an irradiated tissue results in oxidative stress induced damage to the donor cells. In vitro systems [84] document cell contact and humoral mechanisms of induction in non-irradiated cells of molecular biologic changes and biochemical changes induced by the irradiated cells of the microenvironment [85, 86].

While recent work in experimental model systems suggests that administration of antioxidant transgenes such as MnSOD-plasmid liposomes locally to the target irradiated organ, can facilitate improved engraftment [47], it remains to be demonstrated whether sustained engraftment and robust tissue repair will follow. It may be that transient reduction in ROS by MnSOD-PL gene therapy facilitates engraftment of cell populations that can help in the initial repair of tissue integrity, but that surviving recipient stem cells provide the repopulation necessary for sustained organ function [21]. Furthermore, tissue repair of epithelial organs by bone marrow derived stem cell transplantation, may not obviate other life shortening effects of irradiation which may be a consequence of irradiation damage to the microenvironment [48, 83].

5.2 Bone Marrow Origin of Stem Cells for Epithelial Tissues

The bone marrow origin of hematopoietic progenitor cells was first demonstrated in the mouse by Till and McCullough who also established a quantitative approach toward calculation of the frequency and density of multilineage stem cells in the marrow [22]. In vitro colony assays for committed stromal or hematopoietic progenitors were next described [23, 24]. Since these pioneering studies in mouse models of marrow transplantation and correlation to human marrow, mobilized peripheral blood, and cord blood stem cell populations; the concept of a limited and quantifiable number of multilineage progenitor cells from the marrow, capable of reconstituting hematopoietic tissues has been established [25]. The entire field of bone marrow transplantation developed from these pioneering studies. Preparation of the host for accepting bone marrow stem cell engraftment by total body irradiation [23, 25] defined the role for both cells of the hematopoietic microenvironment (bone marrow stromal cells) and humoral cytokines produced by irradiated tissues, in the process [23, 24].

The bone marrow origin of cells of other than the hematopoietic lineage has remained a subject of intense investigation and continues to be controversial. Bone marrow stromal cells, recently renamed mesenchymal stem cells, were demonstrated originally by Friedenstein et al. [92] to have a quanti ìable and limited number [27], and were shown to be transplantable in vivo [23]. Werts et al. [28] first demonstrated the capacity of bone marrow stromal cells to migrate from one non-irradiated site into a heavily irradiated site in the mouse marrow. Anklesaria et al. [29, 30] showed that cell lines derived from bone marrow as well as uncloned populations of marrow stromal cell lines could repopulate serial niches of irradiated target tissue by intravenous injection.

The capacity of bone marrow stromal cells to differentiate to osteoblasts, chondrocytes, adipocytes, as well as supportive tissues of the hematopoietic microenvironment is well established [31, 32]. There is controversy over the subdivisions of marrow stromal cells, and whether there is a true subpopulation that is capable of multilineage differentiation. Alternatively, all bone marrow stromal cells have

been suggested to retain a multilineage differentiation capacity and the term "transdifferentiation" of the marrow stromal cell suggests that these cells have a different capacity for a lateral transfer from one epithelial lineage to another (osteoblast to chondrocyte) [111].

A very important experiment by Terry and Travis [33], first demonstrated that abdominal irradiation of mice could be rendered sublethal by bone marrow transplantation. Among the potential explanations discussed, include the possibility that bone marrow contained cells capable of reconstituting gastrointestinal stem cells. Karus et al. [34] identified epithelial marked cells of donor origin, in mice reconstituted with a single hematopoietic stem cell. In the last 8 years, numerous publications have suggested the possibility that a subset of bone marrow stem cells is capable of reconstituting epithelial organs including liver, GI tract, esophagus, skin, glandular tissues including the pancreas, beta-islet cells of the pancreas, central nervous system, striated muscle, cardiac muscle, lung, and other tissues [35–42]. These studies can be characterized by several degrees of rigorousness: most studies demonstrate histochemical or immunohistochemical markers of epithelial tissue, in the same cells with donor chromosome or histo compatibility markers [34, 35]. A second level of rigorousness involves the serial transfer assay of sorted cells from a first generation recipient to a second generation recipient, documenting the self-renewal capacity of such cells of bone marrow origin [49]. The most rigorous assay for demonstrating bone marrow origin of epithelial tissues, is the use of a functional assay in which biochemical or immune or structural deficiencies in a recipient animal, are corrected by transplantation of bone marrow derived cells [50]. In this latter and most rigorous category of proof of bone marrow origin of epithelial tissues, partial reconstitution of the recipient organ should be demonstrated. An example of rigorous proof is the MDX mouse model of Duchenne muscular dystrophy in which striated muscle cells are deficient in components of contractile proteins and render muscles in a mouse incapable of significant functional activity [51–58]. Transplantation of subsets of muscle derived stem cells from normal donor mice into these animals has shown partial correction of the functional defect [58]. Utilizing bone marrow stem cells in this assay has also shown some positive results; however, even these studies fall short of significant correction of the defect [51–53].

Recent studies have attempted to prove that bone marrow derived cells can either replace defective lung function in (CFTR) cystic fibrosis transmembrane receptor mutant mice [59]; replace defective liver protein synthesis in mouse models of liver protein deficiency [42], or correct cardiac functional defects in mouse models of myocardial damage [53].

The present state of research in this area has gained some support from studies utilizing embryonic stem cell lines. Embryonic stem cell lines, have been demonstrated to differentiate to specific tissue elements in vitro, and subsets of ESCs can be transplanted in vivo forming organ specific tissue foci [60–62]. For applications to tissue engineering, and bioengineering, either embryonic stem cell lines or sorted subpopulations of bone marrow stem cell lines provide an attractive potential resource for repairing tissue. The challenge remains one in which absolute proof of the concept can be demonstrated, and then more importantly that subpopulation of the cells can provide reconstitution of a significant relative volume of the target organ to provide for a functional and quantifiable repair.

A compelling reason to continue research in the area of marrow stem cell reconstitution of irradiation damaged tissue is the observation that cells of bone marrow origin naturally repair radiation damaged tissues in vivo. Bone marrow origin cells have been demonstrated to reconstitute the liver of bone marrow transplant recipients [35]. Allogeneic bone marrow transplant recipients have been shown to have significant replacement of lung tissue by donor derived cell populations [63–69]. Finally, there is evidence that stem cell populations sorted from epithelial organs using the same cell separation techniques, can be transplanted into recipient animals and will reconstitute the same organ, or in some cases the bone marrow itself suggesting that there is a reversed epithelial to bone marrow reconstitution capacity [54–57]. Thus, these data suggest that multilineage epithelial stem cell populations can exist not only in the bone marrow, but in the target organ itself. It remains a major challenge of cell biology to define the parameters of isolation of bone marrow progenitors for epithelial organs, to develop ways of amplifying such cells for transplantation, and then most importantly to demonstrate reproducible engraftment of target organs with bone marrow derived cells leading to functional reconstitution in the recipient.

5.2.1 Repopulation of Recipient Target Organs with Bone Marrow Derived Stem Cell Progenitors: A Double Edged Sword

Since the first demonstration of the use of total body irradiation to prepare recipient animals for bone marrow transplantation, there has been controversy over the role of irradiation in the process [70–73]. Clinical bone marrow transplantation has gone through cycles of evolution of either utilizing total body irradiation, either single fraction or fractionated, for nearly all patients with hematopoietic malignancies, or going to protocols in which irradiation is removed and substituted with use of Busulfan and Cytoxan to reduce the pulmonary toxicity of irradiation. Over the last 30 years, marrow transplant centers have fluctuated back and forth between using total body irradiation as desirable in the preparatory method, or as undesirable because of toxicity. The most recent evolutionary step in this process has been to design a "non-myeloablative" total body irradiation dose [70–73], designed to be low enough to prevent pulmonary toxicity, but high enough to produce microenvironmental changes including upregulation of cytokines and depopulating hematopoietic stem cell niches in the recipient. The cellular and molecular mechanisms of the effect of total body irradiation are still unknown. Table 5.1 demonstrates the hypothesized positive and negative effects of total body irradiation preparing a host for bone marrow transplant.

The irradiation dose dependent "clearance" or "preparation" of a marrow site for transplantation has been demonstrated [29, 30].

Table 5.1. The role of total body irradiation (TBI) in preparing the host for bone marrow transplantation

Putative positive effects of TBI
1. Removal of malignant cells from the marrow
2. Clearing "space" in bone marrow stem cell niches, removing recipient cells and providing for homing of donor cells
3. Inducing stimulatory cytokines from the marrow microenvironment which facilitate engraftment
4. Removing immuno-competent cells from the recipient to prevent graft rejection
Putative negative effects of TBI
1. Damage to the hematopoietic microenvironment, vascular and stromal cells and production of toxic cytokines and ROS
2. By removing differentiated cells stimulation of repopulation by surviving recipient stem cells which compete with donor stem cells
3. Co-morbidity of lung toxicity, GI stem cell toxicity, and toxicity to mucosal stem cells

5.2.2 Bone Marrow Derived Stem Cells in Bioengineering Repair of Irradiated Oral Cavity and Oropharyngeal Mucosa

Animal models of irradiation damage to the oral cavity and mucosa have been published [87, 89]. In these models, single fraction and fractionated irradiation is associated with induction of apoptotic bodies, micro-ulceration, and then sloughing of mucosal tissue. This apoptosis leads to secondary infection, severe damage to the mucosa also leads to severe dysphagia, dehydration, and weight loss. A common acute side effect of radiotherapy of head and neck cancer is mucositis. Salivary gland damage from irradiation has also been well documented [87]. In studies with epitope-tag, hemagglutinin-tagged MnSOD transgene, intraoral administration in the mouse model have been demonstrated to result in transgene penetration to cells in the basal layer of the epithelium, at sites where cycling stem cells are known to reside [89]. Quiescent stem cells in the mucosal basal layer are induced to cycle as part of the repopulation response of irradiated tissue. An hypothesis tested in recent experiments was that MnSOD-PL treatment of the oral mucosa could result in stabilization of the mucosal cells such that post-irradiation cell cycling would be decreased [89]. This would presumably lead to decreased destruction of stem cell populations during the fractionated irradiation program and better healing.

The question of whether bone marrow derived stem cell populations can reconstitute the oral cavity and oral mucosa has recently begun to be addressed. A recent report [90] demonstrated that mouse salivary glands are repopulated with cells coming from the bone marrow. With three-dimensional culture in vitro and in vivo explants, ductal epithelium and branching microscopic ducts were demonstrated to be of bone marrow origin. In one experiment cells were removed from the first generation recipients of bone marrow transplant, stem cell populations iso-

lated and transplanted into a second generation of irradiated mice in which the glandular epithelium was shown to be replaced by cells originally of bone marrow origin [90]. The question of how much bone marrow derived stem cells contributes to repair of the irradiation damaged oral cavity and oral mucosa is not known. Stem cell repopulation in both salivary gland and oral cavity/oropharynx are known to occur and are part of the response to fractionated irradiation, but the question of whether circulating stem cells mobilized from the bone marrow can compete for the repopulation with the in situ epithelial stem cells is not yet known.

Other experiments in radiation protection of the oral cavity using MnSOD-plasmid liposomes in both single fraction and fractionation models have shown significant stabilization of the oral mucosa, and parotid glands [87]. In these experiments, decreased micro-ulceration, decreased weight loss, and improved survival were noted. In addition, increased saliva production by the irradiated oral cavity was demonstrated. Several experimental paradigms have been derived to determine whether bone marrow stem cell populations can replace irradiation damaged oral cavity tissues.

Fibrotic areas in the high dose irradiated sites, that receive in excess of 70 Gy, have been limited to high dose boost areas in programs of IMRT or brachytherapy. The question of whether fibroblasts forming the fibrosis come in part from marrow stromal cells (mesenchymal stem cells) that are mobilized from the bone marrow has not been conclusively demonstrated with the oral cavity or oropharynx, but since similar studies have been carried out in lung [48] and esophagus, it is possible that secondary immobilization of cells involved in oral cavity fibrosis may involve progenitors of bone marrow origin.

5.2.3 Bioengineering for Repair of Irradiation Damage in the Esophagus Using Bone Marrow Derived Stem Cell Progenitors

Recent studies in an experimental model of murine irradiation esophagitis have demonstrated significant preservation of the esophageal lining by MnSOD-plasmid liposome administration [21, 47, 91, 93–98]. Mice receiving either MnSOD-PL prior to single fraction irradiation or every third day during six- or eight-fraction radiotherapy [94] showed improved survival, which was directly related to MnSOD biochemical levels in the irradiated tissue [96]. In the esophagus model, a stem cell population has been isolated from the basal layer of the esophageal mucosa [95]. Using techniques originally designed to isolate stem cell populations from bone marrow, and striated muscle, single cell suspensions of esophagus were sorted for Hoscht/Prodium Iodide or side population cells [95]. These side population cells have been shown to contain quiescent stem cell populations from bone marrow or from smooth muscle [95]. In a second technique a serial preplate method was utilized whereby single cell suspensions of esophagus were plated in tissue culture medium on plastic plates, and then daily for 7 days nonadherent cells removed and placed into a second cell culture [95]. By serial removal of adherent cell populations over 7 days, those cells found to be nonadherent and still in suspension on day 7 had properties of stem cells [95]. In both model systems (side populations and serial preplate) cells were isolated which when injected intravenously into esophagus irradiated mice, homed to the esophagus and formed donor origin foci. Furthermore, in mice which were chimeric for sex mismatched GFP+ bone marrow, esophagus irradiation resulted in migration to the irradiated esophagus of cells of bone marrow origin which produced GFP+ foci in the esophagus [47]. The esophageal stem cells from either primary or secondary recipients in serial transfer demonstrated properties of multilineage differentiation in vitro [98]. Cells formed multilineage colonies containing cells with myeloepithelial morphology, fibroblast morphology, and endothelial morphology [95, 98]. Histochemical staining showed that single cell derived colonies contained cells positive for vimentin, endothelin, and F480 (which was originally used as a macrophage specific marker, but has now been found in peripheral blood multilineage progenitor cells) [95, 98].

The question of whether bone marrow derived stem cell populations can reconstitute and facilitate repair of radiation damaged esophagus has recently been tested. In these experiments (Fig. 5.1) donor male mice containing ROSA marked bone marrow, Y-probe+, G418 resistance marker, and LAC-Z marker were engrafted into female C57BL/6J mice that had received 31 Gy to the esophagus 5 days previously. Unlike bone marrow, esophageal cells undergo apoptosis at 5 days after irradiation suggesting a delayed turnover of irradiation damaged cells in this squamous mucosal organ compared to

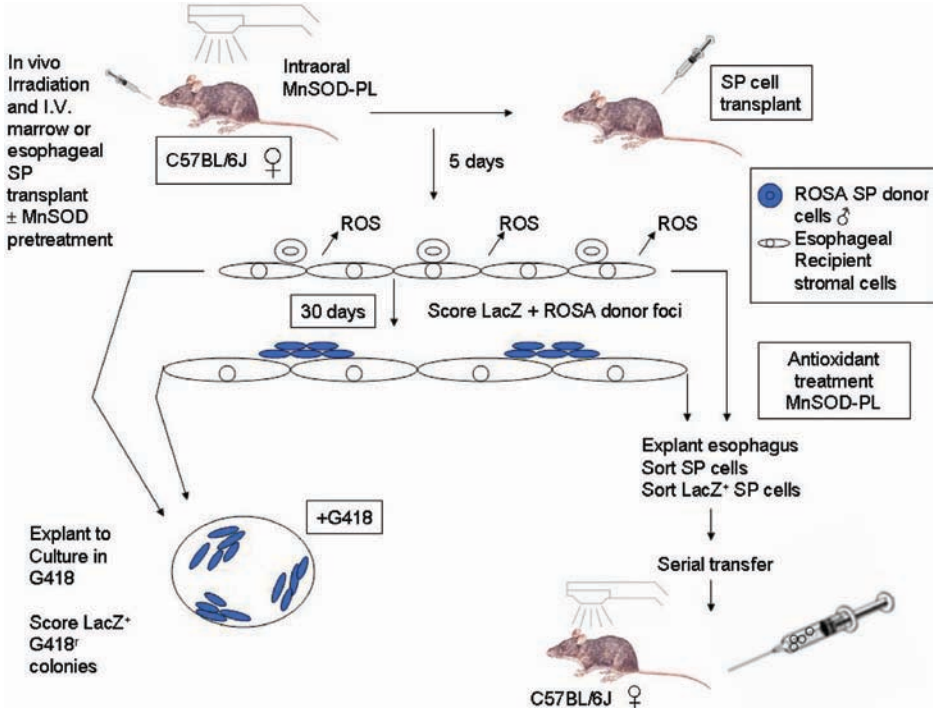

Fig. 5.1. Experimental model system to show donor marrow stem cell origin of esophageal stem cells. ROSA male donor mice have three markers (Y chromosome, G418 resistance, LAC-Z production). Recipient esophagus is irradiated 24 h after intraoral administration of MnSOD-PL, then donor marrow is given intravenously. Esophagus is removed. Cells shown to be donor origin in vivo and in vitro, and donor esophageal side population (stem cells) show self renewal by serial transfer to recipient second generation irradiated esophagus (With permission from Greenberger JS (2008) Gene Therapy Approaches for Stem Cell Protection. Gene Therapy 15:100–108)

the more rapidly proliferating hematopoietic cells in the bone marrow [97].

Removing the esophagus from marrow transplanted mice at serial time points after transplant and out to 21 days demonstrated that a significant contribution to the esophageal repair was attributable to cells that had been injected intravenously and had markers for LAC-Z, Y-probe in vivo, and following explant in culture in the presence of 50 μg/ml G418, demonstrated G418 resistance. Colonies growing in G418 also contained Y-probe and had multilineage differentiation markers [98].

Serial transfer or self-renewal of esophageal progenitor cells that were derived of bone marrow has recently been carried out. SP cells removed from the esophagus of first generation recipient mice at 14 days after marrow injection, were sorted for SP cells, and then either SP or non-SP cells injected intravenously into a second generation of C57BL/6J female mice that receive 31 Gy to the esophagus. The esophagus from the second generation mice demonstrated significant LAC-Z positive foci in situ, which were also Y-probe+, and explant of these cells to culture revealed multilineage G418 resistant esophageal colony forming cells [98].

Whether cells of bone marrow origin which provided esophageal repair, and were capable of serial transfer, are in fact multilineage stem cells remains to be rigorously tested. However, there is striking evidence that in both the first and second generation recipients, administration of MnSOD-PL intraesophageally prior to irradiation, and intravenous injection of bone marrow cells enhanced bone marrow engraftment into the esophagus [98]. These data suggest that irradiation may remove a population of esophageal progenitor or stem cells, eliciting a cell cycling response in the esophageal basal layer, recruiting quiescent stem cells into cycling for the repair process. However, the persistence of irradiation damage effects in the microenvironment may in fact work against the repopulation response [98]. The irradiation induced changes in the esophagus microenvironment have been shown to include continuous production of radical oxygen species (ROS) and inflammatory cytokines [93, 96]. Administration of MnSOD-PL prior to irradiation (and stem cell transplant) has been shown to reduce both ROS production and inflammatory cytokine production [93, 96]. Since bone marrow derived cells engrafted more efficiently into the irradiated esophagus that had been treated with MnSOD-PL, we hypothesized that irradiation cleared the space but that MnSOD-PL "cleans" the space and facilitated engraftment (Fig. 5.2). Another phenomenon associated with bone marrow derived cell migration and engraftment of the esophagus is the phenomenon of cell fusion. Bone marrow derived cells fused to esophageal as well as lung cells providing heterokaryon formation [99].

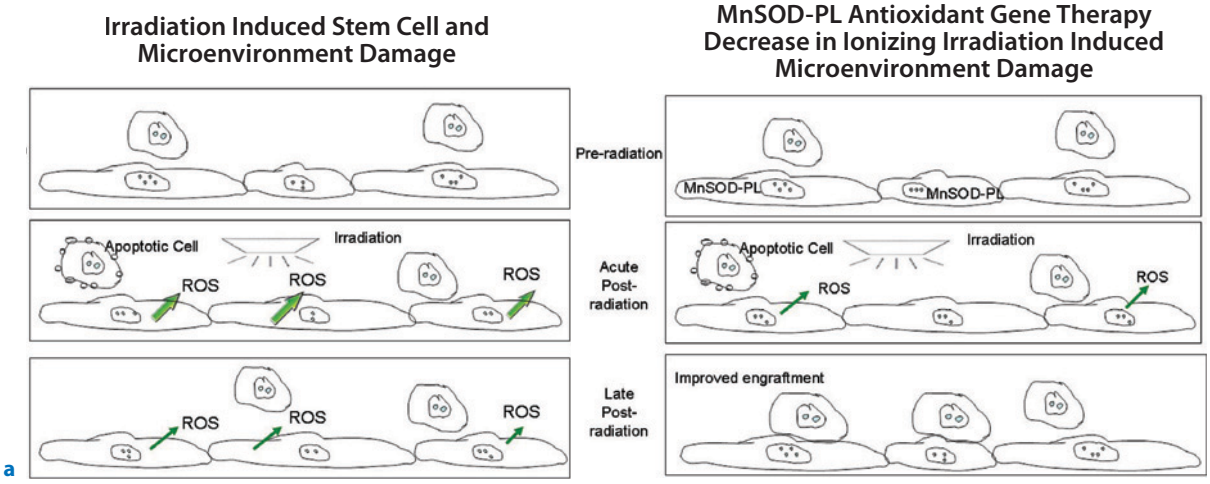

Fig. 5.2a,b. Hypothesis: MnSOD-PL administered to target organ before irradiation reduces both acute and late effects of ionizing irradiation by reducing oxygen species production. Untreated microenvironment (**a**), MnSOD-PL treated microenvironment (**b**)

The repair of mucosal surfaces including those in the oral cavity and esophagus is a complex phenomenon, but appears to involve a repopulation response which can be enhanced by providing additional cells from the circulation [100].

5.2.4 Bioengineering of Irradiation Damaged Lung Through Use of Bone Marrow Derived Stem Cell Progenitors

Radiation protection of the lung by antioxidant gene therapy has been described [101–108]. There has been much work describing the complex biology of the pulmonary stem cell [59, 63–69, 109, 110]. Work of Barry Stripp and Susan Reynolds [109, 110] demonstrated two populations of Clara cell secretory protein positive cells (CCSP) in the mouse bronchioles, one quiescent, and the other cycling. Both these cell populations appear capable of restoring the integrity of chemically damaged bronchial tissue. A cycling CCSP cell subset was removed by Naphthalene treatment and serial histopathologic examination studies of the drug treated bronchioles demonstrated repopulation by a CCSP population that was resistant to Naphthalene [109, 110]. The CCSP+ quiescent cells which resisted Naphthalene treatment, were, however, removed in another transgenic mouse strain (HSV-TK-CCSP), when the mice were treated with Gangcyclovir. This interesting mouse strain has been genetically engineered to have the Herpes virus thymidine kinase gene attached to the CCSP promoter such that its induction by Gangcyclovir results in production of toxic free radical species in both the cycling and quiescent CCSP populations [110]. Recent studies suggested that ionizing irradiation damage to the lung, either single fraction or fractionated, does destroy lung stem cells although rigorous dose response curves have not yet been carried out.

The question of whether bone marrow derived progenitors can reconstitute the irradiation damaged lung is a subject of intense investigation. Recent data [63] indicate that mouse lung when irradiated, can be reconstituted by bone marrow derived cells if animals are supplemented with G-CSF (granulocyte colony stimulating factor) mediated mobilization of bone marrow into the circulation. Confocal microscopic studies of lung sections revealed bone marrow origin cells with lung specific markers including TTF1 in experiments carried out in sex mismatched marrow chimeric mice [63]. However, in these experiments relative numbers of cells of bone marrow origin were very small, less than 5%. Similarly, small numbers of bone marrow derived progenitors were detected in the lungs of cystic fibrosis transmembrane receptor (CFTR) knockout mice, the animal model system for cystic fibrosis [59].

Under baseline conditions irradiated lung may not elicit a significant repopulation or repair response from bone marrow derived cells. However, in recent

experiments, delivering by inhalation technique [104]. MnSOD-Plasmid Liposome antioxidant gene therapy (as was described above for intraesophageal administration of MnSOD to enhance bone marrow derived stem cell repair of the esophagus) increased numbers of bone marrow origin cells were found homing to and proliferating in the irradiated lung. When MnSOD-PL inhalation gene therapy was supplemented by G-CSF administration after radiation, further numbers of bone marrow derived cells were detected in the lungs. G-CSF alone may not be the optimal way to mobilize bone marrow cells into an epithelial organ for purposes of post-irradiation repair. Use of FLK-3 ligand, and VEGF supplementing G-CSF may be a more appropriate way to optimally mobilize stem cell populations from the marrow into the lung. The question of whether significant volumes of lung tissue can be replaced by bone marrow origin stem cells is still unanswered, but techniques are available to facilitate such experiments in several animal model systems.

Less controversial is the clear involvement of bone marrow derived marrow stromal cells (mesenchymal stem cells) in irradiation pulmonary fibrosis as well as the fibrosis associated with bleomycin injury to the lung [48]. Several experimental model systems have shown using intravenous bone marrow injection, or in sex mismatched chimeric mice with mismatched bone marrow, that bone marrow derived cells contribute to the fibrotic response in the irradiated lung. An attractive hypothesis is the possibility that MnSOD-PL antioxidant inhalation gene therapy can ameliorate both the acute and chronic effects of lung irradiation damage through mobilization of repairing stem cells in their early phase and inhibition of mobilization of deleterious ibrotic cells in the late time frame (Fig. 5.3). The ease of administration of inhalation MnSOD-Plasmid Liposomes, which has been carried out in fractionation irradiation experiments [1, 4]. indicates that mice in these chimera experiments could be treated with inhalation MnSOD-PL weekly, during the period of latency between the acute response and late radiation ibrosis response [48].

If bone marrow derived stem cell populations are naturally involved in repairing irradiation damaged epithelial organs perhaps at low levels, then acceleration and enhancement of this response during tissue injury may enhance this component of the irradiation damage response. Furthermore, methods by which to harness this natural pathway and direct it, may involve enhanced marrow mobilization (G-CSF, VEGF, FLK-3 ligand administration), and homing and repopulation by engrafting cells (MnSOD-PL administration to the lung microenvironment). Such methods may prove a realistic strategy for tissue engineering to ameliorate the acute effects of irradiation lung damage.

Recent research efforts have focused on the development of superoxide dismutase mimic molecules. While MnSOD may provide an efficient antioxidant gene therapy approach for organ specific radiation

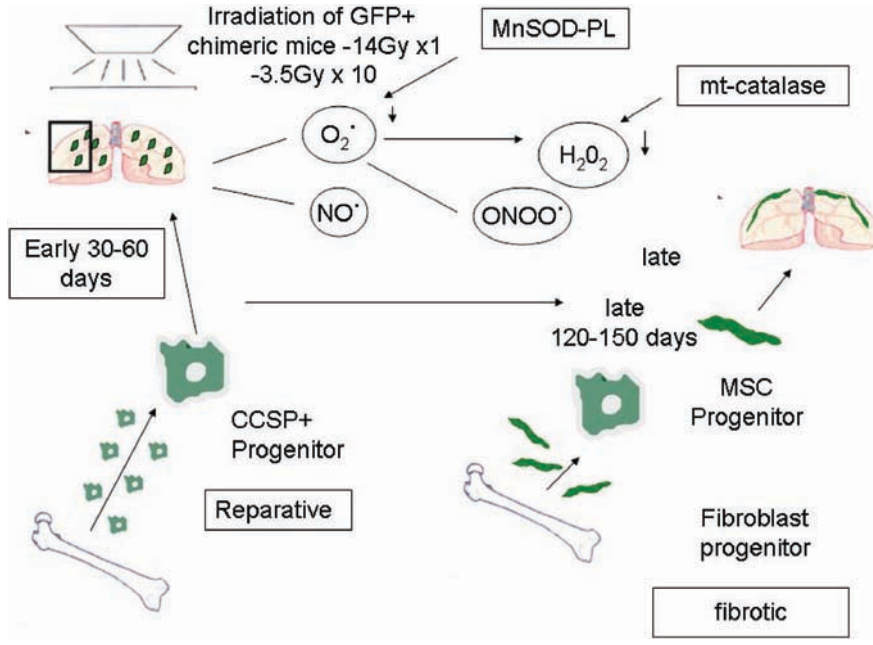

Fig. 5.3. Experimental model system to show bone marrow origin of pulmonary epithelial cells. GFP+ male marrow chimeric C57BL/6J female mice are irradiated to the lungs to 14 Gy in single dose or to 3.5 Gy daily for 10 days. Subgroups received inhalation MnSOD-PL treatments prior to first irradiation fraction, or twice weekly. Early marrow migration of GFP+ male CCSP+ marrow origin cells repopulate the functional lung. Late migration of GFP+ male marrow stromal cells (*MSC*) contribute to fibrosis [112]

protection, systemic protection may require a small molecule analog of MnSOD which could be administered either orally or by skin patch technique to facilitate irradiation repair in multiple organs. Possibly, local administration through inhalation of an aerosolized small molecule-MnSOD mimic may provide a more efficient method of prevention of acute and chronic radiation side effects to the irradiated lung.

5.3 Summary

At the present time, there is no effective way to replace significant components of the microenvironment in irradiated epithelial tissues, except by transplantation of the entire organ, and even here "bystander" damage may be sustained to the transplanted organ as a result of a continuous exposure to humoral factors including long lived free radicals that are produced for prolonged periods after irradiation [48]. Demonstrating the replacement of stem cell populations in epithelial organs, by progenitor cells derived from the bone marrow may provide a first step in understanding the role of the marrow in the pathophysiology of repair of irradiation induced tissue damage and may identify new targets for therapy. While simply transplanting new cell populations into an irradiated microenvironment, and reducing ROS production in the target organ by sustained administration of antioxidants may not reverse irradiation damage, an understanding of the consequences of these therapeutic approaches may provide valuable insights towards ameliorating the late effects of ionizing irradiation damage.

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Development of a Queriable Database for Oncology Outcome Analysis

6

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6.1 Introduction

Clinical trials and oncology data management have undergone considerable change in the past decade. Imaging has become a key tool for clinical trials management and a biomarker for clinical trial validation as imaging technologies improve and become more precise. Images have become extremely helpful in determining staging/eligibility, treatment response, and outcome determination including disease recurrence and progression. In modern protocols, images are often reviewed in real time to validate these points in order to improve compliance to study requirements and create uniform patient populations for clinical trials analysis. Data acquisition and management systems are currently in use to acquire and display images in electronic digital formats for view by both on site and off site radiology reviewers. As clinical trials become more global in focus, the ability for databases to accommodate diverse imaging acquisition strategies will become increasingly important for information review. It will likewise become increasingly important for tools to be created that support facile digital image transfer and review including image annotation clinical trial validation.

Imaging has become the key vehicle in radiation oncology in order to define and validate the fields of radiation therapy to use in clinical trials as well as to determine which areas receive full dose or lower dose of radiation treatment. Radiation therapy planning systems are now fully image based for tumor target de ìnition and sparing of normal tissue. Radiation treatment execution is rapidly becoming image guided in order to accommodate for changes in position and target motion during therapy. The fusion of metabolic and anatomic images may signi ìcantly alter target volume definition at presentation and subsequent imaging on treatment may help validate deformable changes in the target volume as a function of treatment. This may improve tumor targeting and further serve to decrease radiation dose to normal tissue.

Moving forward, clinical protocols are collecting patient serum and tumor tissue at presentation and at later time points. Microarray analyses for genomics and proteomics are Dicom objects and can be thought of as images, therefore the potential exists for these objects to reside side by side with both imaging and radiation therapy objects as part of a queri-

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able database for clinical oncology analysis. Linking this information with patient outcome would greatly facilitate the development of clinical translational science. This can be accomplished with established databases housed within the cooperative groups or by creating prospective patient registries to track treatment administration and patient outcomes.

With improvements in patient outcome documented in many disease sites including breast, pediatrics, and prostate, patients are now available for analysis of normal tissue function. Although the primary clinical endpoint remains tumor control, normal tissue tolerance, both as an acute and late effect of therapy, is becoming of increasing importance in evaluation of the effects of therapy including the genesis of second malignancies which may be treatment driven. In many disease sites we are learning how to adjust and subtract therapies as well as decrease the duration of treatment. For example, in Hodgkin's disease treatment protocols are designed to reflect response to initial chemotherapy with secondary randomization points built into protocols based on chemotherapy treatment response validated by both metabolic and anatomic imaging performed and reviewed in real time. A patient determined to have a rapid early response to chemotherapy will have a shorter course of chemotherapy and possibly not undergo radiation therapy based on the completeness of their response to induction therapy. Outcome images are obtained and incorporated as part of protocol evaluation. This strategy requires real time review of images and radiation therapy treatment objects for protocol compliance. Previous iterations of protocols in Hodgkin's disease have demonstrated a discordance of 37% in site versus central review of images with respect to response and an equal rate of discordance in compliance to radiation therapy treatment schema. This could have considerable in luence on study analysis and outcome. Central real time review of objects has had a considerable impact in improving the discrepancy between site and central evaluation with deviation rates on study now at or below 5% in many studies. Resolving these issues prior to randomization and pre-therapy has become an important aspect of clinical trials moving forward, and promoting these processes is expected to improve uniformity of data for clinical trials. A key objective in improving clinical trials is to promote facile data acquisition strategies and display data in a uniform database format for queriable research. Promoting media for off site clinical review strategies and establishing electronic vehicles for query will be crucial to improving access to data and will signi ìcantly enhance the productivity and quality of our translational science. Integrating information germane to clinical trials including imaging, radiation therapy, genomics/proteomics, treatment, and clinical outcome will be the objective for improving the quality of translational science. Making this information available through a single electronic format will promote clinical investigation for outcome analysis for patients treated for malignancy.

Physicians and scientists participating in the CURED effort are interested in the late effects of therapy management including the effect of treatment on normal tissue function and the generation of second malignancies [1]. The protocols in development by this group intend to capture data including images, radiation therapy treatment objects, microarray, and patient outcome data and house the information in a database that can be queried for analysis. The intent is to also collect data on cancer survivors. These patients may/may not have been treated on previous clinical trials. Integrating this data and objects including outcome into a uniform database is an important objective for this group. Incorporating existing platforms into this effort may provide the appropriate efficiency of scale to provide a robust platform for clinical research.

6.2 Efforts to Date

6.2.1 Quality Assurance Review Center

The Quality Assurance Review Center (QARC) has a long-standing interest in developing processes and initiating process improvements in imaging and radiation therapy for both clinical cooperative group and industry clinical trials. QARC became independently funded through CTEP in 1980 with initial mission focus in radiation therapy planning for both children and adults enrolled on cooperative group protocols. As radiation therapy became image based in target definition, QARC began a process in 1985 for diagnostic image acquisition to validate the fields of radiation therapy. As part of study review investigators required that the diagnostic images and the radiation therapy treatment objects resided

in a side-by-side format for protocol object review. During the past decade institutions preferred to forward objects to QARC via many forms of electronic media and QARC has established a diverse portfolio for data acquisition including images in order to create a facile and all inclusive strategy for data acquisition. Institutions worldwide forward objects in many diverse electronic formats to QARC. This is often dependent on the informatics expertise and equipment at each site. Radiation therapy digital data is submitted to QARC in multiple formats and media including RTOG objects. The submitted objects, including images and radiation therapy treatment data, reside in a uniform format in the QARC database housed in a facile retrievable format. Imaging and radiation therapy objects reside in a side-by-side format for object review. The database is the central aspect of QARC function and serves as the epicenter of activities with each division of QARC using the platform for data management including real time review of objects and world-wide inter-institutional communication for problem solving [1–3].

6.2.2 Database Function

QARC: This report of the QARC Information Systems is organized in the following sections: I. Network Infrastructure; II. Web Interface and Security; III. MAX Database Operating System; IV. Digital Data Management; and V. Data Integrity and Backups.

I. Network Infrastructure

The QARC network includes 17 servers, 46 PCs, 16 laptops and 10 printers. Every node on the network connects to one of ive central switches running at 1 Gigabit per second. The server group consists of Windows 2003 Enterprise Server, one Red All desktop and portable systems at QARC run Windows XP Professional Service Pack 2.

II. Web Interfaces and Security

QARC is responsible for managing its own network security. QARC has full T1 1.5 megabits per second connection to the internet. Security is implemented using Cisco 2821 IOS and Checkpoint NG Firewall. Three "demilitarized zones" (DMZs) allow protected access to QARC information. One public DMZ serves general internet users and the other two DMZ's are used to collaborate with external organizations and Cooperative Groups. Virtual Private Network (VPN) is supported using both Cisco and Checkpoint technologies.

III. MAX Database System

QARC's database, called MAX, is a validated relational operating system with its foundations in SQL server in 2005 Enterprise. It includes query by form functionality that allows any user to intuitively query data on any field or set of fields in the database. MAX includes over 300 tables, over 600

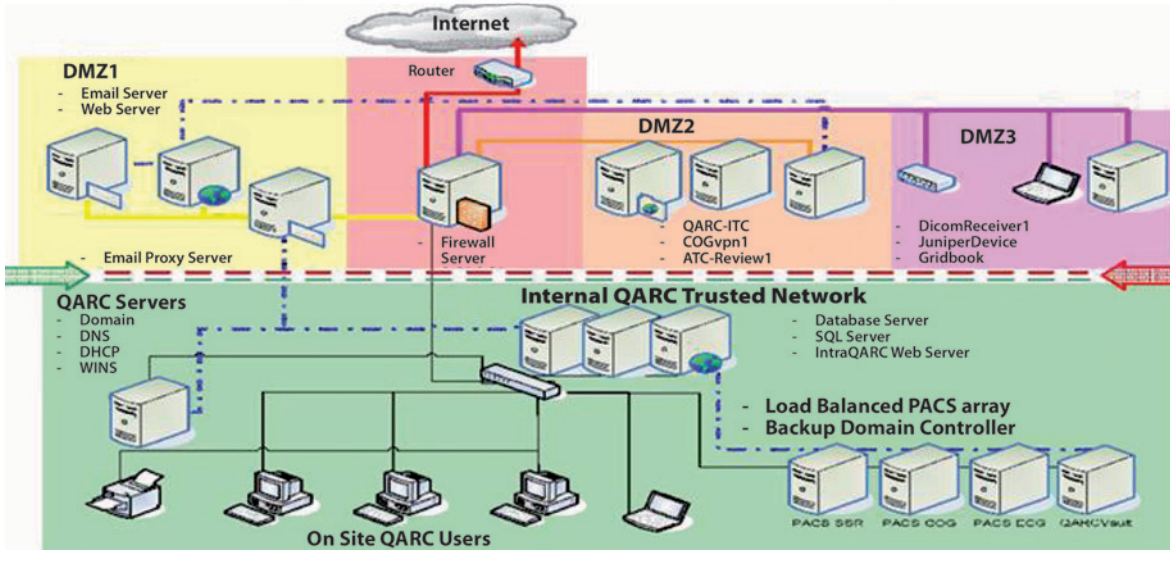

Fig. 6.1. QARC network

and approximately 200 customized reports. There are over 40,000 patient records in MAX. Within the patient record are links to the tables that include information about the cooperative group and institution contacts and benchmarks, protocols, correspondence, DICOM and non-DICOM digital data. MAX features integrated messaging systems which allow users to communicate with each other, the IS Department and with external contacts at all institutions. Patient data is routinely extracted from MAX and sent to the statistical centers of the Cooperative Groups and Pharmaceutical companies via password-protected e-mail and data files.

Internal to MAX, the QKB is an indexed help facility and library tailored for QARC staff. It is a repository of knowledge specific to QARC. MAX creates multiple audit trails of stored data and user activity within the database. QARC has undergone a systematic validation process of its information systems to be compliant with 21 CFR Part 11. A standard operating procedure (SOP) generator has been incorporated into the MAX system and is used to produce the QARC SOPs. QARC follows standard procedures to assure maintenance of compliance, including multiple auditing mechanisms, documentation templates, regular backups of the database and associated files, and strict adherence to a change management processes.

IV. Digital Data Management

The MAX system manages all electronic media received at QARC. Electronic media is divided into three categories in MAX: (a) DICOM imaging which refers to all diagnostic digital imaging that conforms to the DICOM standard and (b) DICOM RT data or RTOG data exchange data which is submitted via FTP, imported into the database and viewed using either the ATC remote review tool (RRT) or the computational environment for radiotherapy research (CERR) program; and (c) eMaterial which encompasses all electronic data that is not DICOM compliant. The growth of the acquisition of digital imaging and electronic data is demonstrated in the graph below. MAX has evolved into a PACS system which resolves patient imaging to patient records, stores out the imaging onto four PACS servers which are built and maintained onsite, and facilitates the transfer and viewing of all digital imaging. There approximately 25,000 diagnostic studies with over 6 million images archived in the QARC PACS.

DICOM Imaging: MAX is fully integrated with a software program, Dicommunicator, which was initially written by Dr. Keith White of Primary Children's Medical Center (Salt Lake City, Utah) and is used at institutions around the world to facilitate the submission of DICOM imaging to QARC. Dicommunicator was donated by Dr. White for use in the clinical trials process in 2000 and has since been managed and developed at QARC. Successful transfer has occurred between QARC and the following PACS systems: Agfa, GE, Integrad Web by Dynamic Imaging, Kodak, Philips, Siemens, Stentor. In addition to 35 installations, major accomplishments include streamlining the interface of the software to increase ease of use and improving the remote installation process.

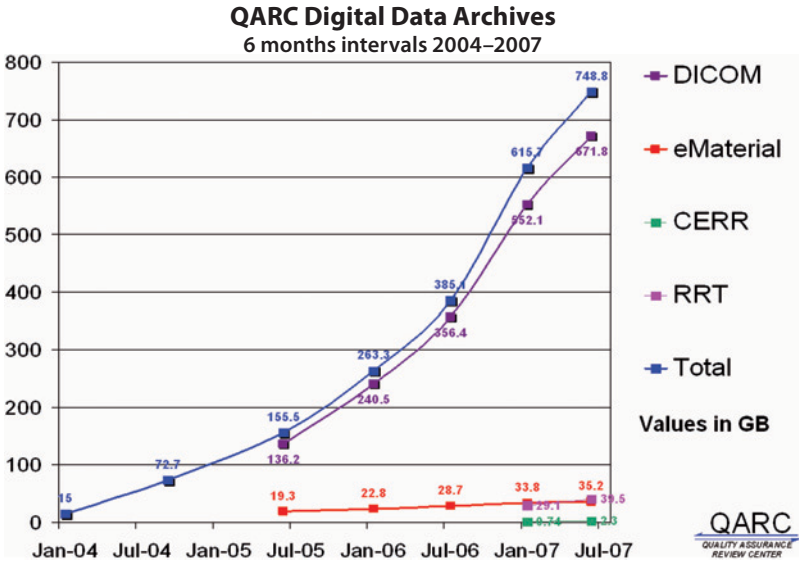

Fig. 6.2. QARC digital data archives

The Dicommunicator software is a critical component in managing digital DICOM data at QARC. Dicommunicator is used to import DICOM studies into the MAX database and match them to the appropriate patient. A CRA reviews this imaging and resolves it to the patient's diagnostic record where it can be launched and viewed by a physician. The workflow process for receiving, identifying, logging and importing digital imaging has become completely digital and is managed through a MAX utility called the "Dicommunicator Import Form Administrator" [4].

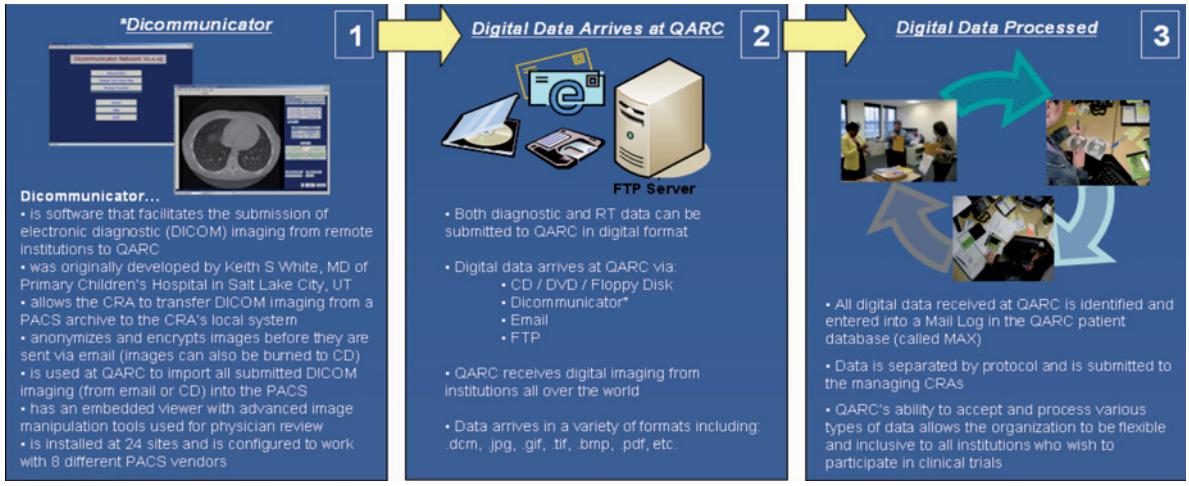

Fig. 6.3. Dicommunicator at site to submit imaging to QARC

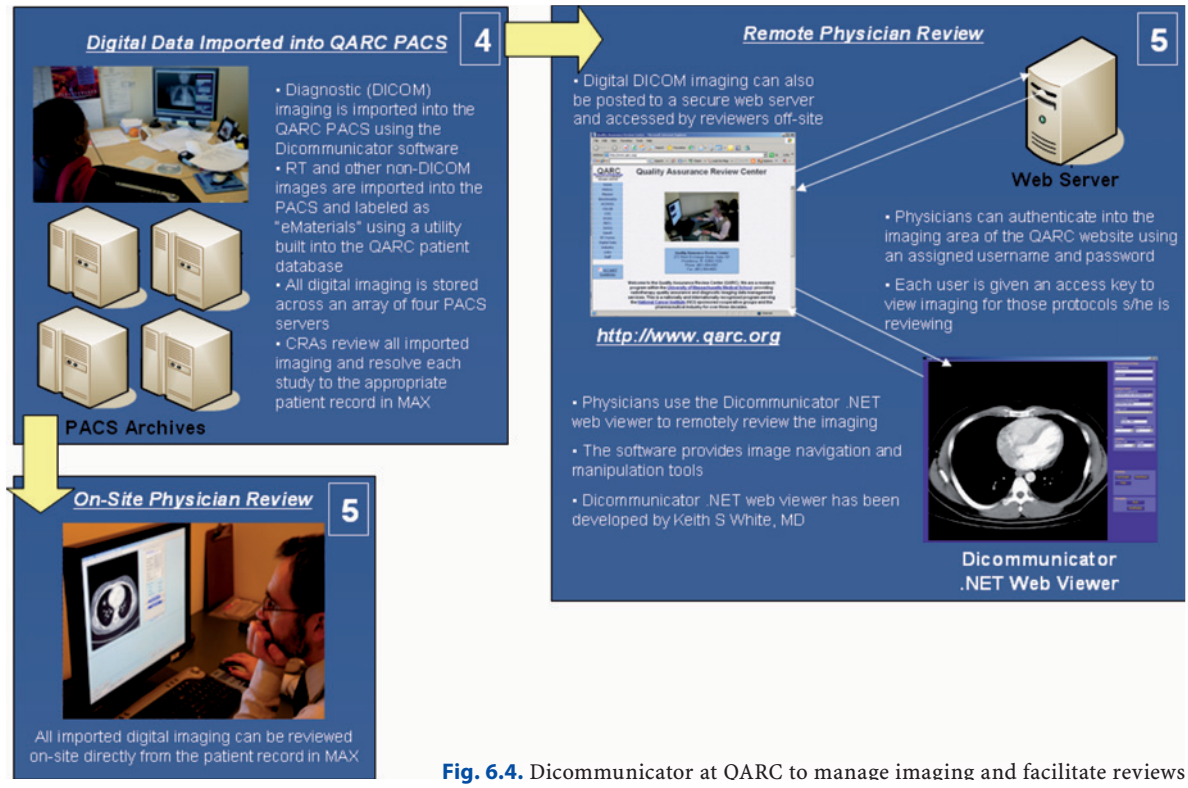

Fig. 6.4. Dicommunicator at QARC to manage imaging and facilitate reviews

V. Data Integrity/Backups

Regular backups of systems and data are realized in a number of ways. Redundant array of independent disks (RAID) is implemented on all servers. Tape Backups are performed on all servers weekly, using Symantec BackupExec Suite 10 and a robotic tape drive. These backups have been successfully utilized in the past to rebuild two servers during tests of QARC's Disaster Recovery Policy.

Peer-to-peer (P2P) routines occur at various times throughout each day, which copy data from servers and PCs to other systems and external hard drives. Database files are written to DVD disks each weeknight. For increased security the backup tapes, external hard drives and DVDs are taken offsite weekly to rotating locations.

6.2.3 Advanced Technology Consortium

The advanced technology consortium (ATC) is composed of the image guided therapy center (ITC), the Radiation Therapy Oncology Group (RTOG), the Radiological Physics Center (RPC), and QARC. The principal investigator of the grant/consortium is Dr. James Purdy, currently the Director of Physics and Vice Chair of Radiation Oncology at the University of California at Davis. The consortium has worked over the past decade to provide uniform credentialing mechanisms for institutions using advanced technology radiation therapy including image guided and intensity modulated therapy in clinical cooperative group trials. The consortium has had signi ìcant success developing a uniform data acquisition strategy for full three-dimensional radiation oncology data sets. Drs. Purdy and Bosch developed RTOG objects in collaboration with CMS planning system (St. Louis, Mo.) and this is the platform used by the RTOG for volumetric data acquisition in all clinical trials involving advanced technology treatment execution. Three-dimensional data sets including dose volume histograms are available for web based protocol review for radiation therapy objects. Aside from their efforts in facilitating publications for primary protocol intent, Dr. Purdy and the ITC group have generated many secondary endpoint publications using the database. Many of these publications have included remote review strategies including re-drawing of unintended treatment objects for radiation target dose definition. ATC members have shared expertise and informatics process improvements. They have collaborated on many projects for process improvement strategies. One of the most successful projects was the introduction of RTOG objects into the QARC portfolio for data transfer. This permitted institutions to forward objects for review in a transparent manner independent of cooperative group membership. QARC, in turn, imbedded RTOG objects into the database housed at QARC. The RTOG objects reside in a side-by-side format with the diagnostic images at QARC linked to each speci ìc patient. Therefore investigators can view, using on and off site mechanisms, both image and radiation therapy objects through the same database. Outcome images can be linked directly to the patient and compared to radiation therapy objects thus facilitating analysis of outcome and late effects of anti-cancer therapy in cancer survivors. One objective moving forward is to migrate the QARC database to ATC partners to share the platform for image and radiation therapy object review, thus creating a uniform platform for object review for investigators in clinical trials [5–9].

6.2.4 American College of Radiology Imaging Network (ACRIN)

ACRIN began as a clinical cooperative group in 1999 with an outstanding portfolio of trials as well as signi ìcant interactions with other cooperative groups. During this period of time ACRIN has established a well tested informatics infrastructure and has become a very strong organization for conducting multi-center clinical trials with a particular focus on cancer screening. ACRIN has developed the infrastructure to promote and support image transfer as well as successfully enhance the quality assurance of imaging. ACRIN developed, in partnership with the American College of Radiology and a private partner, an electronic image transfer mechanism called Preview 32. This system has many of the same features of Dicommunicator at QARC. It has a robust infrastructure that supports image anonymization, transmission, and quality assurance. It has been successfully deployed at more than 150 sites with engagement of PACS and IT infrastructure at participating sites. The system has acquired images on 76,000 patients including more than 13 million images on clinical trials [10, 11].

As part of the process improvement strategy, ACRIN recognized that Preview 32 required effort at the site to maintain; therefore a key focus was to evaluate an alternative image transfer strategy moving forward. Similar to QARC, Preview was built on proprietary software platforms, thus limiting interoperability with the caBIG workspace. As a response to this challenge, ACRIN, in partnership with the American College of Radiology, has developed a new generation of imaging management software. This new platform is called TRIAD. The ACR is providing the programming resources for this effort as the ACR intends to use this platform for education and credentialing strategies moving forward as well as being made available to ACRIN. TRIAD is a thin client web-based system that can be easily installed over the web on computers currently in use at sites and connected to the Internet. The advantage to TRIAD is that it will promote review of in vivo objects image related issues in a direct fashion through the Internet. As will be discussed in the following section, integrating TRIAD into the QARC database is an objective moving forward which will promote and facilitate data review.

ACRIN has developed processes similar to QARC to conduct in house reader assessments through the deployment of diagnostic quality workstations at the ACRIN headquarters local network as well as establish a mechanism for distributed assessments via image transfer to the reader's home institution. These strategies have greatly facilitated review of data for clinical trials and have served to promote the uniformity and quality of the data used for clinical trials analysis.

6.2.5 Virtual Imaging Evaluation Workspace (VIEW)

Imaging has evolved to become an important biomarker for clinical endpoints in for both industry and cooperative group clinical trials [12–14]. A challenge, however, has been to acquire, archive and centrally review, in an independent fashion, the images obtained on cooperative group treatment trials. The NCI has supported formation of a consortium for this purpose. Consortium members include those members of the cooperative groups that had electronic infrastructure in place for digital image transfer and ability to generate the audit trails necessary for regulatory compliance. The consortium consists of ACRIN, QARC, and the imaging core lab of the CALGB housed at the Ohio State University directed by Dr. Michael Knopp. The members of the consortium are in the process of developing an inter-operative IT infrastructure transparent to VIEW members and the cooperative groups served by the consortium. This infrastructure will be 21 CFR Part 11 compliant and compatible with caBIG. The consortium will develop standard operating procedures for data acquisition and assessment of imaging endpoints for cancer clinical trials for both anatomic and functional imaging as well as develop an assessment strategy for newer advanced technologies moving forward. This will include both institutional credentialing for participation in clinical trials as well as the development of standardized quality assurance metrics for reviewer assessment. The consortium will work with the FDA and clinical trials sponsors to develop imaging charters compatible with regulatory guidelines and data management standards. The consortium will further explore strategies to advance the science of alternative imaging strategies and establish database functions that can be mined for queriable research. As these ideas mature and gain strength, integration of imaging databases with both patient outcome data and other therapy objects will further promote the field of translational science. VIEW will play a key role in the integration of imaging science with translational science and further serve to help integrate imaging objectives with clinical research strategies.

6.2.6 CaBIG

In recognition of the need to develop integrative database structure and the paucity of tools to achieve this objective, the National Cancer Institute developed a strategy to move this science forward through the development of the cancer biomedical informatics grid initiative (caBIG) in February 2004. The overarching responsibility of the caBIG initiative was to develop an informatics infrastructure to facilitate exchange of information among clinical cancer centers and other institutions and develop databases for research. The caBIG community is adopting and in some cases, developing informatics standards and policies using an open source approach. The "informatics grid" strategy was established as the vehicle of choice for this purpose using open source software platforms and takes advantage of the power of grid computing and the excellent

open source software developed by this community. One of the most important developments of the caBIG project is the establishment of the in vivo imaging workspace. Eliot Siegel, MD of the University of Maryland (clinical lead) and Paul Mulhern of Booz Allen Hamilton (administrative lead) coordinate this effort. One of the most important participants is Joel Saltz, MD. Dr. Saltz is the chair of Biomedical Informatics at the Ohio State University and a pathologist and computer scientist by training. Dr. Saltz has been instrumental in the development of the in vivo imaging middleware project, which provides inter-operability between computer systems that historically, have not been integrated. One of his responsibilities has been to help develop a software strategy to integrate these diverse systems for data storage into the national archive. Dr. Saltz and his team are responsible for analytical service, security infrastructure, and grid-service platforms compatible with caBIG infrastructure. The in vivo imaging middleware library supports interoperability between caGrid and DICOM data models and exposes DICOM aware data resources as caGrid compliant services.

The informatics toolkit developed by the middleware project focuses on federated data potentially arriving for archival from diverse sources and permits this data to be formatted and reviewed with multiple query functions. The middleware project makes use of the caGRID informatics infrastructure, which will be attractive to the cooperative groups, and quality assurance centers because it can be used to enhance data review and integrate diverse data platforms by exploiting the semantics of the data rather than the representative formats of the data. The transition of legacy data archives to strongly typed grid data services is one of the key thrust areas of the in vivo imaging workspace moving forward. Access to the QARC database was demonstrated via the grid architecture during the 2006 Radiological Society of North American meeting. The effort of integrated metabolic imaging formats with the CALGB imaging core service, directed by Michael Knopp MD, was demonstrated by the caBIG team as well. These are examples of legacy databases with which the in vivo imaging workspace group has begun the process of integration. The next key decision for caBIG, the cooperative group statistical data centers, and the quality assurance centers will be to decide whether caBIG efforts should be the primary data acquisition model or to integrate on the back end with existing systems for data management. Both approaches have merit, therefore an integrated plan may be strategic and provide flexibility for an all inclusive data acquisition strategy including worldwide institutions with diverse informatics

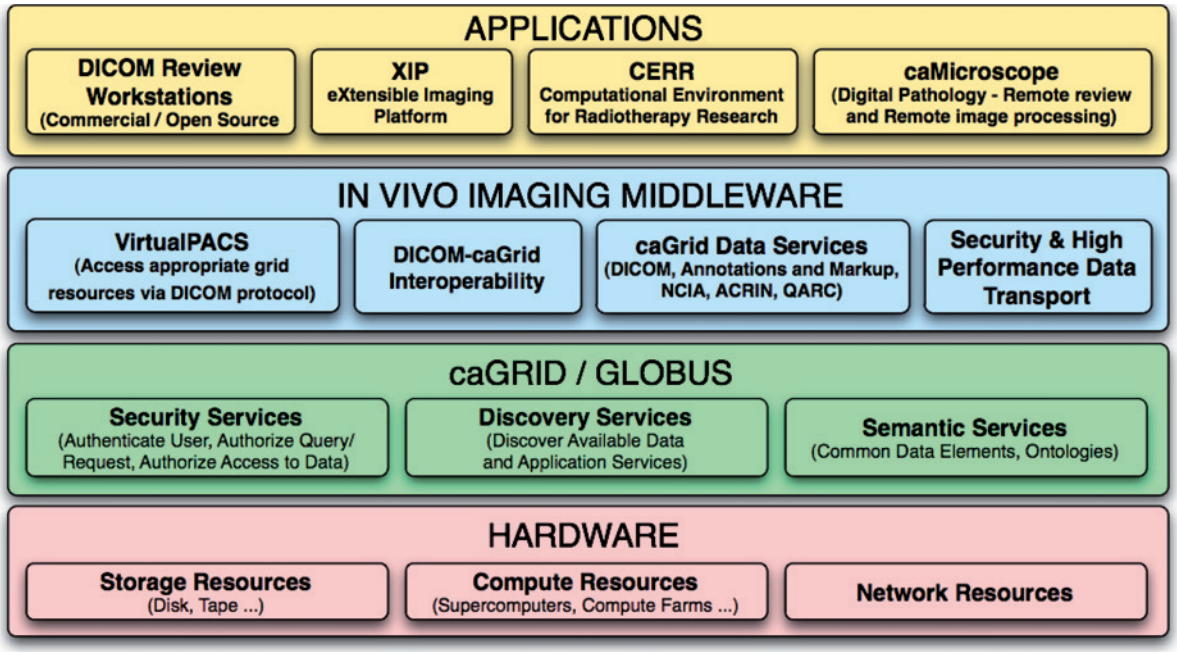

Fig. 6.5. IVI middleware

platforms. This will become especially important as clinical trials become more international in practice [15–19].

6.3 Future Strategies for Cancer Clinical Trials

The key objective for clinical trials is to promote the development of queriable databases for clinical research. Housed within the database available for query should be all information required for the evaluation of translational science including patient characteristics, imaging, radiation therapy treatment objects, treatment information, microarray for genomics and proteomics, and patient outcome. This information currently is available, however it is housed in segregated legacy informatics formats without integration. A strategy for integration is a key objective to improve our clinical science and provide an infrastructure for validating clinical trials in a facile manner.

Clinical trials are becoming more complex in scope and have multiple endpoints embedded into each trial that include images and tissue acquisition and use this information to assess secondary endpoints as part of the study. Often trials require real time review of images and object data as part of the protocol to trigger secondary and tertiary study endpoints. In COG protocol AHOD 0031 (intermediate risk Hodgkin's disease), anatomic and metabolic images are reviewed after two cycles of chemotherapy in order to determine the rapidity of response. This evaluation triggers a randomization to the duration and type of chemotherapy. Images are reviewed again after four cycles of chemotherapy as patients who had rapid early responses to chemotherapy and are deemed in complete response after four cycles are then randomized to involved field radiation therapy or no further treatment. For those who receive radiation therapy on this trial the planned fields are centrally reviewed pre-therapy for protocol compliance. Outcome images are collected on this protocol in order to con ìrm location of relapse and its relationship to previous sites of disease. Real time review of imaging objects is used on many adult and pediatric cancer clinical trials including trials with international participation. At QARC the database is used as the format for site and investigator review of objects in both a real time and retrospective format. Therefore future database strategies must have the nimble functionality to permit real time review of objects and integrative strategies to permit mid-cycle evaluation of data for adaptive statistics. Query function that is diverse is important in order to review data in a complete and thorough manner [3, 4, 20].

For potential protocols for CURED, functionality that is diverse, adaptive, and complete will be very important. Because the focus of CURED will be on patient outcome and normal tissue function, data acquisition must focus on diverse strategies as patient treatment occurred using varied informatics platforms with years of informatics transition. The protocols under development by CURED include the evaluation of breast MR for patients treated for Hodgkin's disease and the use of helical computer tomography of the chest to evaluate the risk of lung cancer in patients treated for Hodgkin's disease. It will be important in these protocols to acquire patient information including imaging, radiation therapy treatment objects, and treatment programs in order to perform appropriate risk hazard analysis for these important secondary events. Likewise it is anticipated that tissue/blood analysis for these patients will also be important, particularly for assessment of germline polymorphisms that might increase the risk of late adverse events. If these patients were treated on previous cooperative group clinical trials, it will be important to link this data, including imaging and biospecimens, from established databases housed in cooperative group data centers. Because imaging and radiation therapy treatment objects on these patients would have been established at diverse time points, it will be important for the integrated database to accept data in multiple formats using a uniform strategy for data review likely through a web based mechanism. For many CURED patients their cancer therapy may have been delivered in a pediatric protocol and the patients are evaluated as adults. Therefore a strategy integrating all data centers for the cooperative groups will be an important feature for this initiative as a long-term strategy [21–24].

Quality assurance groups and data centers have the potential of meeting the objective of integration. It will require cooperation by all interested parties with active use of middleware integration platforms and the caBIG in vivo imaging database system. Each area of interest including imaging, radiation therapy objects, microarray, and patient outcome will require a different approach for data integra-

tion with integrated display available for queriable research on a uniform display platform.

Imaging will be accomplished with integration of the informatics infrastructure between ACRIN, QARC, and the CALGB through the VIEW initiative. ACRIN has developed a strategy for uniform data acquisition with both the PREVIEW and TRIAD systems. This has been a very successful strategy. The image acquisition strategy at QARC has included a uniform image transfer system (Dicommunicator) as well as a platform to accept computer disc and display the objects in a uniform data set. The South West Oncology Group has a partnership with AG Mednet for image transfer and review. This program can be integrated into the developing process as well. Each center involved in the VIEW consortium will be able to receive and accept images from each member. Therefore each member will have both CD and direct image transfer capability. QARC, in turn, will be able to integrate and launch TRIAD through the QARC database for display review.

Display of radiation therapy objects has progressed very well through the Advanced Technology Consortium. RTOG objects are available at QARC and are launched side by side to the image objects for review of protocol patients. These objects are reviewed in real time and in retrospect by QARC staff and investigators either through Webex or distributed through the .net service featured by Dicommunicator. Web based strategies for off site review of radiation therapy objects are currently used by the RTOG. Integration with VIEW investigators will insure image integration with the radiation therapy objects for simultaneous display.

In collaboration with Dr. Foran at the University of Medicine and Dentistry in New Jersey, the Saltz group has developed caGrid enabled infrastructure to support Pathology virtual slide and tissue microarray image management and analysis. This effort is now supported by R01 funding from the National Library of Medicine. This virtual slide infrastructure effort is now over 10 years old; the current virtual image management infrastructure is caGrid compliant and leverages the in vivo imaging middleware library [25, 26].

The Ohio State University houses tissue banks for the COG and the CALGB through the efforts of Drs. Qualman and Jewel. Each has considerable experience in housing microarray data in digital formats as well as establish platforms for distributed review of pathology objects. The next iteration of this strategy will be incorporating the objects into a uniform file format housed with imaging and radiation therapy objects for queriable research. In the first generation of this platform the QARC database will be modified to incorporate pathology objects and this will be further developed through cooperation of caBIG for enterprise function and archive [27–29].

Linking this information with patient outcome will be the next step in establishing the platform for queriable research. The data could be retrieved by the cooperative group from the QARC database for internal use by investigators in the cooperative groups as a first step in the development of this strategy. The QARC database is relational with each cooperative group statistical center; therefore the informatics base for initiating this objective is in place. It can be significantly improved with integration of the caBIG middleware software. For patients treated on cooperative group clinical trials a long-term strategy may include speci ìc protocol information with outcome data potentially made available with permission from the sponsoring cooperative group including data on relapse and normal tissue toxicity. At the appropriate time point and with permission from the cooperative group, the patient outcome data could be made available to the national archive for queriable research linked to the specific patient in the QARC database for review of images, radiation therapy objects, and microarray and other biological data. This may create database utilization across protocols with similar disease site orientation.

As stated previously, patients entered on CURED protocols may/may not have been treated on cooperative group trials. If so, data may exist on the patient that could be mined for outcome analysis. Thus images, treatment objects, and tissue may be housed within the established database. Tissue and images obtained as part of the CURED protocol could be linked to the previous platforms for outcome analysis. Likewise, the database can be used to acquire and display information for CURED patients who were not treated on cooperative group protocols as needed.

Thus cooperation between established centers for quality assurance in clinical trials and cooperative group data centers can lead to a new strategy for clinical translational research. Integrating imaging, radiation therapy objects, biological data, and patient treatment/outcome data may help facilitate patient analysis and help navigate the development of new protocols and initiatives. The database at QARC currently links images and radiation therapy objects in a side-by-side format with a plan to link

microarray objects to this data through cooperation with tissue banks of the cooperative groups. Middleware software generated through caBIG can be used to support this process and perhaps permit links to the established data centers of the existing cooperative groups to permit analysis of outcome events. It is possible that tissue/germline DNA may be obtained on patients treated/evaluated on separate clinical trials housed in separate databases on patients evaluated by CURED. Middleware linkages will facilitate review of these objects and promote interactions between data centers as this may become important for patients on CURED protocols. These patients may have diverse data acquisition platforms for data entry and have been treated at different time points in cancer management, however display in a common uniform file format for queriable research is possible with expertise currently at hand. Promoting this strategy may help facilitate improvements in cancer patient management, improve the outcome for cancer patients and generate new insights into the pathogenesis of cancer that can be tested in the next generation of clinical trials.

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Post-Radiation Dysphagia

7

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CONTENTS

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Swallowing is a complex process that begins with the placement of food in the mouth and ends when the food enters the stomach. It involves voluntary and involuntary stages which are coordinated through several cranial nerves and a multitude of muscles that control the function of the oral cavity, the pharynx (skull base to the lower border of the cricoid), the larynx, hyoid bone, and esophagus [1, 2].

Swallowing is initiated by the stimulation of receptors in the oropharyngeal area. Sensory impulses reach the brain stem through cranial nerves VII, IX, and X, while motor control is exercised through cranial nerves IX, X, and XII. The cricopharyngeal sphincter (CPS) relaxes as the bolus reaches the posterior pharyngeal wall before it reaches the CPS. Cranial nerve V contains both sensory and motor fibers and is important to chewing.

Swallowing physiology consists of three phases [3]:

1. Oral phase (1 s): The oral tongue and teeth reduce the food to a bolus. As the food is transported back toward the pharynx, receptors in the oropharyngeal mucosa trigger the pharyngeal phase.
2. Pharyngeal phase (1 s): During this stage, the velopharyngeal port closes to prevent food from entering the nose. The hyoid bone and larynx begin their forward and superior ascent, the epiglottis is folded down to an inverted position, the tongue base moves toward the posterior pharyngeal wall, and pressure is generated by the top-to-bottom contraction of the pharyngeal constrictor muscles, which push the bolus of food toward the esophagus. Lastly, through laryngeal and hyoid elevation and anterior movement, the cricopharyngeus muscle relaxes, resulting in the opening of the CPS.
3. Esophageal phase: When the CPS opens, the bolus of food enters the upper esophagus and is transported down to the stomach through peristalsis.

Patients with head and neck cancer tend to be elderly. With advanced age, swallowing physiology becomes compromised, resulting in increased bolus "holding", delayed onset of swallow, slower pharyngeal transit time, and reduced generation of pharyngeal pressure [4, 5].

7.1 Evaluation of the Swallowing Mechanism

7.1.1 Objective Evaluation: Instrumental Assessment

- Videofluorography (VFG) is the most commonly used procedure to assess swallowing dysfunctions. VFG, including modi ied barium swallow and esophagogram, can visualize the oral, pharyngeal, and esophageal phases of swallowing. During VFG, the patient is given food in measured volumes and viscosities. Swallowing physiology is viewed in the lateral and anteroposterior planes and temporal measures are made. The duration of physiologic events during the entire swallow can be measured as they change during swallows of boluses of various volumes and viscosities. Oropharyngeal residue and aspiration can be quanti ied. Oropharyngeal swallow efficiency (OPSE), a global measure of the safety and speed of swallow, is calculated by measuring the total oral and pharyngeal transit time of the bolus divided by the percentage of the bolus swallowed [6–9].
- Manometry, in which the patient swallows a soft tube containing pressure sensors, measures pressures generated in the mouth, pharynx, and esophagus during swallowing. Manometry is used primarily to measure pressure changes in the esophagus and has value for studying oropharyngeal swallowing dysfunctions [10, 11].
- Functional endoscopic evaluation of swallowing (FEES) provides views of the laryngopharynx different from those seen with VFG. This procedure, which is easy to perform, uses iberoptic endoscopy (FE) to view mucosal and anatomical integrity, pharyngeal residue, swallowing with sensory testing, and aspiration [12]. Wu et al. [13] have discussed the advantages and disadvantages of using the fiberoptic endoscope vs VFG to evaluate patients with swallowing disorders.
- Ultrasonography can be used to study tongue physiology during swallowing [14]. However, this procedure has no value for assessing other phases of deglutition.

7.1.2 Objective Evaluation: Observer-Assessed

Several tools are available to assess short- and long-term cancer treatment-induced swallowing dysfunctions. Common Terminology Criteria for Adverse Events (CTCAE) are frequently used to assess acute toxicity. Late toxicities can be assessed using the Radiation Therapy Oncology Group (RTOG)/European Organization for Research and Treatment of Cancer (EORTC) criteria and the Subjective Objective Management Analytic (SOMA) scale [15–17].

7.1.3 Subjective Evaluation: Patient-Reported Quality of Life

Some of the instruments used to assess quality of life (QOL) in patients with head and neck cancer, including swallowing dysfunctions, include: the University of Washington Quality of Life tool (UWQOL) [18]; the M.D. Anderson Dysphagia Symptom Inventory (MADSI-HN) [19]; the EORTC-QLQ H&N [20]; the Performance Status Scale for Head and Neck Cancer patients (PSS-H&N) [21]; the Radiation Therapy Instrument Head and Neck (QOL-RTI/H&N) [22]; the Functional Assessment of Cancer Therapy-H&N (FACT-H&N) [23]; and the Head and Neck Radiotherapy Questionnaire (HNRQ) [24]. While these instruments all measure some aspects of head and neck cancer–related QOL, it is not clear which one best applies to the assessment of swallowing dysfunctions in patients with head and neck cancer and to various treatment modalities.

7.2 Baseline Swallowing Function in Patients with Head and Neck Cancer

Pauloski et al. [25] compared 352 patients with head and neck cancer with 104 controls. Pretreatment, 59% of patients complained of dysphagia. On VFG

study, the majority of these patients had functional study suggesting inconsistency in perception of swallowing and actual swallowing ability. However, compared to controls, patients had significantly longer oral and pharyngeal transit time, greater oropharyngeal residue, and lower swallowing efficiency. Swallow function worsened with increased tumor stage and in patients with oral and pharyngeal lesions compared to those with laryngeal lesions. It is not clear if the swallowing decrement was the result of tumor infiltration of muscles and nerves or of pain and ulceration.

7.3 Swallowing Disorders Induced by Radiation Alone

Radiation-induced late toxicities, including swallowing disorders, are unevenly distributed, with some patients exhibiting more cellular radiosensitivity. Con licting data have emerged related to target-tissue sensitivity (fibroblast vs DNA repair capacity vs lymphocytic chromosomal damage) and late radiation damage [26–28]. Denham et al. [29] have categorized radiation-induced normal tissue injury as direct, indirect, and functional, in addition to resulting from genetic susceptibility. A number of tumor and radiation variables [30–35] also in ıuence the incidence of late damage. Several investigators have documented cervical and pharyngeal fibrosis and laryngeal dysfunction resulting in swallowing disorders following standard or accelerated radiation schemes [36–41]. Therefore, it is essential to understand the biomechanics of swallowing disorders and to know the anatomical organs that are critical for swallowing and the radiation dose–volume relationship of these organs in order to prevent and reduce the incidence of swallowing disorders and devise effective rehabilitation techniques [42].

Radiation of the head and neck area produces xerostomia and short-term laryngopharyngeal edema, resulting in acute dysphagia. Radiation-induced swallowing disorders can manifest months to years later as a result of extensive ibrosis and vascular and neural damage [43]. The biomechanics of these disorders have been studied by Lazarus et al. [44] using VFG in a group of patients with dysphagia 10 years following radiation. These patients demonstrated a number of oropharyngeal motility disorders, including reduced tongue-base contact with the posterior pharyngeal wall, reduced laryngeal elevation, and compromised vestibule and true vocal cord closure. These disorders resulted in pharyngeal residue, which was aspirated after swallow. Despite different tumor sites, all patients exhibited similar altered biomechanics, which most likely resulted from the large radiation doses and volumes that encompassed the orolaryngopharyngeal area during treatment. In 1995, a similar observation was made by Dejaeger et al. [43] who used manofluorography in a patient 5 years following radiotherapy for a pharyngeal carcinoma. Kendall et al. [45, 46] used VFG to evaluate 20 patients with head and neck cancer previously treated with radiation. These patients were able to maintain nutrition and none complained of dysphagia. When compared with 60 normal subjects, all 20 patients demonstrated abnormal swallow mechanics and prolonged oropharyngeal time for all bolus sizes. The onset of aryepiglottic fold closure relative to the onset of swallow was delayed and there was a trend toward delayed hyoid elevation. In this study, there was a trend toward earlier opening of the upper-esophageal sphincter relative to the arrival of the bolus, most likely as a compensatory mechanism. Patients with base-of-tongue cancer had worse swallow mechanics compared to patients with pharyngolaryngeal cancers.

To assess pharyngeal dysfunctions following radiation, Wu et al. [12] used iberoptic endoscopic examination in 31 patients with dysphagic nasopharyngeal cancer, with a mean follow-up of 8.5 years following radiation. They observed pharyngeal retention (93.5%), post-swallow aspiration (77.4%), atrophic changes in the tongue with or without fasciculation (54.8%), vocal cord paralysis (29%), velopharyngeal incompetence (58%), delay or absence of swallow reflex (87.1%), and poor pharyngeal constriction (80.6%).

Jensen et al. [47] made a similar observation using functional endoscopic evaluation of swallowing (FEES) and EORTC-QOL questionnaires in 25 patients with pharyngeal cancer treated with radiation alone and followed up for a minimum of 2.5 years (mean 5 years). Of these patients, 83% had subjective swallowing complaints. The most frequent objective finding was reduced sensitivity in the oropharynx (94%) and reduced range of motion at the tongue base (79%). Pharyngeal residue appeared in 88% of these patients, 59% experienced laryngeal penetration, and 18% aspirated. Penetration and aspiration were observed primarily with thin liquids. All of the six patients with aspiration were smokers.

7.4 Surgery and Radiation-Induced Swallowing Dysfunctions

Surgery-related swallowing disorders usually occur during the first few months following surgery. Abnormal swallowing biomechanics depend on the site of surgery, the extent of resection, and the type of reconstruction [1, 48, 49].

Pauloski et al. [50, 51] studied the effect of postoperative RT in a surgical resection-matched patient population. Patients receiving postoperative radiation had increased oral transit time, greater pharyngeal residue, lower OPSE, and shorter duration of cricopharyngeal opening. Patients without postoperative radiation demonstrated improvement in swallowing ef ìciency up to 12 months post-surgery, while patients with adjuvant radiation did not show any improvement in swallow function.

7.5 Chemoradiation-Induced Swallowing Dysfunctions

Over the past two decades, the intensity of treatment using chemotherapy and radiation has increased, resulting in better tumor control and organ preservation. However, acute and late toxicities have also increased [52, 53]. Forastiere et al. [54] reported RTOG 91-11 data where 1 year post-treatment only 9% of patients treated with radiation alone needed liquid or soft food, compared to 23% of patients in the chemoradiation group.

In two separate studies, Lazarus et al. [55, 56] reported that the swallowing mechanics in head and neck cancer patients treated with concomitant chemoradiation protocols and compared the results with age-matched controls. A large percentage of patients exhibited abnormal swallow mechanics similar to those seen in patients treated with radiation alone; also seen were reduced tongue-base movement toward the posterior pharyngeal wall, reduced tongue strength, reduced laryngeal elevation, lower OPSE, an increased number of swallows needed to clear the bolus, and a high incidence (89%) of aspiration of liquids.

Neuman et al. [57] compared the effects of treatment using high-dose intra-arterial cisplatinum with radiation vs intravenous chemotherapy and radiation. A large percentage of patients exhibited abnormal swallow measures that were similar in both groups, except that the intra-arterial group exhibited less aspiration with 1–3 ml of liquids.

Eisburch et al. [31], Kotz et al. [58], Mittal et al. [30], and Nguyen et al. [59] have also reported a high incidence of oropharyngeal dysfunctions following concomitant chemoradiation. Most of these patients exhibited some degree of aspiration, base-of-the-tongue weakness, pharyngeal residue, reduced laryngohyoid movement, decreased epiglottic inversion, swallow reflex delay, and velopharyngeal incompetence. Upper esophageal stricture was also observed in several patients.

Disorders resulting from radiation and chemotherapy can affect both oral and pharyngeal phases of swallowing. Swallowing biomechanics are altered as a result of reduced lingual manipulation and propulsion of bolus, reduced tongue strength, delayed triggering of the pharyngeal motor response, impaired tongue-base motion, pharyngeal contraction, laryngohyoid motion, laryngeal vestibule closure, and cricopharyngeal opening [60].

Over time (3–12 months), these disorders may reduce in severity [61], although this is not always the case. A substantial number of patients experience deterioration of swallowing function over time as a result of vascular damage and fibrosis [44, 62–64]. This discrepancy highlights the importance of objective evaluation of swallow physiology before and at several points following treatment. Additional research is needed on the measurement and treatment of swallowing disorders after various chemoradiation protocols [3]. The confounding effects of tumor site and stage, pre-existing dysphagia, dental status, smoking history, and other comorbidities also need to be studied.

Dysphagia resulting from radiation and chemotherapy could also be a result of stricture formation in the hypopharyngeal or upper esophageal area. There is evidence to support the increased incidence and severity of stricture formation following treatment with radiation plus chemotherapy compared to radiation alone. Laurell et al. [65] reported a 3.4% incidence of stricture formation in head and neck cancer patients treated with radiation alone compared to an incidence of 21% observed by Lee et al. [66] in a group of patients treated with radiation plus chemotherapy. This study reported a higher incidence of stricture formation in patients receiving hyperfractionation, in patients with hypopharyngeal primary tumors, and in women.

7.6 Organ at Risk and the Dose–Volume–Effect Relationship

Laryngopharyngeal disorders resulting in late dysphagia and aspiration are not regimen-specific and are the result of edema and fibrosis [51]. To correlate the relationship of radiation dose–volume–effect, it is critical to know the relative importance of the organs involved in swallowing physiology. Pauloski et al. [67] used VFG to evaluate the "organ at risk" in 170 patients. Laryngeal elevation, tongue-base retraction, and the cricopharyngeal opening consistently predicted patients' ability to swallow different food consistencies. Eisbruch et al. [68] reviewed the literature and identified the structures that, if damaged, could potentially cause abnormal swallowing physiology. From their study of 26 patients assessed with VFG, direct endoscopy, and CT scan they identi ied pharyngeal constrictors (PC) and glottic and supraglottic larynx (GSL) as dysphagia- and/or aspiration-related structures (DARS). In a prospective study, Feng [69] established the dose–volume–effect relationship for DARS and the esophagus in 36 patients with stage 3/4 head and neck cancers treated with chemoradiation. For intensity-modulated radiation therapy (IMRT) dose optimization, planning treatment volume (PTV) was excluded from the target organ (swallowing structures, major salivary glands, and oral cavity). However, the entire organ was used to establish the dose–volume–effect relationship. A strong correlation was observed between the mean dose to DARS and dysphagia endpoints. Aspiration was observed when the PC mean dose exceeded 60 Gy and the dose–volume threshold was V40 = 90%, V50 = 80%, V60 = 70%, and V65 > 50%. For aspiration to occur, the GSL dose–volume threshold was V50 > 50% (> 50% of volume receiving 50 Gy). For stricture, a mean dose of ≥ 66 Gy and a dose–volume threshold of V50 = 85%, V60 = 70%, and V65 = 60% for PC was observed. For stricture formation to occur, no relationship between mean dose to the GSL and esophagus was observed. The mean dose to the PC and esophagus was correlated with liquid swallowing, while only the mean dose to PC was correlated with solid swallowing on patient-reported and observer-rated swallowing scores.

In a retrospective study of 25 patients managed with radiation alone, Jensen et al. [47] studied the dose–volume–effect relationship using FEES and the QOL questionnaires EORTC C30 and H&N 35. In this study, radiation dose to base-of-tongue and PC did not correlate with swallowing endpoints. However, doses < 60 Gy to the supraglottic area, larynx, and upper esophageal sphincter resulted in a low risk of aspiration.

Dornfeld et al. [70] reported on 27 patients with head and neck cancer who were treated with IMRT radiation + chemotherapy and free of disease for at least 1 year following treatment. Swallowing difficulties and the type of diet tolerated (Diet Score) decreased progressively with radiation doses > 50 Gy to the aryepiglottic folds, false vocal cords, and lateral pharyngeal walls near the false cord.

Levendag et al. [71] reported on 81 patients with oropharyngeal carcinoma treated with three-dimensional CRT or IMRT with or without brachytherapy ± chemotherapy. A significant correlation was observed between the mean dose to the superior and middle pharyngeal constrictor muscles and patient complaints of severe dysphagia. A median dose of 50 Gy predicted a 20% probability of dysphagia. This probability increased signi icantly beyond a mean dose of 55 Gy, with an increase of 19% associated with each additional 10 Gy to superior and middle constrictors.

Doornaert et al. [72], using RTOG and EORTC-QOL questionnaires in 81 patients with head and neck cancer, correlated the mean dose to the pharyngeal wall structures (PWS), including mucosa and pharyngeal constrictor muscles and swallowing outcome. They reported a steep dose–effect relationship beyond 45 Gy to PWS and concluded that a mean dose of 45 Gy is the optimal threshold dose for predicting swallowing difficulties.

Coglar et al. [73], in a study of 96 patients with head and neck cancer treated using IMRT ± chemotherapy, observed no aspiration when the mean radiation dose to the larynx and inferior pharyngeal constrictor was ≤ 48 Gy and ≤ 54 Gy, respectively. A dose–volume effect was observed. At V50 = 21% for the larynx and V50 = 51% for the inferior constrictor, no aspiration or stricture were observed. No stricture was observed if the mean dose to the inferior constrictors was kept below 54 Gy. The mean dose to the larynx did not correlate with stricture formation.

O'Meara et al. [74] retrospectively reviewed the data of head and neck cancer patients treated with two-dimensional radiation + concurrent chemotherapy. They observed an association between the median dose to the inferior hypopharynx (pharyn-

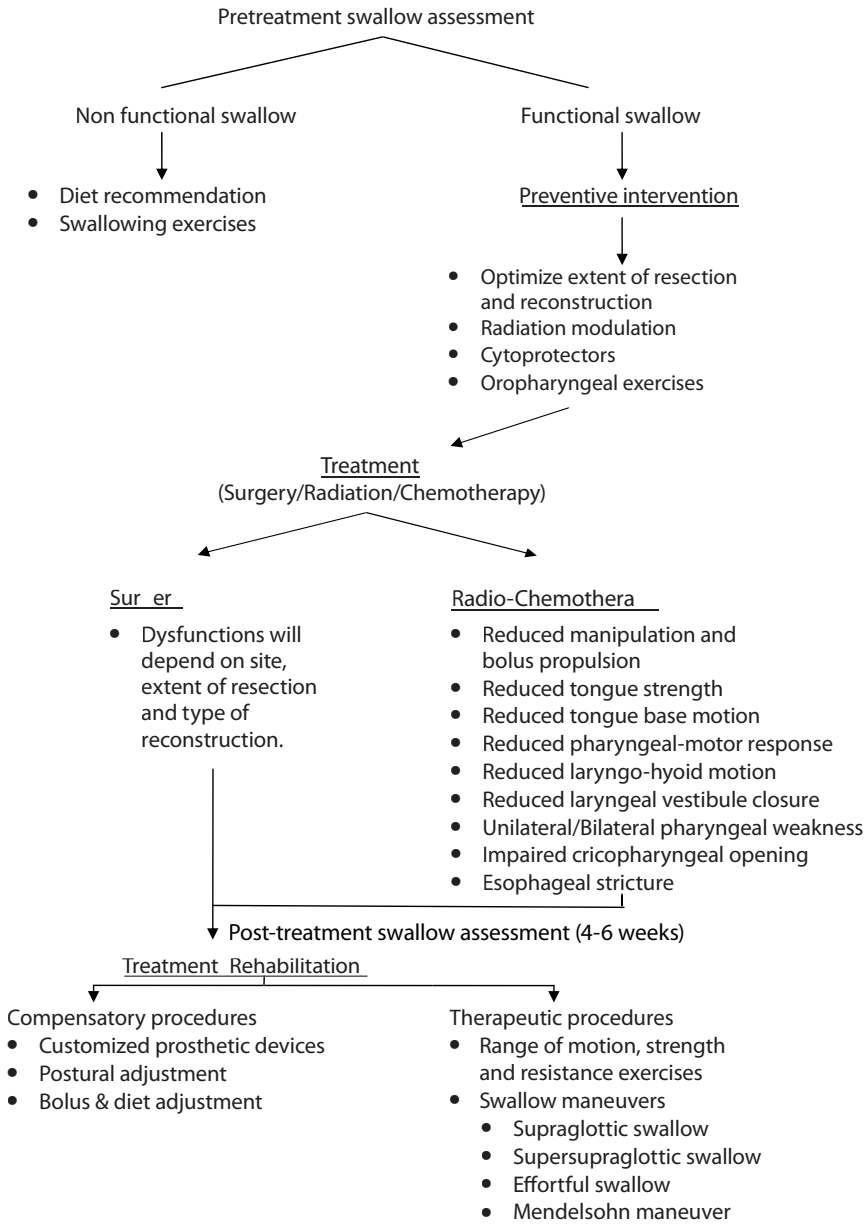

Fig. 7.1. Algorithm for the evaluation and treatment of swallowing dysfunctions in patients with head and neck cancers

goesophageal inlet) and severe late toxicity (grade ≥3 pharyngolaryngeal dysfunction). The incidence was 46%. The median dose to the inferior hypopharynx was 58 Gy among patients with severe late dysphagia, compared to 50 Gy in patients without severe dysphagia.

There is a paucity of dose/volume data about hypopharyngeal/upper esophageal stricture in head and neck cancer patients treated with radiation + chemotherapy. Laurell et al. [65] compared radiation dose–volume data in 22 patients with proximal esophageal stricture vs 22 reference patients with no stricture following radiation. They recommend a mean dose of <65 Gy to the first 2 cm of proximal esophagus and a mean dose of <60 Gy to the first 5 cm of proximal esophagus as a tolerance dose below which the incidence of esophageal stricture is low. However, further studies are needed to establish the dose-modifying effect of chemotherapy given concurrently with radiation.

Table 7.1. Organs at risk and dose–volume relationship above which swallowing dysfunctions increases significantly

Reference	No. of patients	Critical organs	Dose (Gy)		Volume data (%)			End point	Evaluation method
			Mean	Median	V50	V60	V65		
[68, 69] • IMRT • RT + Chemo	26/36	Larynx			50			Aspiration	VFG
		PC	>60		80	70	50	Aspiration	
		PC	>66		85	70	60	Stricture	
[73] • IMRT • RT ± Chemo	96	Larynx	<48[a]		21			Aspiration and stricture	VFG
		IC	<54		51				
[72] RT ± Chemo	81	Pharyngeal Mucosa and constrictors	45					QOL	• RTOG • EORTC C-30 & H/N 35
[74] 2DRT + Chemo	148	Pharyngoesoph-ageal inlet		50				Grade 3 + Pharyngoe-sophageal dysfunction	RTOG late toxicity
[71] 3DCRT/IMRT ± Brachy ± Chemo	81	Superior and middle constrictors	55					• Grade ≥ 3 • EORTC • PSS – H&N • MDADI	• RTOG • QOL • QOL
[70] • IMRT • RT ± Chemo	27	Aryepiglottic fold False cord Lateral pharyngeal Wall near false cord	50					• Diet score • H & N QOL • Weight loss • PEG Tube	• QOL • Clinical Assessment
[47] • 3DCRT • RT alone	25	Larynx Upper esophageal sphincter	60					• Aspiration • QOL	• EORTC • QOL • FEES

[a] No correlation with stricture formation
PC, pharyngeal constrictors; IC, inferior constrictor; FEES, functional endoscopic evaluation of swallowing

Table 7.1 summarizes some of the published data for organs at risk and the radiation dose–volume relationship resulting in objective and/or subjective swallowing disorders. The differences between dose–volume–effect relationship and organs at risk between various studies could be due to lack of uniformity in dose reporting, differences in organ contouring, use of 2D vs 3D data for treatment planning, dose optimization, and the endpoints used to measure swallowing dysfunctions.

7.7 Preventive Intervention to Reduce Swallowing Disorders

There are no large phase 3 trials to suggest that prevention is possible. However, several single-institutional studies suggest that with the use of physical, technical, and biochemical agents and exercise of the oropharyngeal muscles it is possible to reduce the incidence and severity of swallowing disorders [42].

7.8 Radiation Modulation

Using current technologies, it is possible to reduce the radiation doses and the volume of critical structures involved in swallowing without compromising the target. Eisbruch et al. [68] identified the larynx and PC as playing an important role. By decreasing radiation to these structures using IMRT, they were able to reduce the incidence of aspiration and swallowing disorders [69]. Similarly, Mittal et al. [30] were able to decrease swallowing disorders with the

use of static multisegmental IMRT. The incidence of early and late feeding tube placement was also decreased with IMRT [75]. However, Milano et al. [76] and Garden et al. [77] observed no difference in swallowing disorders with the use of IMRT. Garden [78] and Chao [79] also observed no difference in the need for a feeding tube when IMRT was used.

IMRT is time-consuming and organs at risk need to be defined to prevent excessive doses to the larynx and postcricoid esophagus, which can be spared using conventional radiation techniques with a small midline shield [80]. The use of this technique is also supported by the dosimetry study of Fua et al. [81]. They were able to decrease the pharyngoesophageal axis (PEA) mean dose from 55.2 Gy to 27.2 Gy using junctional IMRT (J-IMRT) with a midline shield as opposed to whole-field IMRT (WF-IMRT). The incidence of dysphagia and the duration of a feeding tube requirement were significantly less when the J-IMRT technique was used. However, the PEA can be classified as a dose-avoidance structure during WF-IMRT in order to decrease the radiation dose to the PEA. Further studies are needed to identify the dose–volume relationship to the critical organs and neuromuscular systems involved in swallowing.

7.9 Oral Feeding vs Feeding Tube

More than 70% of patients treated with intensive concurrent chemoradiation for head and neck cancer required a feeding tube by the end of treatment [66, 82, 83]. At 1 year following treatment, at least 20% of patients still required a feeding tube to supplement their oral intake [70, 82, 83]. The use of a prophylactic feeding tube is controversial [84]. Rosenthal et al. [85] support the use of oral feeding to the maximally tolerated food viscosity as long as possible even if the patient already has a feeding tube. Gillespie et al. [86] reported a worse swallowing outcome in patients who had not had oral intake for more than 2 weeks. Patients should continue oral intake to reduce the risk of long-term tube dependency and dysphagia [87]. Rosenthal et al. [85] and Mikhal et al. [88] suggest that a nasogastric (NG) feeding tube decreases the need for esophageal dilatation vs a percutaneous endoscopic gastrostomy (PEG) tube. They hypothesized that the NG tube serves as a stent to prevent stricture formation. However, when using the NG tube, it is necessary to take care to avoid trauma to the PEA. We also support continual oral feeding so long as it does not compromise the patient's nutritional status and increase the risk of aspiration.

7.10 Cytoprotectors

Amifostine (WR 2721) is the most commonly used cytoprotector for reducing the incidence of xerostomia and mucositis [89–91]. However, there is no data to support its role in decreasing late swallowing disorders. Further studies are needed.

7.11 Oropharyngeal Exercises

Oropharyngeal exercises are designed to improve swallowing biomechanics by increasing the excursion of swallowing organs and strengthening the musculature involved in deglutition. Range-of-motion exercises are available for the oral tongue, base of tongue, and the hyoid-laryngeal complex [1, 3, 92]. Isometric resistance exercises are used to strengthen the tongue, jaw, larynx, and lips [93]. Some exercises can facilitate opening of the upper esophageal sphincter [94].

7.12 Therapeutic Intervention to Improve Swallowing Disorders

Rehabilitation of swallowing disorders should be started as soon as possible following treatment. Logemann [1] suggests less benefit with delay in swallowing therapy. Waters et al. [95] report that the extent of swallowing dysfunctions at 6 months post-treatment predicts long-term function. Kotz et al. [96] recommend treatment for oropharyngeal dysphagia as soon as possible post-chemoradiation. Rehabilitation techniques can be categorized broadly as compensatory and speci ic therapy procedures.

Compensatory procedures are used to manipulate the bolus flow and reduce aspiration, which can be accomplished by:

- Postural adjustments such as chin down, head tilt, head back, and head rotation toward the weak side; lying down can be effective in decreasing aspiration [97, 98].
- Bolus adjustment and dietary modification [1, 99–101]; patients with decreased awareness of food in the oropharyngeal area may benefit from a larger bolus, while patients requiring multiple swallows to clear a bolus may benefit from smaller bolus size.
- Customized oropharyngeal prosthetic devices [102–108] should be used to compensate for the loss of tissues during surgery; these devices will result in the reduction of oral residue and efficient clearance of food from the oral cavity to the oropharynx.

7.13 Therapy Procedures

Oropharyngeal exercises to improve strength and excursion range of the oral tongue, base of tongue, and the laryngohyoid complex will improve oral transit time, decrease pharyngeal residue, improve oropharyngeal swallowing efficiency, and decrease the risk of aspiration [92, 101, 109, 110]. Therapy exercises developed by Shaker et al. [94, 111] have been shown to improve dysphagia resulting from dysfunction of the upper-esophageal sphincter.

Patients can also be taught voluntary maneuvers to improve OPSE and prevent aspiration during deglutition. Supraglottic swallow protects the airway by holding the vocal folds closer together [1]. Super-supraglottic swallow prevents aspiration by facilitating closure of the laryngeal vestibule [112]. The effortful swallow causes increased pressure in the oral cavity and pharynx, resulting in better bolus clearance [112]. The Mendelsohn maneuver works by increasing the opening of the upper-esophageal sphincter during swallow [113, 114].

Optimum rehabilitation requires pre- and post-treatment assessment of swallowing physiology and observation of modified barium swallow (MBS) changes while various therapeutic swallow procedures and maneuvers are implemented. Patient education techniques such as biofeedback using FE, MBS, and surface electromyography have been used to help patients learn various exercises and improve swallowing dysfunctions [115, 116]. Several randomized trials are in progress to assess the outcome of various treatment strategies to improve dysphagia [117].

7.14 Summary

Swallowing is a complex process involving neuromuscular coordination of several oropharyngeal muscles and cranial nerves. Swallowing physiology can be studied using VFG, FE, and oropharyngeal manometry. Patients' symptoms and treatment toxicity can be assessed using validated QOL measures and observer assessment.

Dysphagia is often present before treatment of head and neck cancer begins.

Radiation, surgery, and chemotherapy result in anatomical and functional changes in swallow mechanics. Radiation-induced xerostomia results in increased bolus transit time. Primary radiation-induced neuromuscular dysfunction causes increased oropharyngeal transit time, incoordination of bolus movement through the oropharynx, reduced tongue-base contact with the posterior pharyngeal wall, impaired laryngohyoid movement, poor vestibule and true vocal cord closure, and impaired upper esophageal sphincter function. These abnormalities result in pharyngeal residue and aspiration.

The use of radiation therapy following surgery exacerbates the damage caused by surgical resection. Surgery-related swallowing disorders usually manifest during the first few months after surgery. The additional effects of RT on swallowing are the result of neuromuscular damage, fibrosis, and uncoordinated oral and pharyngeal phases of swallowing.

Patients treated with concomitant chemoradiation protocols show a higher incidence of swallow disorders compared to patients treated with RT alone [118]. Most orolaryngopharyngeal dysfunctions are similar to those observed in patients treated with RT alone. It is not possible to quantitate the roles played individually by RT and chemotherapy in causing swallowing disorders.

Preliminary clinical and dosimetry data characterize the radiation dose–volume and toxicity relationship of some of the critical structures involved

with pharyngeal swallowing. Several studies are in progress to expand this knowledge.

Further studies are needed to tease out the confounding effects of patient age, tumor location and stage, pre-existing dysphagia, dental status, xerostomia, smoking history, and other comorbidities.

Several preventive and therapeutic strategies can be implemented to reduce the incidence or prevent some swallowing disorders. Pre- and post-treatment assessment of the swallowing physiology of all head and neck cancer patients is essential to best implement these measures.

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Lithium as a Differential Neuroprotector During Brain Irradiation

8

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CONTENTS

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8.1 Introduction

Radiotherapy remains a major treatment modality for primary and metastatic brain tumors, as well as leukemia and lymphoma involving the central nervous system (CNS). Cranial irradiation is also used as prophylaxis for patients at high risk of involvement of the brain by neoplasia, for example small-cell lung cancer. Despite important advances in diagnosis and therapy of malignant solid tumors, brain metastases continue to present signi ıcant problems for clinicians attempting to prevent progression of disease and limit morbidity associated with therapy. Indeed, up to two thirds of all brain metastases cause clinical symptoms [1, 2]. In addition, there is evidence that the overall incidence of brain metastases is increasing because of improved systemic therapy for cancer [3]. Further, the survival rates of children with both primary [4–7], and secondary [3] brain tumors are increasing.

Although important advances have been made, cranial radiation therapy continues to present significant problems for clinicians attempting to prevent and limit morbidity associated with the therapy. The radiation dose that can be administered safely to the tumor is limited by the potential toxicity on normal surrounding CNS tissue. The primary factors influencing the likelihood of developing complications include the volume of normal brain tissue treated, the total radiation dose, and the fractionation schedule. The likelihood of brain damage also increases in the young [8, 9, 10–14], especially < 5 years old, and the elderly [10]. Furthermore, the use of concurrent or sequential chemotherapy can significantly affect the incidence and severity of radiation-induced toxicity. In addition, the tumor burden often impairs cognitive function, making it difficult to assess accurately the separate effect of radiation. These side effects impact dramatically the quality of life and

are associated with decreased education and increased unemployment when these treated children reach adulthood [8, 15, 16]. Moreover, the cognitive defects occurring after radiation therapy seem to be progressive and irreversible [17, 18].

A full understanding of the potential complications such as cognitive impairments associated with cranial irradiation is needed to develop novel strategies for toxicity prevention.

8.2 Neurotoxicity from Brain Radiotherapy

The complications of radiation therapy are usually divided into acute effects that can occur during the course of radiation, early-delayed effects that appear 2–4 months after radiation, and late effects that can develop more than three months after the initiation of radiation therapy. Complications that might occur many months or even years following cranial irradiation are generally not important as a consideration for patients with brain metastases or grade IV primary brain tumors, where a median survival in the range of 6–12 months is expected. Late effects are a more important consideration for patients with a much longer life expectancy (e.g. low-grade glioma patients or pediatric patients with acute lymphocytic leukemia) [19, 20]. The distinction between early and late complications is also important since acute and early-delayed complications are usually reversible while late reactions often are not. Finally, a recent study also suggests that the ability to focus and sustain attention in children with primary brain tumors changes during the 6- to 7-week period of cranial irradiation and the subsequent 5 years, and remain relatively stable over time after irradiation [21]. The following is a brief review of potential radiation-induced side effects, with an emphasis on late neurocognitive injury.

8.2.1 Acute Complications

Acute encephalopathy generally occurs within 2 weeks of the onset of irradiation. The disorder is usually mild, characterized by headache, nausea, drowsiness, fever, and sometimes worsening of neurological signs [22]. Exceptionally, the encephalopathy is severe in patients who already had a syndrome of intracranial hypertension at the onset of treatment (multiple metastases, large posterior fossa tumor) or who received high-dose fractionation. In these cases, patients may develop a clinical picture of brain herniation [22]. Radiation-induced breakdown of the blood–brain barrier with increased intracranial pressure is incriminated in the pathogenesis of the syndrome. Acute encephalopathy is generally most severe following the first radiation dose and gradually lessens in severity thereafter.

8.2.2 Early-Delayed Complications

Early-delayed complications occur between 2 weeks and 3–4 months after the completion of radiotherapy and may take several forms:

- The somnolence syndrome develops in many patients (particularly children) who have received whole brain or large volume irradiation [23]. It is mainly characterized by hypersomnia, drowsiness, irritability and sometimes headache and fever [24]. At this stage, neuropsychological evaluation often demonstrates attention deficits and alteration of recent memory functions. The somnolence syndrome usually resolves spontaneously within 2–3 weeks. Corticosteroids reduce the duration of the syndrome and may prevent its development [25, 26].
- In about 15% of patients, early-delayed complications simulate local tumor recurrence. Patients may complain of recurrent focal symptoms, with headache and/or recurrence of pretreatment neurologic symptoms and signs. Improvement occurs spontaneously, but steroids accelerate its resolution [27].
- A severe leukoencephalopathy with cognitive dysfunction is a very rare early-delayed complication of cerebral irradiation, which may be transient or persistent. Reversible defects in memory also occur, and do not necessarily predict long-term impairment [28, 29].

8.2.3 Delayed Complications

Delayed complications occur 3 months to many years after completion of radiotherapy. Unlike early-delayed reactions that are usually reversible, late

reactions are generally irreversible. The likelihood that the irradiation will induce delayed damage to the nervous system depends on many factors including the total dose delivered to the nervous system, the dose delivered with each treatment, and the total volume of nervous system irradiated. On the brain, a dose of 60 Gy delivered with 1.8- to 2-Gy fractions represents the upper limit of the "safe dose". Other factors that in luence tolerance of the nervous system include the length of survival after completion of radiation therapy, the presence of other systemic diseases that enhance the side effects of irradiation (e.g., diabetes, hypertension), concomitant chemotherapy and other unidentifiable host factors [30]. Cognitive dysfunction/leukoencephalopathy and radionecrosis are the main delayed complications of brain irradiation.

8.2.3.1 Neurocognitive E fects

Differentiating adverse effects of cranial irradiation upon neurocognitive function from the effects of the underlying malignancy can be very difficult. Within the last decade, prospective studies have been performed to characterize accurately the effects of cranial irradiation upon neurocognitive function [31]. It is important for such studies to include long follow-up whenever feasible, because impaired neurocognitive function may become evident as a late effect of treatment [32]. Important data on the impact of radiotherapy on neurocognitive function has been derived from studies in adults treated with prophylactic cranial irradiation and low-grade gliomas, as well as in pediatric patients. Indeed, a large follow-up study of children who survive acute lymphoblastic leukemia [33] showed that men and women in the irradiated group had higher-than-average unemployment rates compared to the adults who didn't receive brain radiotherapy in their childhood (15.1% vs. 5.4% and 35.4% vs. 5.2%, respectively).

8.2.3.1.1 Cognitive Dysfunction/Leukoencephalopathy

Radiation-induced cognitive dysfunction and leukoencephalopathy without necrosis are becoming the most frequent complications in long-term survivors [34]. This clinical "entity", also called "diffuse radiation injury" or "radiation-induced leukoencephalopathy" differs from radionecrosis in clinico-radiological aspects as well as in pathology [35]. The most dramatic complication is dementia, but there is also evidence that radiotherapy can induce a less severe encephalopathy leading to subtle neuropsychological impairment [28, 36].

8.2.3.1.2 Radiation-Induced Dementia

Progressive "subcortical dementia" represents the main clinical characteristic of this disorder. At a late stage, severe cognitive deterioration is typically characterized by a severe intellectual loss, with predominant fixative memory impairment, difficulties in focusing attention, emotional lability and apathy. Productive phenomena such as delirium or hallucination are typically absent. Signs of cortical involvement, like aphasia, apraxia or agnosia, are unusual. As a consequence of preserved insight, depression is frequent, but antidepressants do not improve intellectual performance. Gait disturbances, ranging from mild retropulsion to severe ataxia, are constant features, as is incontinence at later stages [37]. The course is characterized by progressive deterioration (80% of cases), more rarely by stabilization and exceptionally by a lasting improvement. Patients become bedridden over a few weeks to months and usually die 1–48 months after the onset of symptoms. There is no effective therapy. There are at least four factors that affect the risk of developing cognitive dysfunction/dementia:

1. Radiation schedule: the risks of cognitive dysfunction are very low with "safe" doses of whole brain irradiation.
2. Volume irradiated: virtually absent for patients undergoing focal conventional radiotherapy alone (volume of radiation excluding the temporal lobes).
3. Concurrent chemotherapy: the frequency of cognitive dysfunction/dementia is increased in patients treated with radiotherapy and concurrent chemotherapy, at least when methotrexate is used.
4. Age: elderly patients appear to be much more sensitive to the diffuse neurotoxicity of radiotherapy. The pathological substrate for intellectual decline has not been clearly identified, but all authors found predominant involvement of the white matter, in agreement with neuropsychological and radiological findings. Diffuse white matter spongiosis, multiple miliary foci of necrosis, and demyelination with severe loss of oligodendrocytes have been reported.

8.2.3.1.3 Mild or Moderate Neuropsychological Impairment

This complication may occur in children (after prophylactic treatment of acute leukemia or irradiation for primary brain tumor) and in adults (after prophylactic irradiation for small cell lung cancer or in long-term survivors of primary or secondary brain tumors). The symptoms generally occur within 4 years of irradiation and are mainly characterized by attention deficits, memory dysfunction and immediate problem solving ability [38, 39]. The clinical course is usually characterized by slow decline of neuropsychological scores without decrease in performance status, but spontaneous stabilization may also occur. Intellectual dysfunctions with signi ıcant reductions in overall intelligence quotients (IQ) score have been observed in survivors of childhood acute lymphocytic leukemia in relation to therapy central-nervous-system prophylaxis consisting of cranial irradiation and intrathecal methotrexate [13]. Several factors were found to be closely associated with a lower IQ score in long-term survivors of childhood acute lymphoblastic leukemia, including a younger age at the time of radiation (in both verbal IQ and full-scale IQ) [40]. Subgroup analysis further showed a correlation between sex, age at the time of radiation, dose of cranial radiation, concomitant intrathecal methotrexate therapy, and duration of therapy with a lower level of intellectual function. Similar decreased IQ scores were found in long-term survivors of cerebellar medulloblastoma treated with surgery and irradiation, especially those children younger than 8 years at time of radiotherapy [41]. These deficits can potentially affect learning, academic performance, as well as employment rate [13, 33].

8.2.3.2 Radiation Necrosis

This disorder is a serious complication that usually begins 1–3 years after completion of radiation therapy. The symptoms depend upon the location of the lesion, and generally recapitulate those of the brain tumor or consist in new focal neurological signs simulating a tumor de novo. Radiation necrosis is caused by vascular endothelial cell damage, resulting in ìbrinoid necrosis of small arterial vessels, and therefore in focal coagulative necrosis and demyelination of the brain parenchyma [42]. These patients do well when the area of radiation necrosis is resected. Corticosteroids usually produce prompt symptomatic improvement in most patients, and there are reports of prolonged responses after corticosteroid therapy without surgery at the price of a frequent dependence on corticosteroids. Anticoagulants and hyperbaric oxygen therapy have also been reported to provide bene ìt [43, 44]. Radiation necrosis, however, is uncommon with doses of 60 Gy or less using conventional fractionation [45, 46]. Radionecrosis is more likely to occur when high doses per fraction are administered and possibly with concurrent chemotherapy [47].

8.2.3.3 Other Adverse Consequences

Include vascular abnormalities (lesions of large blood vessels) [48], and endocrinopathies [49–51].

8.3 Mechanisms of Brain Injury

An increasing body of evidence suggests that radiation-induced brain injury is a continuous, multifactorial, and dynamic process. Pathology in the brain has been attributed to vascular injury, inflammation, and apoptosis (thoroughly reviewed in [52]). Discussion of all these mechanisms is beyond the scope of this chapter. We will particularly focus on the potentially reversible mechanisms of radiation-induced brain injury. In particular, the proposed mechanisms of cognitive de ìcits due to cranial irradiation will be discussed.

Clinical studies reveal that radiation-induced damage to the hippocampus plays a significant role in the cognitive decline seen in patients who have undergone cranial irradiation. The hippocampus is one of two sites in the mammalian forebrain that exhibit active neurogenesis even in adulthood and is the critical neurologic center for learning and memory [53, 54]. Problems with learning, memory, and spatial processing that have been observed in patients after cranial irradiation are related to hippocampus injury. In support of this, radiation to the hippocampus is associated with more pronounced cognitive deficits [55–58].

Ionizing radiation induces a number of cellular responses including apoptosis and cell cycle checkpoints. Radiation-induced hippocampal injury has been linked to increased apoptosis of neuronal pro-

genitor cells within the subgranular zone of the hippocampus. This appears to involve an increase in the proapoptotic proteins p53, Bax, and caspase-3 by radiation [54, 59–61]. Interestingly, little to no apoptosis is seen in other areas of the cerebrum [62]. In addition to the increased neuronal cell apoptosis, decreased neurogenesis within this subgranular zone has been reported [59, 62–66]. This is secondary to the induction of p53-mediated cell cycle checkpoints by radiation and is associated with increased levels of phosphorylated p53 and the cell cycle regulating protein p21 [54]. Finally, decreased microvascular angiogenesis and increased microglial activation have been attributed to the loss of these pre-seen following cranial irradiation [65]. Taken together, these data suggest that radiation-induced hippocampal cell apoptosis and inhibition of neurogenesis contribute to the cognitive decline seen in patients who have undergone cranial irradiation.

8.4 Potential Neuroprotectors

In an effort to decrease neurotoxicity and improve patient quality of life following cranial irradiation, pharmacologic agents which exhibit radioprotective effects have been rigorously investigated. Since radiation-induced cellular damage has been attributed primarily to the adverse effects of free radicals, agents with direct free radical scavenging properties were studied as radioprotectors. The best known radioprotectors are the sulfhydryl compounds, such as cysteine [67] and cysteamine [68]. However, these molecules are considered to be toxic at the doses required for radioprotection, and result in significant side effects, including nausea and vomiting. Another well studied compound is amifostine [69], which has been approved by FDA as a radioprotector [70]. Indeed, amifostine, a synthetic thiol, has been used in clinical trials and it protects normal tissue, such as oral mucosa, from the unwanted effects of radiation more than tumour cells. Several mechanisms of action were proposed, including free radical scavenging, hydrogen transfer, inducing hypoxia and stabilizing DNA through direct binding [70–72]. Despite encouraging data, the use of amifostine is limited for the protection of the central nervous system. In addition, several side effects occurring with amifostine are dose-limiting and prevent maximal radioprotection [69, 73–75], including hypotension, hypocalcaemia, nausea and vomiting, sneezing, and mild somnolence. In recent years, numerous promising agents such as immunomodulators, haemopoietic growth (i.e. oxymetholone, 5-androstendiol) and stimulating factors (i.e. EPO, interleukins), synthetic chelators and natural antioxidants (i.e. glutathione, melatonin, vitamin E) have been examined for their ability to attenuate radiation-induced injury [76–85]. So far, their clinical benefits and neuroprotective efficacy remain limited.

Therefore, there is still a need to identify novel and effective compounds to protect the brain from radiation-induced injury. One such compound is lithium, a drug which has been widely used in the treatment of bipolar mood disorder [86]. Evidence suggests that lithium protects the brain against a variety of insults to the brain such as stroke and oxidative stress [58, 61, 86–90]. However, the mechanisms of the neuroprotection by lithium are not well defined. The signal transduction pathways perturbed by lithium include phosphoinositide turnover and activation of the wnt pathway via inhibition of GSK-3. In addition, lithium suppresses pro-apoptotic proteins p53 and Bax while enhancing pro-survival proteins Bcl-2 and Akt [87, 91]. Importantly, lithium reduces brain damage in animal models of neurodegenerative diseases and stroke [92]. These actions by lithium make it an attractive candidate as a neuroprotector during cranial irradiation.

8.5 Mechanisms of Lithium-Mediated Neuroprotection Against Radiation-Induced Apoptosis

Given the neuroprotective effects of lithium, the potential of lithium as a neuroprotector during cranial irradiation was investigated using preclinical models. A decrease in radiation-induced cell death was demonstrated with a 7-day lithium prophylaxis prior to the initiation of radiation in HT-22 neuronal cells as well as in neurons within the subgranular zone of irradiated mice [61]. As shown in Figure 8.1, lithium improved clonogenic survival of irradiated HT-22 neurons but not D54 glioma cancer cells. Speci ìcally, analysis of apoptosis via annexin V staining in these cells reveals a protection from radiation-induced apoptosis by lithium (Fig. 8.2). In irradiated

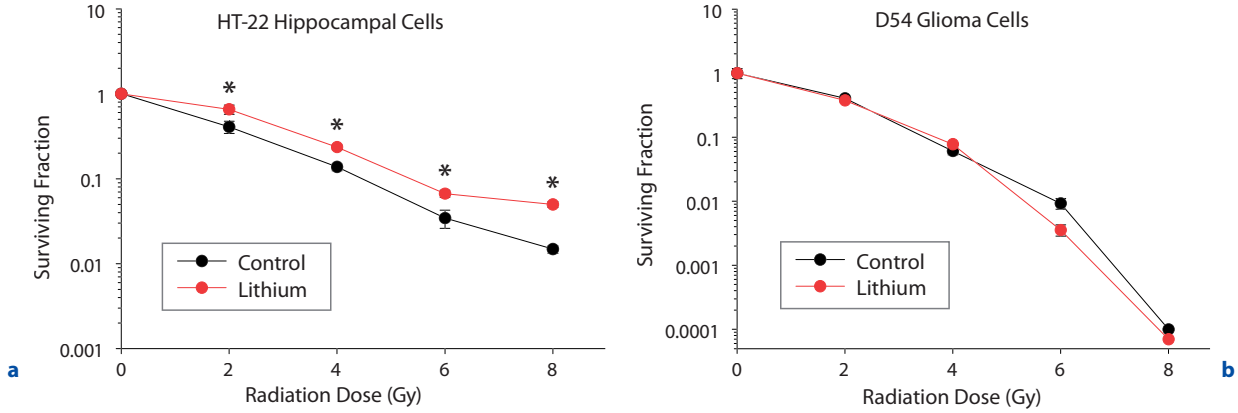

Fig. 8.1a,b. Lithium protects normal hippocampal neurons (**a**) but not D54 glioma cancer cells (**b**) from radiation-induced cell death. HT22 normal hippocampal neurons and D54 glioma cancer cells were treated with either vehicle or 3 mmol/L lithium chloride for 7 days followed by various doses of radiation. Colony-formation assays were subsequently assessed in these cells. Surviving fractions for each dose of radiation are shown relative to irradiated cells not exposed to lithium (* $p < 0.05$). (Adapted with permission from [61])

mice, lithium protects hippocampal neurons in the subgranular zone from apoptosis as evidenced by TUNEL staining (Fig. 8.3). Thus, lithium appears to decrease radiation-induced apoptosis of neurons.

More importantly, lithium also improved cognitive performance in irradiated mice. In these experiments, mice were subjected to Morris water maze studies, which involve a circular pool filled with water with an attached clear square platform beneath the water. The platform could either be visible via marking with a black flag or hidden by removing the flag and clouding the water with white paint. The study began 6 weeks following irradiation with or without lithium prophylaxis by placing the mouse into the water, and the length of time to find the platform in water was measured (average latency time). At 8 days after training, the average latency time was measured again. As shown in Figure 8.4, radiation treatment signi ìcantly increased the average latency time compared to unirradiated mice. This increase in latency is attenuated by lithium. These results indicate that lithium indeed protects hippocampal cells and neurons within the subgranular zone against radiation-induced apoptosis and preserves cognitive functions in irradiated mice compared with mice treated with radiation alone.

Optimal neuroprotection by lithium is achieved when administered for 7 days prior to irradiation. This suggests an epigenetic change by lithium. To assess the effects on gene expression by lithium during neuroprotection, microarray analysis was performed. Over 30,000 genes were examined in the

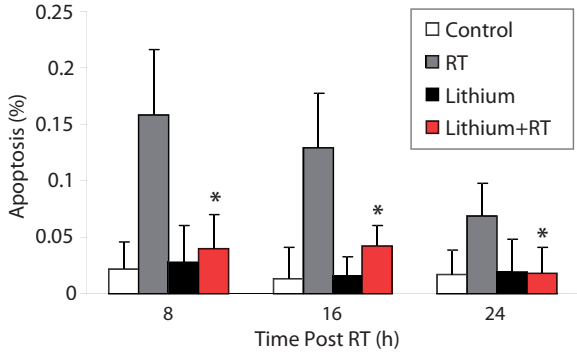

Fig. 8.2. Lithium-mediated protection of normal hippocampal neurons involves reduction of radiation-induced apoptosis. HT22 normal hippocampal neurons treated with vehicle or 3 mmol/L lithium chloride for 7 days were subjected to sham or 3-Gy irradiation. Morphologic nuclear condensation of cells was subsequently assessed via DAPI staining and analysis under microscopy. Percentage of apoptotic cells is shown (* $p < 0.05$). (Adapted with permission from [61])

HT-22 neuronal cells following 7 days of lithium. Interestingly, lithium induced a greater than two-fold expression of genes involved in anti-apoptosis signaling, neurogenesis, and DNA repair. Conversely, lithium also suppressed greater than two-fold the expression of proapoptotic genes. Verification of several of these genes confirmed microarray results. Specifically, the mRNA of the antiapoptotic proteins decorin and NAIP are induced by lithium, and a similarly increase in the protein levels of decorin and NAIP can be observed.

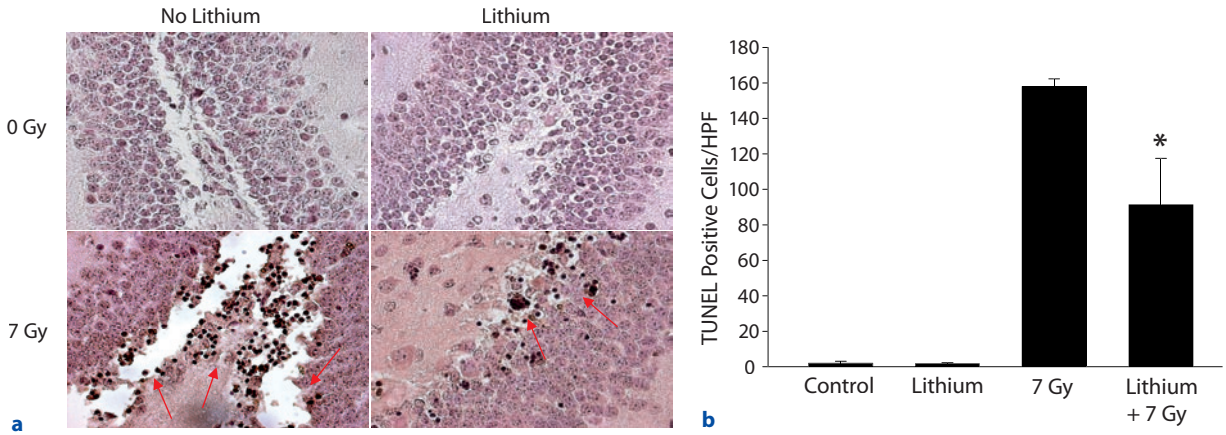

Fig. 8.3a,b. Lithium protects hippocampal neurons in vivo from radiation-induced apoptosis. (**a**) Representative histologic hippocampal sections from mice treated with and without lithium, with and without radiation. (**b**) Quantitation of apoptotic cells. Two-week-old C57/BL/6J mice pups were treated with daily i.p. injections of lithium chloride (40 mg/kg) or PBS. On the 7th day of lithium treatment, the pups were treated with 7 Gy of cranial irradiation or sham irradiation. At 10 h later, the animals were sacri ìced and brains were fixed and coronally sectioned. Apoptosis of cells was assessed via TUNEL staining (* $p < 0.05$). (Adapted with permission from [61])

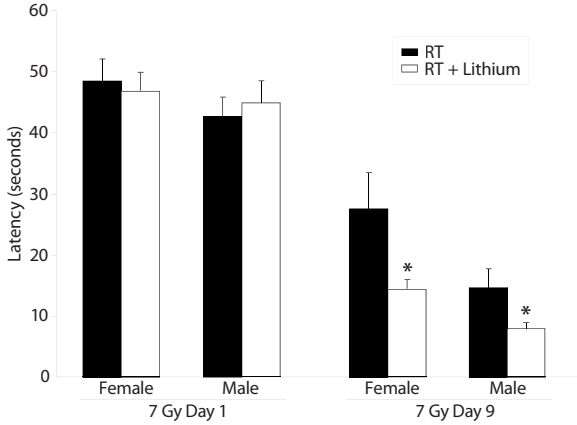

Fig. 8.4. Lithium attenuates radiation-induced cognitive decline in irradiated mice. Cognitive function following cranial irradiation was studied in C57/BL6 mice. One-week-old pups were treated with daily i.p. injections of lithium chloride (40 mg/kg) or PBS for 7 days. On the 7th day of lithium treatment, the pups were treated with 7 Gy of cranial irradiation or sham irradiation. At 6 weeks later, the animals were studied using hidden Morris water maze testing. Average latency times in male and female mice are shown in irradiated animals with or without lithium prophylaxis at day 1 and day 9 of testing (* $p < 0.05$). (Adapted with permission from [61])

To further characterize the molecular mechanism by which lithium protects neurons from radiation-induced apoptosis, several other proteins involved in the apoptotic pathway were analyzed in response to lithium. Lithium treatment correlates with activation of the pro-survival Akt pathway and enhances the levels of the antiapoptotic proteins bcl-2 and β-catenin [61]. Interestingly, lithium also suppresses the proapoptotic pathways [61]. Decreased levels of bax, whose function has been shown to be required for radiation-induced apoptosis [93], are observed with lithium. Lithium also inhibits the proapoptotic enzyme glycogen synthase kinase-3β (GSK-3β). GSK-3β activity can be regulated via activating phosphorylation at Tyr216 or inhibitory phosphorylation at Ser9. Lithium increases Ser9 phosphorylation and concomitantly decreases Tyr216 phosphorylation [61]. This is associated with accumulation of β-catenin and cyclin D, two downstream targets of GSK-3β. As the GSK-3β pathway is known to be involved in cell survival and proliferation, radioprotection by lithium could be due to lithium-mediated inhibition of GSK-3β. Our preliminary results con ìrm this hypothesis, as inhibition of the GSK-3β pathway using small molecule inhibitors or genetic manipulation using dominant negative GSK-3β reproduces the neuroprotective effects by lithium [94].

Our microarray results also suggest a potential role of DNA repair pathways in lithium-mediated neuroprotection. This is intriguing as the most critical lesion induced by radiation is the DNA double strand break (DSB) [95]. As few as one unrepaired DSB is lethal to the cell. Preliminary results suggest that the neuroprotective effects by lithium involve enhanced DNA repair of radiation-induced DSBs (personal communication, Fen Xia, MD, PhD). In

support of this possibility, inhibition of DNA repair pathways with small molecule inhibitors attenuates lithium-mediated neuroprotection.

The actions of lithium are multifactorial but seem to converge with the inhibition of radiation-induced apoptosis. However, the exact defining mechanism to achieve neuroprotection remains to be fully elucidated. The potential of GSK-3β inhibition as a target for neuroprotection is an interesting alternative to lithium and will be discussed later in this chapter.

8.6 Future Directions

8.6.1 Clinical Phase I Trial of Lithium

Based on the promising pre-clinical results, and given that long-term neurotoxicity following cranial radiotherapy is a significant clinical problem that affects the quality of life of cancer survivors, a lithium clinical phase I trial is currently ongoing. This study tests the safety and toxicity of lithium as a neuroprotective agent during cranial radiotherapy. Patients with histologically confirmed extracranial primary malignancy and associated brain metastases received lithium treatment one week prior to as well as during whole brain radiotherapy. The dosing of lithium started at one-half (300 mg BID) of the maximal dose (300 mg QID) indicated for mood disorders. Whole brain irradiation consisted of a total of 3,000 cGy over ten fractions. Preliminary data suggests that lithium can be safely administered concurrently to whole-brain radiation therapy with minimal grade 3 toxicity, although the dosage needs to be adjusted accordingly to serum levels of lithium. A phase II clinical trial is currently planned to study functional MRI and neurocognitive function in adult patients receiving prophylactic cranial irradiation.

8.6.2 Potential Disadvantages of Lithium

Although lithium is available in multiple oral preparations (i.e. carbonate or citrate), several critical variables can limit its potential in clinical setting. First, lithium has a relatively low therapeutic index, requiring careful blood level monitoring in light of the well known toxicity of lithium [96]. Side effects from lithium are common but generally mild, especially when used for a short treatment course for radioprotection. Lithium's early potential adverse effects are gastrointestinal discomforts (nausea, vomiting, diarrhea, stomach pain), muscular weakness, thirstiness and frequent urination, feelings of being dazed, sleepy, and tired, and hand tremor [96], which can sometimes disappear in certain patients. After several days of treatment this group of early side effects normally subsides. Late side effects of lithium can occur in longer therapy, including hand tremor, constant thirst and abundant urine excretion. Another major late side effect is thyroid enlargement, which is caused by lithium's perturbation of thyroid functioning.

In addition, the therapeutic action of lithium is delayed, requiring 5–7 days prophylaxis prior to the initiation of radiation therapy to reach steady-state concentrations [97]. Lithium is renally excreted after ~ 24 h, and is a function of renal sufficiency [98]. Finally, the therapeutic efficacy of lithium may rely on the "dirty" characteristics of its multiple mechanisms of action, which non-specificity can be viewed as a potential weakness.

8.6.3 GSK-3β Inhibitors

GSK-3β belongs to a family of glycogen-synthase kinases, which is a multifunctional serine/threonine kinase implicated in multiple biological processes including apoptosis [99–102]. GSK-3β is highly enriched in the brain and has been implicated in central nervous system dysfunctions including Alzheimer's disease [103], schizophrenia [104], dopamine-associated behaviors [105], bipolar disorders [106], and Parkinson's disease [107]. Our previous data support a role of GSK-3β inhibition in lithium-mediated neuroprotection against radiation-induced apoptosis. Consistent with this hypothesis, speci ìc inhibition of GSK-3β 16 h prior to irradiation or using dominant negative GSK-3β significantly attenuated radiation-induced apoptosis in hippocampal neurons [94].

Given lithium's requirement for a 7 day prophylaxis, its narrow therapeutic window, and its lack of specificity, the use of GSK3β inhibitors as neuroprotectors provide clear advantages over lithium. Prophylaxis with GSK3β inhibitors can start as early as

16 h (vs. 7 days for lithium) prior to starting cranial radiation, eliminating the need to wait 1 week prior to initializing radiation treatment that is necessary with lithium. In addition, increased speci ìcity can be achieved using these inhibitors, potentially decreasing the side effects of neuroprotectors. Further investigation with these inhibitors is warranted.

8.6.4 Summary

There is strong pre-clinical rationale to support the use of lithium as a protector of radiation-associated injury to the brain. Lithium inhibits GSK3 and blocks radiation-induced apoptosis in hippocampal neurons without protecting cancer cells. Lithium also improved neurocognitive function in mice treated with whole-brain irradiation.

A phase I clinical trial currently evaluates the feasibility of neoadjuvant and concurrent lithium treatment in patients receiving whole-brain radiotherapy. Preliminary results suggest that lithium is well tolerated in patients with brain metastases treated with cranial irradiation, and support future clinical studies to evaluate the efficacy of lithium as a neuroprotector.

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Risks and Surveillance of Second Malignant Tumors in Prostate and Bladder Cancer Survivors

9

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CONTENTS

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9.1 Introduction

The relative proportion of men surviving prostate cancer has increased as a consequence of a combination of aggressive screening, resulting in an earlier stage cancer at presentation, and improvements in treatment. Administrative databases, such as the Surveillance, Epidemiology, and End Results Program (SEER) database, have been useful in determining the incidence of second malignant tumors in patients treated with either external beam radiotherapy or interstitial seed implants. What cannot be determined, however, from a review of administrative databases is whether second malignant tumor development was a result of treatment or other causes such as genetic susceptibility resulting in increases in tumor development. Guidelines, such as the National Comprehensive Cancer Network (NCCN) guidelines, offer little guidance as to which surveillance tests are appropriate for patients once they are considered "cured" of their cancer.

This chapter will review the incidence of second malignant tumors in patients with prostate and bladder cancer and attempt to develop principles for second malignant tumor surveillance.

9.2 Risk of Second Malignant Tumors after Prostate Cancer

An increased risk of second malignancies after curative radiotherapy has been reported with other cancers, such as breast cancer, but the literature is mixed as to whether an increased risk is observed for patients undergoing radiation for prostate cancer [1–3]. Single institution reports with small num-

bers of patients have concluded an increased rate of second malignancies is not seen after radiotherapy while other mostly large studies using administrative data bases such as SEER, have concluded otherwise [4–8]. The overall incidence of second malignant tumors may be low and second malignant tumor development could be related to anatomic location for some malignant tumors, such as bladder or rectal cancer being in close geographic proximity to the radiation field [8].

9.3 Rectal Cancer

Studies using the SEER database have reported an increased incidence of rectal cancer after external beam radiation for prostate cancer. Brenner et al. [7] were among the first to use SEER data, from 1973–1993, to report an increased risk of rectal cancer after radiation when compared to surgery. The higher risk of rectal cancer development after radiotherapy for prostate cancer increased for patients surviving ≥ 10 years.

More recently, Baxter et al. [9] reported an increase in colorectal cancer in irradiated sites but not in the remainder of the colon from an analysis of SEER registry data from 1973–1994. The adjusted hazards ratio for rectal cancer development was 1.7 (95% CI, 1.4–2.2) when compared to the surgery only group. The analysis was limited to men 18–80 years old and only men with invasive, non-metastatic microscopically confirmed cancer. In addition only men alive 5 years after diagnosis were included and excluded men who had developed rectal cancer within the 5-year period after the completion of radiotherapy. The investigators used the International Classi ication of Disease Oncology 2 (ICD 0 2) to classify the location of the rectal cancer in relation to the radiation field. This method is imprecise at locating the second malignancy in relation to the radiation field as field sizes are not specified in the SEER database.

In another study using SEER data from patients treated between 1973 and 1999, Moon and colleagues [8] reported a statistically significant increased risk of rectal cancer in men treated with external beam radiation therapy as their only form of therapy [adjusted odds ratios (OR) 1.6; 95% CI, 1.29–1.99]. Men receiving radiation of an unspecified type had higher odds of secondary rectal cancer (OR, 2.34; 95% CI, 1.50–3.65). Interestingly, men receiving implants, either alone or in combination with external beam radiation did not have significant different odds of secondary cancer occurring at any of the 20 most common sites reported. This could be a result of less exposure of normal tissues to radiation by the implant and lower external beam doses used when combined with prostate seed implants. Similarly, Liauw et al. [10] also did not find an increased risk of rectal cancer in patients treated with either brachytherapy alone ($n = 125$) or combined with external beam radiation ($n = 223$). But small numbers of patients in this study may limit the ability to detect a small increased risk of second cancer if one does exist. In addition, an analysis of over 1351 consecutive patients treated with brachytherapy alone ($n = 652$) or combined with external beam radiation ($n = 699$) found an equal distribution of colorectal cancer before ($n = 23$) or after brachytherapy ($n = 25$) [11]. The majority of the cancers (73%), however, were diagnosed within 5 years of brachytherapy with a peak incidence 1 year after implant. The contribution of external beam radiation to the development of colorectal tumors could not be made as the authors did not separate the incidence of second malignant tumors by treatment.

A similar finding of increased risk of colorectal cancer was reported by Pickles and Phillips [12] from patients with prostate cancer treated with external beam radiation in British Columbia between 1984–2000. They, however, included second primary tumors starting 2 months after the conclusion of the radiation which could have overestimated the true incidence of second malignant rectal cancers.

In contrast, an increased risk of rectal cancer after prostate radiotherapy was not reported by Kendal and colleagues [13] using SEER registry data for patients treated between 1973 and 2001 controlling for age effects and differences between the surgical and untreated cohorts. Cancers of the rectosigmoid colon were excluded in this study because the authors stated the rectosigmoid colon would not uniformly fall within the usual radiation treatment fields used for prostate cancer treatment. The rate of rectal cancer development was more than twice that of surgical controls, similar to the rate Baxter et al. [9] reported. Furthermore, Brenner [14], in an editorial to the Kendal et al. [13] article, highlighted differences between the Kendal and the Baxter analyses, which showed an increased risk of rectal cancer. A number of single institution studies have not detected an in-

creased risk of rectal cancer development after prostate radiation suffering from insufficient number of patients and lacking statistical power to show an increased risk of rectal cancer development [4–6]. The large database studies are summarized in Table 9.1.

9.4 Bladder Cancer

The bladder's close proximity to the prostate makes it an organ at signi ìcant risk for development of a second malignancy after radiotherapy. A 77% increase in risk of bladder cancer development > 10 years after completion of treatment compared to patients with prostate cancer treated with surgery was reported by Brenner et al. [7]. Neugent et al. [15] only found an increased risk of bladder cancer, and not other cancers, after radiotherapy when an analysis of the SEER database was performed. Neugent et al. did not restrict their analysis to patients < 60 years old as Brenner et al. did which may account for not finding an increased risk of other malignancies. Levi at al., using a smaller database, did not find an increased risk of bladder cancer after radiotherapy for prostate cancer. Moon et al. [8], using SEER data, also found an increased risk of bladder cancer in patients treated with radiotherapy. Interestingly, men who had an interstitial implant, alone or in combination with external beam radiotherapy, did not have an increased incidence of second malignant tumors. Total bladder dose may be important etiologic factor in bladder cancer development after radiotherapy since patients receiving an interstitial implant receive a lower external beam dose when combined with an implant.

Cigarette smoking was identified as the single most important etiologic factor for bladder cancer development after 1421 men were treated with radiotherapy for prostate cancer [16]. In another analysis, patients treated with radical prostatectomy were approximately half as likely to have post-treatment bladder cancer compared to patients who underwent radiation [17]. A second bladder cancer, however, was defined as a cancer developing only 30 days after the end of radiation compared to 5 years in SEER database studies. Patients receiving radiation were 1.59 times more likely to develop bladder cancer compared to patients receiving any other treatment [17]. Similar to Wegner et al., patients who smoked cigarettes had an independent two-fold increase in the risk of bladder cancer over non-smokers. Chrouser et al. [18], in an analysis of patients receiving radiotherapy for prostate cancer at the Mayo Clinic, found a higher rate of bladder cancer development after external beam radiotherapy in patients who also smoked cigarettes.

The biologic behavior of bladder cancer developing in patients receiving radiotherapy compared to de novo bladder cancer has not been studied extensively. Bladder cancer screening programs may be beneficial for patients having undergone radiotherapy for prostate cancer if the cancers that develop after radiotherapy are more aggressive and are potentially less curable compared to de novo bladder cancers. In an analysis of the University of Miami cystectomy database, Bostrom et al. [19] found most bladder cancers in patients with a history of radiation for prostate cancer presented as locally advanced tumors and had poorer survival than age and stage matched controls. Chouser et al. [18] found while patients developing bladder cancer after radiotherapy had fewer recurrences, 20% had progressed to invasion compared to 10% progressing to invasion in the sporadic population. The pertinent large database studies are outlined in Table 9.2.

9.5 Sarcoma

Post-radiation sarcomas usually develop many years after the completion of radiation and are located in the high dose radiation region. Single institution studies have reported the development of post-radiation sarcomas within the radiation field many years after prostate cancer irradiation [20–22]. Large database analyses have also reported an increased risk of sarcoma development. Pickles and Phillips reported an increased risk of sarcomas comparing patients receiving radiation to those not receiving radiation with a relative risk of 2.49 but did not mention where the sarcomas developed in relation to the radiotherapy ìeld [12]. In addition, Brenner et al. [7] reported an increased risk of sarcomas developing within the treatment field in patients receiving radiation when compared to patients not receiving radiation but did not find a significant difference in sarcoma development outside the radiation field when comparing the two groups.

Table 9.1. Second rectal cancers after radiation for prostate cancer

Author	Data Type	Patients	Second malignant tumor development risk	
Brenner et al. [7]	SEER	122,123	OER (RT compared to surgery) ≥ 5 years 35%; 95% CI (–1,86) ≥ 10 years 105%; 95% CI (9,292)	$p = 0.06$ $p = 0.03$
Baxter et al. [9]	SEER	85,815	HR 1.7;	$p < 0.0001$ compared to surgery
Kendal et al. [13]	SEER	237,773	RR (RT compared to surgery) 0–10 years – 2.16; 95% CI (2.0–2.33) > 10 years – 15.62; 95% CI (12.01–19.83)	
Pickles and Phillips [12]	British Columbia Registry	39,261	SIR (RT compared to no RT) > 5 years 32% > 10 years 53%	
Moon et al. [8] EBRT EBRT/Iimplant Implant	SEER	297,069	AOR 1.6; 95% CI (1.29–1.99) 1.59 0.3	$p < 0.05$
Neugut et al. [15]	SEER	141,761	RR < 5 years 0.7 (0.5–0.9) 5–8 years 0.8 (0.5–1.2) > 8 years 0.8 (0.4–1.3)	

OER, observed to expected ratio; HR, hazard ratio; RR, relative risk; AOR, adjusted odds ratios; EBRT, external beam radiation therapy; RT, radiation therapy; SIR, standardized incidence ratios

Table 9.2. Second bladder cancers after radiation for prostate cancer

Author	Data Type	Patients	Second malignant tumor development risk	
Brenner et al. [7]	SEER	122,123	OER (RT compared to surgery) ≥ 5 years 55%; 95% CI (24,92) ≥ 10 years 77%; 95% CI (14,163)	$p = 0.0001$ $p = 0.01$
Liauw et al. [10]	Single institution	348	OER 5–10 years 2.33 (0.6–4.06) 10.1–20 years 2.35 (0.05–4.66)	
Boorjian et al. [17]	CaPSURE	9,780	HR 1.59; 95% CI (0.97–2.6)	
Pickles and Phillips [12]	British Columbia Registry	39,261	SIR (RT compared to no RT) > 5 years 8% > 10 years 89%	
Moon et al.[8] EBRT EBRT/implant Implant	SEER	297,069	AOR 1.63; 95% CI (1.44–1.84) 1.08 1.4	$p < 0.05$
Neugut et al. [15]	SEER	141,761	RR < 5 years 1.0 (0.8–1.2) 5–8 years 1.3 (1.0–1.7) > 8 years 1.5 (1.1–1.2)	

OER, observed to expected ratio; HR, hazard ratio; RR, relative risk; AOR, adjusted odds ratios; EBRT, external beam radiation therapy

9.6 Lung Cancer

Although not geographically in close proximity to the prostate, SEER database analyses have reported an increased incidence of lung cancer in patients with prostate cancer treated with radiation. Brenner et al. [7] and Moon et al. [8], both using SEER data, found a statistically significant radiation-associated increase relative risk in lung cancer after radiotherapy for prostate cancer. An increasing risk of lung cancer correlated with increasing survival time, meaning the longer the patients lived the greater the chance they would develop lung cancer. Levi on the other hand, using a smaller database, did not find an increased risk of lung cancer in patients with prostate cancer treated with radiation. Pickles and Phillips [12] reported a signi ìcant increased risk of tumors of the pleura in men with prostate cancer receiving radiation. What is not explicitly stated in their report is what is meant by tumors of the pleura and whether this also includes lung cancer.

A history of tobacco abuse could be one explanation of the higher rate of lung cancer in men with prostate cancer treated with radiation. There was, however, no difference in the proportion of men who smoked or ever smoked between men who had surgery or radiation in a Canadian case controlled study [23]. In an attempt to explain the increased risk, Brenner et al. [7] hypothesized given the treatment techniques of the time the lung would have received 0.6 Gy, almost two orders of magnitude less than the bladder and rectum would have received. The increased incidence of lung cancer may be as a result of the low radiation dose which is supported by the increase in relative risk of lung cancer development from 5% in the 0–5 year time period after radiation compared to 42% $\geq$ 10 years after radiation.

9.7 Male Breast Cancer

Male breast and prostate cancer are hormonally driven cancers, therefore sharing a common thread. A 5% coincidence of male breast cancer with prostate cancer exists but it is important to distinguish between metastatic spread of cancer [24]. A few single institution case reports have documented male breast cancer being diagnosed a number of years after the diagnosis of prostate cancer in patients receiving hormone therapy leading to speculation of the role of estrogen in the development of male breast cancer [25, 26]. None of the reports using SEER information, however, reported an increase in male breast cancer after prostate radiation [7, 8]. An increased risk of male breast cancer after a diagnosis of prostate cancer was found in a review of the Swedish Cancer Registry [27]. The mode of treatment was not mentioned in the report and development of male breast cancer was hypothesized to be related to estrogen therapy the men may have received although some of the increased risk could be related to a BRCA2 mutation in some patients [28].

9.8 Pancreas Cancer

Like lung, the pancreas is not located in geographic proximity to the prostate therefore it can be difficult to explain the exact mechanism for second malignancy development in the pancreas as a result of radiotherapy for prostate cancer. Using SEER data from 1973 to 1990, Neugut et al. [29] found an increased relative risk (1.2, 95% CI 1.1–1.3) of pancreas cancer as a second malignancy in men who were treated for prostate cancer. This has been the only study, SEER based or single institution, to report an increase in pancreatic cancer in patients with prostate cancer. Inclusion of synchronous pancreatic tumors may be the reason for the finding since the authors only excluded the first 6 months after diagnosis. This may point to a role of other etiologies such as a relationship to other tobacco-related malignancies or an undiscovered genetic susceptibility in a certain patient population.

9.9 Leukemia

Using SEER data, Neuget et al. [15] reported a radiotherapy associated risk of acute myelocytic leukemia of 0.1% in 10 years. The mean bone marrow dose for 14 prostate cancer patients treated with conformal

radiotherapy ranged from 3.5 Gy to 7.7 Gy [30]. This dose range is similar to that calculated for patients treated with radiation for ankylosing spondylitis and for women with cervical cancer treated with radiation [31, 32]. An increased risk of 1% in the 10 years following radiation was reported for patients with ankylosing spondylitis treated with radiation and 0.3% for women with cervical cancer treated with radiation. A higher risk of leukemia within the first 5 years was not detected by other researchers when compared to atomic bomb survivors, but Brenner et al. did find a non-significant 5% increased relative risk for leukemia during the first 5 years after diagnosis [5, 7, 12]. The impact of treatment with intensity modulated radiation therapy (IMRT) on the development of second malignant tumors including leukemia will need to be investigated in the future since IMRT spreads lower doses of radiation over greater areas of non-cancerous tissue [33, 34].

9.10 Surveillance Strategies for Prostate Cancer Patients Treated with Radiotherapy

Surveillance strategies should target patients at increased risk for second malignancy development and start approximately 5 years after completion of treatment. It is unclear whether screening should occur only in patients who are without evidence of biochemical failure or should all patients regardless of disease status be screened. Screening recommendations should be developed for detection of lung, rectal and bladder cancer since these are the tumors determined to be more prevalent after treatment for prostate cancer. More aggressive screening recommendations should be considered for patients who have abused tobacco because the incidences of lung and bladder cancers are higher in this patient population. The data are not clear at this point, which screening tests would be best for identification of second malignant tumors in patients > 5 years from the end of treatment. Urine cytology is a non-invasive test while cystoscopy visualizes the entire bladder but is invasive. Chest X-rays are an inexpensive method of evaluating the lungs but may not be able to detect smaller tumors compared to a high resolution CT scan. Further studies are necessary to determine the most appropriate screening tests in this patient population.

9.11 Second Malignant Tumors after Treatment for Bladder Cancer

An increased risk of second tumors has also been reported after bladder cancer treatment [35–42]. Secondary upper urinary tract tumors were reported in 3.1% of patients following treatment of primary transitional cell carcinoma of the bladder [37]. The mean latency between initial treatment and development of bladder cancer was 80 months and a significant difference in the development of a second malignant tumor was not seen regardless of initial treatment. A higher incidence of lung cancer, standardized incidence ratio (SIR) of 1.3 among males and 2.6 among females, was noted in a report of > 10,000 patients treated for bladder cancer in Finland [39]. An increased SIR was also found for larynx caner in males, SIR 1.7, and kidney cancer in females, SIR 3.6. A greater risk of second cancers was seen among patients < 60 years of age at the time of diagnosis of the bladder cancer compared to patients > 60 years of age. Using the same patients, Salminen et al. [38] investigated the role of tobacco-related products in the development of second malignancy and found 44% of the second malignancies were smoking related. Lung cancer was the most frequent occurring second malignancy followed by larynx cancer in men, SIR 1.67, and kidney cancer in women, SIR 3.55. An excess risk was noted up to 20 years after diagnosis. In an analysis of patients with early stage bladder cancer treated on a prospective trial of bacillus Calmette-Guerin, Herr et al. [40] found 21% of patients developed upper tract tumors after a median interval of 7.3 years. The majority of the cancers were invasive with ⅓ of patients dying of the upper tract tumors. Multiple primary superficial bladder tumors increased the risk of upper urinary tract cancers in an analysis of > 1500 patients treated for superficial bladder tumors [41]. The increased risk of upper tract tumors probably represents the multifocal nature of this disease as opposed to the adverse effects of treatment. Fabbri et al. [42] found a relatively constant high incidence of prostate cancer and kidney cancer in patients with bladder cancer over time [42]. The authors, however, included cases of second cancer diagnosed within 6 months after the diagnosis of bladder cancer making it difficult to know if the cancers were synchronous or metachronous.

Second malignant tumors developing after treatment for bladder cancer may not be related to the treatment received, as was seen in patients treated with radiotherapy for prostate cancer, but may be related to the etiologic agent causing the bladder cancer initially. Post-treatment follow-up strategies should be monitor not only for bladder cancer recurrence but also other tobacco-related malignancies.

9.12 The Effect of Technology on Second Malignant Tumor Development after Prostate Radiotherapy

Technologic advances such as improved target localization by CT treatment planning and sophisticated treatment planning algorithms have improved the ability of radiation oncologists to shrink the treatment field for patients with prostate cancer undergoing radiotherapy. Normal tissue exposure from radiation would be decreased as radiation oncologist move away from conventional radiotherapy to three dimensional conformal radiotherapy (3-D CRT).

Radiation technique and delivered dose cannot be determined from SEER data and only one paper mentioned radiation technique in reporting second malignant tumors of the bladder after radiotherapy for prostate cancer [18]. The importance of radiation dose and field size can be inferred from the studies not reporting an increased risk of second malignancy in patients having an interstitial implant as part of the management for their prostate cancer. These patients received external beam radiation doses lower than that received by patients treated with external beam radiotherapy alone. The volume of tissue receiving high radiation doses should decrease as treatment progresses from 3D CRT to IMRT. The result of this, however, will be a larger volume of normal tissue exposed to lower doses of radiation. In addition, total body exposure will be increased, secondary to the increased number of monitor units used to deliver the modulated treatment, due to leakage radiation [34].

Hall and Wuu have estimated IMRT is likely to almost double the incidence of second malignancies compared with conventional radiotherapy with the number being higher for younger patients. Kry et al. [33] estimated a conservative maximal risk of fatal secondary malignancy associated with six IMRT and one conventional treatment plans for prostate cancer. Depending upon treatment energy and type of linear accelerator, IMRT treatments required 3.5–4.9 times as many monitor units to deliver the prescribed treatment compared to the conventional plan. The conservative maximal risk of a fatal second malignancy varied depending upon beam energy and linear accelerator.

The use of proton beam therapy in the treatment of prostate cancer is increasing with an increasing number of proton beam centers planned for the United States. The risk of second malignancies in patients treated with proton beam therapy is hypothesized to be lower than for patients treated with X-rays because of the unique physical properties of the proton beam. An incidence rate of 82 second cancers per 100,000 person-years was reported in patients with ≥5 years of follow-up who received proton therapy [43]. The contribution of proton beam therapy to the development of second malignancies could not be determined from this study since all patients who developed second malignancies had a combination of photons and protons. Further studies are needed with the expected increase in the use of proton beam therapy in the future.

9.13 Cost-E fectiveness of Screening for Second Malignant Tumors

Currently, there are no studies evaluating the cost-effectiveness of screening for second malignant tumors after prostate cancer radiation. Studies evaluating the cost-effectiveness of surveillance strategies for breast cancer survivors have been recommended while CT screening for lung cancer in Hodgkin's lymphoma survivors may increase overall and disease-free survival, especially for patient who smoke cigarettes [44, 45].

Any screening program should utilize non-invasive means for detecting a tumor with as low as a false negative rate as possible. General screening for many of the urologic malignancies, however, do not meet the criteria for a successful cancer screening program namely, high prevalence, availability of a sensitive and specific screening test, ability to detect clinically important cancers at an early stage, and cost-effectiveness [46]. A screening program for bladder cancer in a high risk population utilizing a urinary dipstick for hematuria, the nuclear matrix

proten-22 (NMP-22)test (BladderChek, Matritech, Inc., Newton, MA) voided urine cytology and a molecular cytology test (UroVysion, Abbott Molecular Inc., Des Plaines, Il) detected a 3.3% rate of bladder cancer [47]. The authors reported the most efficient screening tool was the combination of UroVysion, cytology and urinary dipstick testing. Urinary NMP-22 was found to be a cost-effective marker for early detection of bladder cancer in patients with hematuria or other indications for risk of bladder cancer [48].

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Cardiotoxic Effects of Radiation Therapy in Hodgkin's Lymphoma and Breast Cancer Survivors and the Potential Mitigating Effects of Exercise

10

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CONTENTS

10.1 Introduction

Radiation-induced cardiovascular disease can compromise the quality of life of cancer survivors. Aerobic exercise training is an intervention that offers the potential to modify the expression and possibly the physiologic severity of cardiac injury. In this chapter, we review evidence supporting this approach to helping our growing survivorship population.

Historically the heart was considered to be resistant to radiation injury. However, cardiovascular disease resulting from radiation therapy that incidentally includes portions of the heart is now known to initiate, augment, or precipitate a spectrum of sequelae. Numerous reports over the last four decades document the cardiac damage which can accompany treatment for Hodgkin's lymphoma (HL) and breast cancer (BC). The delayed sequelae stemming from this cardiovascular disease dramatically increases the potential loss of productive life years among these cancer survivors by increasing morbidity and mortality [3, 4]. Most dramatically, this is illustrated by the fact that cardiac disease is the third leading cause of death in HL survivors treated with mediastinal radiotherapy, ranking below only disease recurrence and secondary cancers [7, 33, 53]. Just as importantly, cardiopulmonary compromise may be related to the persistent fatigue that is often seen in cancer survivors [42, 61, 67]. After years of concern that exercise was dangerous to people in the general population who had heart failure, it has now been shown to be safe and to improve cardiac function and fatigue. However, it is not clear whether exercise regimens are safe and effective for improving fatigue and cardiac function in cancer survivors treated with thoracic radiotherapy.

The purpose of this discussion is to review the potential cardiac sequelae of chest irradiation, the evidence linking cardiac function to fatigue and quality of life and finally the published evidence showing exercise is safe and effective in patients and survivors treated with chest irradiation. Because the most common cancers treated with chest radiotherapy with a significant number of survivors are HL and BC, we focus on the evidence from these two populations.

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10.2 Cardiopulmonary Sequelae

All of the structures of the heart including the pericardium, myocardium, valves, conduction system, and coronary arteries can be damaged by radiation therapy. Therefore the spectrum of radiation-induced cardiac disease is quite broad and includes both direct (Table 10.1) and indirect effects (Table 10.2) [1]. This range of possible manifestations draws largely from reports on HL survivors, while the risk in BC survivors has only been unequivocally demonstrated for ischemic disease through epidemiologic studies of myocardial infarction risk and perfusion scan data. The latter potentially also relates to the risk of restrictive cardiac changes affecting function. Furthermore the risk in BC survivors is likely restricted to patients treated with radiotherapy that included the internal mammary nodes on the left side.

Hodkin`s: Three reports representing a combined 4553 survivors treated between the 1960s and 1990 with mediastinal radiotherapy demonstrated that cardiovascular disease (CVD) accounted for between 9.4% and 16% of all deaths [3, 4, 6, 33, 53]. Absolute excess mortality from cardiovascular disease ranged from 11.9 to 48.9 per 10,000 patient years. This excess CVD mortality is predominantly caused by the increased risk of fatal myocardial infarction. Studies of HL survivors treated with mediastinal radiotherapy have estimated that they are 2.2 to 7.6 times more likely to die from MI than the general population [31, 53, 49]. This risk becomes statistically signi ìcant 5–10 years after treatment [7].

A recent update of a Dutch cohort of 1474 HL survivors, treated before age 41 between 1965 and 1995, established that CVD incidence as well as mortality is increased [6] among this group. HL survivors had a three- to five-fold increased incidence of CVD compared with the general population, with a 24%

Table 10.1. Spectrum of radiation-induced cardiovascular disease. (Modi ìed from [2] with permission)

Manifestation	Comments
Pericarditis	1. During therapy: Associated with mediastinal tumor and some chemotherapy agents such as cyclophosphamide [83] 2. Post-therapy: Acute effusion, chronic effusion, pericarditis, constrictive pericarditis. Seen with high doses of RT and large volumes of heart within the RT field [83]
Myocardial fibrosis	1. Fibrosis secondary to microvasculature changes [83] 2. Frequently with normal left ventricular dimensions, ejection fraction and fractional shortening as measured by radionuclide scan or echocardiogram [50] 3. Progressive, restrictive cardiomyopathy with fibrosis may occur. This can lead to pulmonary vascular disease and pulmonary hypertension [50] 4. Diastolic dysfunction may occur alone as well as with systolic dysfunction [50]
Coronary artery disease	1. The structural changes in the coronary arteries associated with radiation therapy are essentially the same as those of ordinary atherosclerosis [83] 2. Premature fibrosis may accelerate atherosclerosis [12, 86] 3. Distribution of arteries affected tends to be anterior with anterior weighted RT 4. ↑ Rates of silent ischemia (see autonomic effects) [31]
Valvular disease	1. Predominantly mitral valve and aortic valve [15] 2. ↑ Regurgitation and stenosis with ↑ time since therapy [15]
Conduction system / arrhythmia	1. Complete or incomplete right bundle branch block is suggestive of right bundle branch ìbrosis [80] 2. Initial conduction abnormalities may progress to complete heart block and cause congestive heart failure, requiring a pacemaker [80] 3. Complete heart block rarely occurs without other radiation-associated abnormalities of the heart [80]
Autonomic dysfunction	1. Frequent cardiac dysfunction with tachycardia, loss of circadian rhythm and respiratory phasic heart rate variability [32] 2. Signs listed in #1 are similar to a denervated heart. This raises the question of whether such changes in survivors are related to autonomic nervous system damage [32] 3. ↓ Perception of anginal pain [32]

Table 10.2. Indirect effects of mediastinal radiation on the cardiovascular system. (Modified from [2] with permission)

Manifestation	Comments
Mediastinal fibrosis	↓ Success of cardiovascular surgery [38]
Lung fibrosis	Chronic, restrictive and can be progressive [19]
Scoliosis and ↓ skeletal muscle	↓ Cardiovascular and lung function [19]
Thyroid	Usually hypothyroid [74] Affects cardiovascular function and lipid pro ile. May cause pericarditis
Thoracic duct fibrosis	Chylothorax-late onset and extremely rare [85]

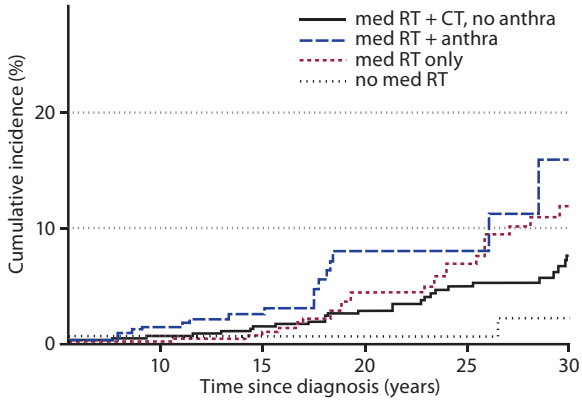

Fig. 10.1. Cumulative incidence of all CVDs combined by treatment group with death from any cause as competing risk (including CVDs) (reproduced from [6]). *med RT*, Mediastinal RT; *CT*, chemotherapy; *anthra*, anthracyclines; *MI*, myocardial infarction; *AP*, angina pectoris; *CHF*, congestive heart failure

cumulative incidence after a median follow-up of 18.7 years. The relative risk for myocardial infarction (MI) and congestive heart failure (CHF) was 3.6 and 4.9, respectively, which translated into 35.7 excess cases of MI and 25.6 excess cases of CHF per 10,000 patient-years. The researchers estimated that HL therapy accounted for 66%–80% of all CVD events in survivors (Fig. 10.1).

Breast: Relative risk estimates of fatal MI after left-sided radiotherapy for BC range as high as 2.2 compared with women who were treated for right-sided BC [71]. Further evaluation has revealed that the increased risk appears limited to those who received the highest dose-volumes of cardiac radiation, which would be women who had their internal mammary lymph nodes irradiated. Convincing evidence that ischemic heart disease is correlated with RT was reported in the Early Breast Cancer Trialists' Collaborative Group meta-analysis of randomized clinical trials. The update in 2005 showed an increased relative risk (RR) of mortality from heart disease among women treated with RT versus no RT (RR = 1.27) [18]. Long-term cardiac outcomes from randomized clinical trials of post-mastectomy RT have been reported [48, 76]. These reveal an increased risk of cardiac mortality (RR ≈ 2.5) in association with left-sided IMN RT. Retrospective population-based investigations have compared mortality endpoints by laterality or by left-side RT versus surgical controls. Two recent investigations showed an increased risk of cardiac mortality (HR ≈ 1.5) among left versus right-side cancers treated with RT in the 1970s, but no apparent increased risk with more modern treatments [22, 28]. However, most studies have demonstrated a significant increase in CAD and/or non-fatal MI associated with left compared to either right-sided RT or no RT [11, 43].

Studies using clinical imaging of the heart underscore the danger of prematurely concluding that the newer methods of RT pose no risk to the heart. Investigators from several institutions have evaluated the heart in patients treated for BC with cardiac perfusion imaging. Researchers at Duke University have accumulated the largest series of patients. Between 1998 and 2001, 114 patients with left-sided BC underwent pre- and serial post-RT single photon emission computed tomography (SPECT) gated cardiac perfusion scans. Studies published on this cohort of patients demonstrate that: (1) radiotherapy to the left chest wall/breast using modern techniques causes perfusion defects in 50%–63% of women 6–24 months post-RT [34]; (2) that the incidence of perfusion defects is associated with the volume of left ventricle irradiated; (3) that the perfusion defects generally persist 3–5 years post-RT [87]; and (4) that the perfusion defects are associated with abnormalities in regional wall motion, subtle reductions in ejection fraction, [52] and episodes of chest pain [87].

Based on the available evidence from research with HL patients, which may translate for BC patients as well, the severity and types of manifestations depend upon the presence of risk factors such

as higher total dose (> 35–40 Gy), higher fractionated dose (≥ 2.0 Gy per day), increased volume of heart exposure, relative amounts of radiation delivered to specific parts of the heart, lack of subcarinal blocking, tumor located next to the heart, younger age at exposure, length of time since exposure, type of radiation source, use of adjuvant cardiotoxic chemotherapy, and additional known risk factors for cardiovascular disease [3, 4]. While certain cardiac sequelae have been greatly reduced by the use of modern techniques, such as acute pericarditis, the effect on the incidence of other events such as myocardial infarction is not as clear.

It should also be noted that both clinically discernible cardiac complications and subclinical abnormalities with cardiac screening modalities such as echocardiogram or nuclear perfusion imaging occur in Hodgkin's and BC survivors after radiation therapy with the presence of asymptomatic abnormalities being much higher among these survivors [73]. The clinical manifestations of radiation-induced heart disease are listed in Table 10.3. The symptoms of a particular cardiac abnormality in survivors is similar to the same problem in the general population. One significant exception may be the fact that survivors treated with chest irradia-

Table 10.3. Signs, symptoms, evaluation and treatment of patients at risk for late effects of thoracic radiotherapy. (Modi ied from [19] with permission)

Late effects	Treatment [a]	Signs and symptoms	Screening and diagnostic tests	Management and intervention
Pericarditis	> 35 Gy	Fatigue, dyspnea on exertion, chest pain, cyanosis, ascites, peripheral edema, hypotension, friction rub, muffled heart sounds, venous distension, pulses paradoxus, Kussmaul's Sign	Electrocardiogram Chest X-ray, Echocardiogram	Pericardiocentesis Pericardiectomy
Cardiomyopathy (Myocardial disease)	> 35 Gy or > 25 Gy and anthracycline	Fatigue, cough, dyspnea on exertion, peripheral edema, hypertension, tachypnea, rales, tachycardia, murmur, extra heart sounds, hepatomegaly, syncope, palpitations	Echocardiogram and/or radionuclide ventriculography – Evaluate diastolic and systolic function	Education regarding risks of: alcohol, isometric exercise, smoking and other drug use, pregnancy, and anesthesia Afterload reducers, beta-blocker, antiarrhythmics, diuretics, digoxin Cardiac transplant
Coronary heart disease	> 30 Gy	Chest pain, dyspnea, diaphoresis, hypotension, pallor, nausea, arrhythmia	Exercise or dobutamine stress test with radionuclide perfusion imaging, or echocardiography (Frequency depends on risk factor profile and symptoms)	Risk factor modi ications including diet and conditioning regimens Cardiac medications and lipid lowering agents Coronary artery bypass graft or angioplasty
Valvular disease	> 30 Gy	Cough, weakness, dyspnea on exertion, new murmur, rales, peripheral edema or any other sign of congestive heart failure	Echocardiogram Cardiac catheterization	Ampicillin prophylaxis for dental or surgical procedures Replacement of valve
Arrhythmia		Palpitations, light-headedness, syncope	Electrocardiogram and 24-h ECG Evaluation for other abnormalities	Pacemaker

[a] Treatment: cumulative radiation exposure of the mediastinum at this level or higher clearly indicates increased risk for the speci ic complication and thus the need to screen for it; however, the complication may also occur at lower doses

tion are more likely to suffer asymptomatic myocardial ischemia and infarction. This would be due to the fact that radiation has been shown to damage the nerves innervating the heart as suggested by the increased risk of autonomic dysfunction in these survivors [5]. The increased risk of myocardial infarction and its devastating consequences, along with the fact that it is more likely to occur without warning in this population, makes it that much more important to screen for traditional risk factors for coronary heart disease, such as hypertension and hypercholesterolemia, and treat them aggressively.

Additionally, the cardiomyopathy and cardiac failure associated with thoracic radiation is signi ìcantly different than that occurring most commonly in the general population and that due to anthracycline therapy. The last two are associated with a dilated cardiomyopathy and characterized by systolic dysfunction. In contrast, because radiation causes ìbrosis of the myocardium, restrictive cardiomyopathy characterized by diastolic dysfunction, predominates in survivors treated with RT alone. Although clinically evident heart failure is rare in survivors treated with radiotherapy alone, studies evaluating survivors with imaging technologies show that subclinical changes are common and may be progressive. The concern is that these subclinical diastolic abnormalities may progress over the long-term to systolic dysfunction, congestive heart failure or both. In either case, one of the most common, albeit non-specific, symptoms of heart failure is fatigue

10.3 Fatigue and Its Relationship to Cardiopulmonary Sequelae

10.3.1 Cancer Related Fatigue

The most prevalent problem reported by all cancer survivors is fatigue; commonly referred to as cancer-related fatigue (CRF) [16, 37, 41, 42, 46, 61, 67, 77]. Cancer survivors report that CRF is first noticed with diagnosis. This CRF worsens during treatment and can persist for months and even years after treatments are complete [37, 41, 42, 61, 67]. It is common to see CRF persist even when the survivor's cancer is undetectable or in remission [37, 41, 42, 61, 67].

CRF is reported by 60%–100% of patients, with 41% or more reporting severe CRF (a score > 7 on an 11-point Likert scale where 0 = no CRF and 10 = CRF incapacitating) during treatment [17, 41, 46, 55, 36, 37, 63]. As many as 81% of cancer survivors report that CRF persists, with 17%–38% reporting persistent CRF at 6 months or longer after completing treatment as severe [9, 42, 61, 63, 67, 75]. This persistent and sometimes severe CRF may be a strong clinical indicator of portending cardiovascular disease resulting from radiation therapy to the chest.

CRF is typically de ìned as a multifaceted, subjective, physiological state characterized by persistent, overwhelming exhaustion and a decreased capacity for physical and mental work. The nature of CRF in cancer survivors makes it a symptom that is dif ìcult to de ìne well. In general, CRF is differentiated from the fatigue experienced by healthy individuals because of its severity, its impact on the quality of life of cancer survivors, the fact that it is not alleviated by rest, and its association with cardiovascular toxicity resulting from therapeutic radiation to the chest [3, 42, 61, 67]. The impact of CRF is far reaching because of its day-to-day impact on quality of life. Cancer survivors endure tremendous distress as a result of CRF because of the inability to prevent or alleviate this debilitating side-effects and the co-morbidities like cardiovascular disease that are associated with its presence [3, 42, 61, 67, 77].

10.3.2 Fatigue in HL Patients and Possible Associations with Cardiopulmonary Status

In HL survivors, fatigue is one of their most common complaints, with one investigator finding that 30% reported symptoms consistent with chronic fatigue, compared to 12% of the general population [51]. Recent studies from the Netherlands [60], Norway [39, 40] and the Dana Farber Cancer Institute [69] have reaffirmed that fatigue, quality of life, or both, are worse in HL survivors than in the general public or sibling controls. In HL survivors, fatigue may be associated with other known late effects such as hypothyroidism, cardiovascular disease, pulmonary dysfunction, muscle atrophy and/or psychological sequelae [69].

In the Norwegian cohort, about half of the subjects were assessed 8 years earlier [51]. Subjects with chronic fatigue at baseline continued to have worse fatigue than those who did not. The association between B-symptoms at diagnosis and chronic fatigue revealed in the original study was also reaffirmed in the larger sample [39], and suggests a relationship with the cytokines responsible for systemic symptoms ("B") in HL. A second report from this cohort suggested chronic fatigue in HL survivors was more related to physical problems than psychological [40].

At least two studies have demonstrated a possible association between fatigue or quality of life and cardiac status. In the Dana Farber study, multivariate analysis revealed that self-reported cardiac disease, history of tobacco use, psychiatric conditions, and low exercise frequency were all associated with worse fatigue [69]. Adams et al. [5] in a study of 48 survivors of adolescent or young adulthood HL treated with mediastinal radiotherapy showed that the physical composite score (PCS) on the SF36 quality of life scale correlated with peak oxygen consumption on exercise stress testing, a measure of cardiopulmonary function. Results suggested that cardiopulmonary function explained 30% of the variation in PCS (r-squared = 0.30, $p < 0.001$) and 13% of the variation in fatigue (r-squared = 0.13, $p < 0.021$). Unpublished data by this group demonstrated the same correlation with PCS measured a median 5 years later. This relationship is unsurprising because fatigue may be an early clinical indicator of heart failure, especially heart failure due to diastolic dysfunction which often does not present in the classic manner of systolic heart failure.

10.4 Exercise Interventions for Fatigue and Cardiopulmonary Function Among Cancer Survivors

10.4.1 Rationale for Exercise Interventions in Cancer Survivors Treated with Mediastinal Radiotherapy

Although evidenced-based guidelines exist for the treatment of heart failure and myocardial infarction, the treatment of diastolic heart failure in general is not well studied and no reports exist that specifically address radiation associated cardiac disease.

Consequently, prevention is the clearest approach to "treating" radiation-induced cardiotoxicity. By definition, prevention suggests that effective interventions need to be initiated and continued prior to, during and after radiation therapy. Modern radiotherapy techniques decrease the dose-volume of the heart irradiated and probably decrease the risk of most types of cardiac disease. Modern techniques include using three-dimensional treatment planning, a linear accelerator as a radiation source, equally weighted anterior/posterior portals each treated daily, and a conformal blocking to minimize cardiac exposures. However, these methods do not eliminate cardiovascular sequelae. Thus effective non-invasive and non-pharmacologic therapy targeting improvements in cardiorespiratory function could prevent or delay the onset of cardiovascular disease. Through this mechanism or independently by reducing CRF, exercise might signi icantly reduce the symptoms and co-morbidity burden among survivors of HL and BC.

Exercise training leads to several adaptive responses involving both central (i.e., heart and lungs) and peripheral (e.g., vascular function, skeletal muscle oxidative capacity) cardiovascular systems. Central adaptations, such as reductions in left ventricular cavity dilation, increased ejection fraction, improved stroke volume and cardiac output and New York Heart Association functional class, are strongly associated with enhanced global cardiovascular functioning, improvements in quality of life and clinical symptoms, and an overall survival benefit in patients with cardiovascular disease [10, 27, 30].

In the general population, regular exercise has been shown to reduce the risk of myocardial infarction [25] and is accepted as an important intervention to reduce the risk of second heart attacks [81]. It is also an important therapy to delay mortality and improve quality of life in patients with congestive heart failure [44]. Exercise regimens have also demonstrated success in improving fatigue (or quality of life) in populations with diseases other than cancer including chronic heart disease [29, 68].

The improvements from exercise regimens in cardiac function and fatigue in other disease populations therefore supports evaluating exercise as a way to improve both in cancer populations and in particular those at risk for cardiac toxicity from radiotherapy to the chest.

10.4.2 Exercise Interventions: Safety and Efficacy in Cancer Survivors

Preliminary data suggest that exercise may be an effective therapeutic intervention to reduce CRF and improving cardiorespiratory function among survivors of HL and BC. For the purpose of this review and according to the National Cancer Institute, "an individual is considered a cancer survivor from the time of diagnosis, through the balance of his or her life." This de ìnition includes cancer patients receiving treatment as well as those who have completed their therapy among the cohort considered cancer survivors.

It is important to de ìne and differentiate between the terms physical activity and exercise because they are often used interchangeably and inappropriately. Physical activity is defined as any skeletal muscle movement that causes an increase in energy expenditure above a resting basal metabolic rate and encompasses a wide variety of lifestyle and occupational activities [8]. Exercise is de ìned as physical activity performed in an organized manner (e.g., a specific frequency, intensity, duration, and mode) with the intent of improving health-related outcomes, including cardiovascular ìtness, muscular strength, body composition, depression, anxiety, sleep, cognition, and fatigue [8]. In this chapter, the term exercise or physical exercise is used to describe any physical activity intervention designed and delivered with the aim of improving CRF or cardiovascular function.

Physical exercise is a behavioral intervention with the potential promise of mitigating the acute CRF experienced by cancer patients during treatment, the persistent CRF they experience after treatments are complete, and the cardiorespiratory impairment associated with treatments such as radiation therapy [62, 64, 65]. Mustian and colleagues [67], Jacobsen and colleagues [45], Galvao and Newton [26], Knols and colleagues [47], Stevinson and colleagues [82], Schmidtz and colleagues [78], and McNeely and colleagues [54] recently summarized the evidence from randomized controlled clinical trials regarding the benefits from physical exercise interventions implemented with adult cancer survivors during and after treatment. The outcomes that were examined included CRF, emotional distress (e.g., depression, anxiety), quality of life, aerobic capacity, muscular strength, flexibility, body composition, functional capacity, and immunological parameters. In total, 15 of these studies assessed CRF and/or cardiorespiratory function as a primary or secondary outcome among Hodgkin's and/or BC survivors and employed a randomized, controlled clinical trial experimental design. The current article restricts discussion to these 15 randomized controlled clinical trials. See Table 10.4 for a complete summary of these studies.

Of these studies, 12 included BC patients/survivors in the sample, while only three studies included HL patients/survivors. Unfortunately, the randomized clinical trials that included HL patients/survivors evaluated them in a mixed cancer population, and subgroups were not large enough to examine HL patients/survivors independently regarding the influence of exercise on CRF and cardiovascular function. Oldervoll and colleagues [70] examined the in luence of an aerobic exercise intervention among a non-randomized sample of HL survivors and found that fatigue, physical functioning and maximal aerobic capacity were significantly improved by a home based aerobic exercise program of 20 weeks. These results did not differ based on whether the survivor reported chronic and severe fatigue at baseline or no fatigue at all. Unfortunately, this pilot study by Oldervoll and colleagues was a one-arm study, with no randomization and included a small sample of 15 survivors without chronic fatigue, and nine survivors with chronic fatigue in the exercise intervention. Additionally, cardiac and/or pulmonary function were not evaluated [70].

The results of these 15 studies provide preliminary evidence suggesting that exercise is safe and well-tolerated by cancer survivors with a wide range of diagnoses. The safety evidence is primarily generated by a priori evaluation of adverse events stemming from the exercise intervention. The tolerability evidence is primarily developed by a priori assessment of adherence and compliance with the prescribed exercise intervention. These studies also suggest the results are similar for patients throughout the cancer care continuum; during and after surgery, chemotherapy, radiation therapy, hormone therapy, and even salvage therapy. One study also suggests that low-intensity and seated exercise is safe and well-tolerated by women with metastatic BC. This growing body of research suggests that exercise interventions involving moderately intense (55%–75% of heart rate maximum) aerobic exercise (e.g., walking and cycling) ranging from 10–90 min in duration, 3–7 days/week are

Table 10.4. Exercise, cancer-related fatigue and cardiorespiratory function among cancer survivors

Reference	Sample	Treatment	Type of exercise	Results[a]
[56]	Breast cancer patients w/stages I and II diagnoses *n*=14	Chemotherapy	Home-based walking 4–5×/week for 10–45 min @ self-paced % HR maximum with support therapy for 4–6 months	↓ **Fatigue** ↑ **Walking ability in exercisers compared to controls**
[57]	Breast cancer patients w/stages I and II diagnoses *n*=50	Radiation therapy	Home-based walking 4–5×/week for 20–30 min @ self-paced % heart rate maximum for 6 weeks	↓ Fatigue ↑ Walking ability in exercisers compared to controls
[24]	Mixed solid tumor or lymphoma survivors *n*=62	Post high-dose chemotherapy and autologous peripheral blood stem cell transplant	Supervised bed ergometer cycling daily for 15 min @ 50% heart rate reserve during hospitalization (~ 2 days/week)	↓ **Fatigue in exercisers** ↑ **Fatigue** ↓ **Vigor in controls**
[58]	Breast cancer patients w/stages I–III diagnoses *n*=52	Chemotherapy and radiation therapy	Home-based walking 5×/week for 30 min @ self-paced % heart rate maximum for 6 months	↑ **Functional capacity** ↓ **Fatigue in high vs. low exercisers**
[79]	Breast cancer stages I–II *n*=123	Chemotherapy, radiation therapy and hormone therapy	Self-directed exercise (5×/week for 26 weeks), supervised exercise (3×/week for 26 weeks plus 2 days of home exercise) and usual care (no exercise program but advise on general wellness and exercise)	↑ **Aerobic capacity among the participants in both the self-directed and supervised exercise groups compared to the usual care participants**
[13]	Breast and colon cancer survivors *n*=21	Post surgery, chemotherapy and/or radiation therapy	Low-intensity aerobic exercise (25%–35% heart rate reserve), moderate-intensity aerobic exercise (40%–50% heart rate reserve) and usual care (no exercise) 3×/week for 10 weeks	↑ **Aerobic capacity** ↓ **Fatigue in the two exercise groups compared to the usual care group;** no significant differences between the two different exercise intensity conditions
[21]	Post-menopausal breast cancer survivors *n*=53	Post-surgery, chemotherapy and/or radiation therapy with no hormone therapy	Individually tailored and supervised upright cycle ergometer training 3×/week for 15 weeks versus no-exercise control	↑ **Peak oxygen consumption, peak power output, oxygen consumption at the ventilatory equivalent for oxygen, carbon dioxide and power output at the ventilatory equivalent for oxygen among exercisers compared to controls**
[20]	Cancer survivors *n*=108	Post-surgery, chemotherapy and/or radiation therapy	Group psychotherapy plus exercise versus group psychotherapy alone for 10 weeks	↓ **Fatigue** ↑ Aerobic capacity **among group psychotherapy plus exercise compared to psychotherapy alone**
[72]	Breast cancer *n*=24	Post-treatment within 5 years	Home-based walking at a moderate intensity (55%–65% maximum heart rate) 2–5×/week for 10–30 min for 12 weeks	↓ **Fatigue in exercisers compared to controls**

Table 10.4. Continued

Reference	Sample	Treatment	Type of exercise	Results[a]
[23]	Women and men with mixed solid tumors *n*=72	Post-surgery for a solid tumor, post-chemotherapy and post-radiation No current treatment	Supervised stationary cycling 5×/week for 30 min @ 80% MHR using interval training at 50 RPM	↓ **Fatigue** ↓ Dyspnea among exercisers
[35]	Metastatic breast cancer *n*=38	Chemotherapy	Home-based seated exercise program 3×/week using a videotape	↓ **Fatigue in exercisers compared to controls** ↓ **Physical function in exercisers compared to controls**
[84]	Mixed cancers (lymphomas, breast, gynecologic, testicular *n*=111	Post-chemotherapy	Supervised home-based flexibility training combined with two exercise sessions per week for 30 min minimum for 14 weeks (mode of activity was patient choice) at a slightly strenuous intensity (13–15 on rating of perceived exertion scale; anchored from 6–20) compared to controls who received no exercise intervention but were told to continue with the normal amount of physical activity they would usually do	↑ **Aerobic capacity among the exercisers compared to the non-exercisers** with no significant differences between groups in fatigue
[14]	Breast cancer *n*=22	Chemotherapy or radiation therapy	Supervised mixed aerobic and resistance exercise 2×/week for 10–20 min at 60%–75% of maximum heart rate for 12 weeks	↓ **Fatigue in exercisers compared to controls**
[59]	Breast cancer stages 0–III *n*=119	Chemotherapy or radiation therapy	Home-based walking for 15–30 min 5–6×/week at 50%–70% maximum heart rate for 6 weeks for radiation patients and 3–6 months for chemotherapy patients versus usual care controls	↓ **Fatigue in fully compliant exercisers compared to controls**
[66]	Breast cancer *n*=21	Post-treatment 2–24 months	Supervised tai chi chuan for 60 min at moderate intensity 3×/week for 12 weeks	↑ **Walking ability in exercisers compared to controls**

[a] Results in bold print indicate a $p \leq .05$.

consistently effective at either reducing or halting the progression of CRF and improving cardiorespiratory function in Hodgkin's and BC patients during and after treatment. Campbell and colleagues [14] were the only group to use both aerobic and anaerobic exercise (resistance exercise). Although the overall results were positive, the study sample was small and, at this time, it is not possible to differentiate the effects of aerobic and anaerobic exercise in this study. Therefore, the evidence supporting the safety and efficacy of anaerobic exercise for improving CRF or cardiovascular function is nonexistent among HL and BC survivors.

Recent meta-analyses by Schmitz and colleagues [78], and Jacobsen and colleagues [45] suggests that the evidence for exercise as an effective therapy for

managing CRF is consistently positive. The effect sizes (ES) are small [e.g., weighted mean ES = 0.13, 95% confidence interval (CI): −0.06 to 0.33 during treatment; weighted mean ES = 0.16, 95% CI: −0.23 to 0.54 post treatment]. This indicates the need for developing more targeted and effective exercise interventions.

Although the extant exercise and cancer control literature provides consistent support for the efficacy of exercise interventions in managing CRF during and after treatment, the investigations must be considered to be preliminary. The studies have small sample sizes. The studies as a body of research lack consistency in the type and amounts of exercise utilized. These limitations make it impossible to apply the results and effectively develop standards of care regarding tailored exercise prescriptions to safely meet the needs of these survivors. Additionally, the measures used to assess CRF and cardiovascular function as well as the type of control groups used are inconsistent. This makes interpreting ìndings and drawing conclusions across studies difficult. Appropriate statistical and follow-up analyses were not always used (e.g., intent-to-treat analyses in randomized controlled trials) which makes comparisons based on regimen and methods of exercise intervention difficult to ascertain.

Despite these limitations, this growing body of research provides consistent preliminary support for the safety of exercise interventions for HL and BC survivors across the entire cancer care continuum. This growing body of literature also suggests that early intervention with exercise during radiation may effectively prevent or reduce acute, chronic and late occurring CRF as well as improve cardiorespiratory function and prevent or delay the onset of cardiovascular disease as a consequence of radiation therapy to the chest among Hodgkin's and BC survivors. However, caution is warranted when considering an exercise prescription among this population of cancer survivors because there are many cardiovascular complications and a comprehensive subclinical assessment of cardiovascular status in long-term cancer survivors with different manifestations has not been performed. Patients should be carefully monitored by physicians and exercise physiologists since some are at risk for heart-rate-related hemodynamic instability, conduction problems, ischemia, and other complications.

10.5 Conclusion

In conclusion, radiation therapy to the chest is associated with premature heart disease among Hodgkin's and BC survivors. Heart disease is associated with reduced quality life years and increased mortality. Exercise is a non-invasive and non-pharmacological intervention that shows promise in preventing or delaying the onset of premature heart disease in survivors of HL or BC. Systematic and thorough evaluation of CRF in this population might identify patients with covert or apparently compensated cardiac disease which is causative for this condition. Most significantly, it may identify the survivors who will bene ìt the most from early implementation of therapeutic exercise interventions targeting the reduction of CRF and improvement of cardiorespiratory function. Ultimately, exercise might prevent or delay the onset of heart disease after radiation therapy in this vulnerable population.

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Biodetection and Biointervention:

Cytokine Pathways as a Rationale for Anticytokine Interventions Post-Radiation

11

Mitchell S. Anscher, Paul R. Graves, Ross Mikkelsen, and Zeljko Vujaskovic

CONTENTS

11.1 Introduction

Normal tissue tolerance limits the dose of radiation that can be used to treat most malignancies [78]. Despite the fact that many cancers present as large masses that require high doses of radiation to control, physicians are forced to limit the dose and volume irradiated in order to prevent the development of potentially serious, life threatening or fatal complications. Consequently, cure rates for some of these malignancies, such as lung cancers or malignant brain tumors, are distressingly low. Recent advances in our understanding of the molecular events underlying the pathogenesis of radiation-induced normal tissue injury has opened up the possibility of biologically-based interventions to prevent, mitigate or treat these complications. This work has also stimulated efforts to develop strategies to stratify patients according to risk of injury as a means to individualize therapy and improve the therapeutic ratio.

11.2 Molecular Mechanisms of Radiation Injury

It has been known for decades that the biologic response to ionizing radiation begins immediately after the first exposure with the generation of reactive oxygen/reactive nitrogen species (ROS/RNS) [73, 77, 90]. More recently, researchers have described how these immediate biochemical events rapidly trigger a series of genetic and molecular phenomena leading to clinically and histologically recognizable injury [11, 22, 25, 47, 53, 55, 62, 66–69, 96, 108]. This response to radiation is dynamic and involves a number of mediators of inflammation and fibrosis produced by macrophages, epithelial cells, and

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fibroblasts. These events appear to be sustained for months to years beyond the completion of therapy [40]; however, the mechanisms responsible for maintaining the injured phenotype, until recently, have remained unknown [74, 108].

The molecular processes responsible for radiation induced normal tissue injury have been, perhaps, most extensively studied in the lung (Fig. 11.1). As previously stated, the initial tissue damage from radiation is generated by direct action of ROS on DNA. This interaction causes tissue injury including endothelial cell damage with an increase in permeability, edema and fibrin accumulation in the extracellular matrix. Endothelial cell damage plays an important role in this process, and recent evidence suggests that the capillary endothelial cell may be the first cellular element to be damaged by RT [85]. This is followed by an in lammatory response with macrophage accumulation and activation. Macrophages, a rich source of proinflammatory and

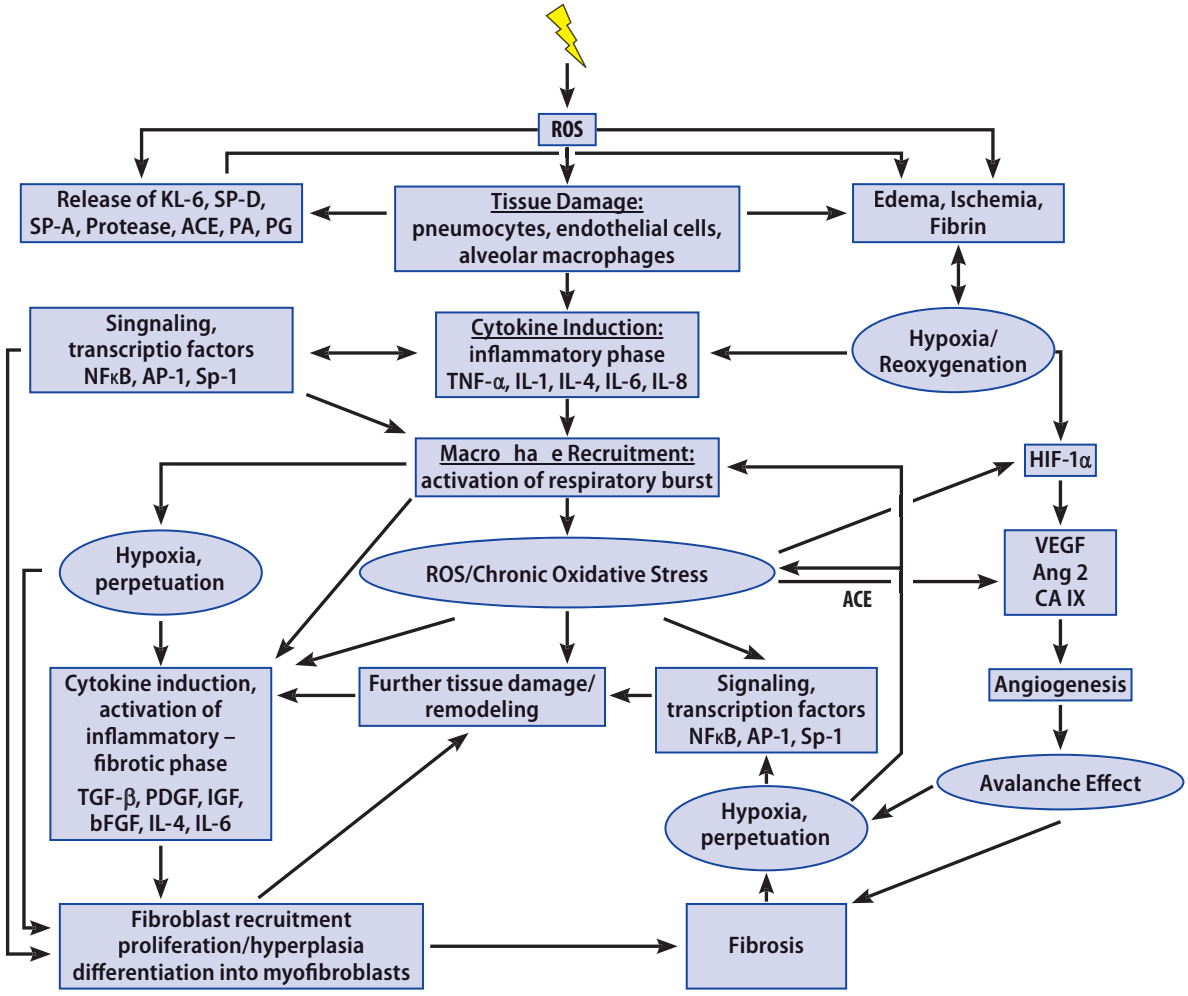

Fig. 11.1. Simplified model of processes involved in the pathogenesis of radiation-induced lung injury. As noted in the diagram, each event has the potential to influence several other processes. Exposure to ionizing radiation initiates a cascade of cytokines and growth factors. Proinflammatory cytokines promote an influx of macrophages and inflammatory cells, which are stimulated to produce ROS, proinflammatory and pro ìbrotic cytokines. ROS serve as redox regulators of transcription factors, which further stimulate induction and activation of cytokines and growth factors. In addition, vascular changes, as well as an increase in oxygen consumption by activated macrophages, contribute to the development and perpetuation of hypoxia and chronic oxidative stress, leading to the non-healing tissue response of chronic radiation injury. *ACE*, angiotensin converting enzyme; *PA*, plasminogen activator; *PG*, prostaglandins; *Ang2*, angiotensin II; *CAIX*, carbonic anhydrase IX; *HI ˙*, hypoxia inducible factor; *PDGF*, platelet derived growth factor; *IGF*, insulin-like growth factors; *bFGF*, basic fibroblast growth factor. (Reproduced with permission from [37])

profibrotic cytokines, along with other inflammatory cells are recruited to an area of injury or evolving in ìammation. The majority of macrophages in lung are derived from circulating monocytes that enter the lung in response to inflammation. Both vascular changes as well as an increase in oxygen consumption (due to macrophage activation) contribute to the development of hypoxia [38]. Hypoxia further stimulates production of ROS, proin ıammatory, profibrogenic and proangiogenic cytokines. This perpetuates tissue damage leading to fibrosis via TGFβ1 production and stimulates angiogenesis via vascular endothelial growth factor (VEGF) production. In an attempt to respond to the proliferative stimulus of VEGF, endothelial cells die due to previously accumulated radiation damage. Hypoxia therefore continuously perpetuates a non-healing tissue response leading consequently to chronic radiation injury [6, 108].

Many of these molecular mediators of normal tissue injury are proteins, which can be measured both in tissue and blood. The ability to quantify the expression of these proteins, in the normal and diseased state, led to attempts to use them as predictors of risk of normal tissue injury after radiation therapy [4, 5, 26, 30, 44, 83, 106]. Until recently, each protein had to be quantified individually using methods such as antibody-based enzyme-linked immunosorbent assays (ELISA) or bioluminescence assays, which are laborious and time consuming [72]. Advances in bioassay technology now permit researchers to quantify multiple proteins simultaneously from the same sample in a rapid and reproducible manner [64]. This technology will greatly enhance the ability to construct protein expression profiles for individual patients and determine whether these patterns of protein expression can improve our ability to predict risk of injury from radiation therapy [51]. Along these lines, blood and tissue banks stocked with samples from patients irradiated for various malignancies will become invaluable resources for normal tissue injury research.

11.3 The Importance of Transforming Growth Factor β in Radiation-Induced Injury

The most widely studied of the potential mediators of normal tissue injury after radiation therapy is transforming growth factor-β1 (TGFβ1). TGFβ has multiple functions that are important in the development of excess fibrous tissue, one of the hallmarks of late radiation injury. TGFβ is chemoattractant for fibroblasts and also promotes differentiation of immature fibroblasts into myofibroblasts, which leads to increased production of collagen and extracellular matrix [92, 93]. TGFβ also decreases production of matrix-specific proteases and increases production of protease inhibitors, resulting in decrease collagen degradation, with a net result of increased fibrous tissue formation [45, 75]. In addition to being autocrine stimulated, TGFβ expression is also stimulated by hypoxia, which further promotes collagen formation [50, 79].

Recent evidence, indeed, confirms that TGFβ1 is important in the pathogenesis of radiation induced normal tissue injury. Rubin et al. [95] reported that alveolar macrophages obtained from bronchial lavage specimens from irradiated rabbits demonstrated increased production and release of TGFβ1 as compared to macrophages from normal lungs. These authors suggested that the fibroblast proliferation and extracellular matrix production found after irradiation are controlled by growth factors that are released from parenchymal cells following radiation exposure. Anscher et al. [3] demonstrated that TGFβ1 expression increased in a dose-dependent manner in the liver of rats following irradiation and that this increase in TGFβ1 expression correlated with the extent of connective tissue production. Barcellos-Hoff [12, 13] has shown that free radicals produced following exposure to ionizing radiation can directly activate TGFβ1. Thus, radiation therapy can both increase local expression and activation of TGFβ1, resulting in increased ìbrosis formation in irradiated tissues. As further evidence to support the role of TGFβ in radiation injury, mice lacking Smad 3 (part of the TGFβ signal transduction pathway) have been shown to be resistant to radiation-induced fibrosis [36], suggesting that targeting the TGFβ pathway might be a useful strategy to prevent radiation injury (see below).

Moreover, the local activation of TGFβ1 in tissues may be an important component in sustaining the process of abnormal wound healing long after the exposure to radiation has ended. For example, active TGFβ1 both recruits and activates macrophages to secrete inflammatory and ìbrogenic cytokines, including TGFβ1 itself [9, 91]. This autoinduction of TGFβ1 is important in maintaining levels of TGFβ1 in wound healing. Following radia-

tion, however, this process contributes to overproduction of collagen and inhibition of epithelial cell proliferation, increased local oxygen consumption by activated macrophages, and decrease oxygen delivery due to microvasculature injury creating an hypoxic environment [74] which further perpetuates normal tissue injury. In addition, sustained overproduction of TGFβ may contribute not only to chronic ìbrosis, but also reduce the effectiveness of cancer therapies [20], and possibly contribute to the development of radiation-induced malignancy (see below).

11.4 Using Plasma TGFβ Levels to Predict Injury Risk

Plasma TGFβ1 levels recently has been used to try and identify patients at risk for the development of normal tissue injury after exposure to chemotherapy and/or radiotherapy. In patients who develop radiation induced lung injury, Fu et al. [40] found sustained elevations in plasma TGFβ1 level for as long as 2 years after treatment. In contrast, patients who did not develop symptomatic lung injury did not exhibit sustained elevations in circulating plasma TGFβ1. In animal experiments, long term overexpression and activation of TGFβ1 has been demonstrated in tissue as well [62, 109, 110]. Thus, elevations in plasma TGFβ1 months after radiation exposure appear to reflect the presence of significantly dysregulated wound healing in the irradiated tissues. In contrast, the absence of sustained elevations of circulating TGFβ1 levels appear to reflect a more normal wound healing process. Thus, sustained elevations of plasma TGFβ1 following radiation exposure may be a useful means to identify patients at risk for late radiation-induced injury, including radiation-induced malignancy. Other investigators, however, have not found plasma TGFβ to be a reliable identi ìer of patients at increased risk for normal tissue injury after cancer therapy [15, 30, 83]. These discrepancies may be due to a number of factors, including differences in techniques used to measure TGFβ, differences in patient populations under study, and the fact that these series contain relatively small numbers of patients with treatment-related injury, thus the power to detect a difference between groups is not large [2].

11.5 The Role of Other Cytokines in Radiation-Induced Injury

A growing body of evidence points toward a complex web of protein interactions as being important in the pathogenesis of radiation injury (see Table 11.1 and Fig. 11.1). For example, Huang et al. [57] have found that IL-7, a cytokine that enhances T cell function and IFN-γ production, inhibits both TGFβ production and signaling, and protects against the development of bleomycin-induced pulmonary fibrosis, a model very similar to radiation injury. Fedorocko et al. [35] showed that radiation exposure could increase cytokine production both directly (IL-6, TNF-α) and indirectly (GM-CSF), either by locally acting paracrine or endocrine effects or as a result of systemic effects of early proinflammatory mediators such as IL-1 or TNF-α. There is no doubt that protein production is a dynamic process, which will change as a result of cancer treatment. Hong et al. [56] have documented temporal and spatial changes in the expression of proinflammatory cytokines (TNF-α, IL-1α and IL-1β) following thoracic irradiation in mice. Given the impact that radiation has on the expression of these, and other, proteins in tissue, and that these changes in tissue protein expression might be reflected in changes in plasma protein levels, it is reasonable to postulate that it may be possible to quantify an individual patient's in ıammatory status by measuring candidate protein levels in the blood.

11.6 Using Other Markers to Predict Radiation-Induced Injury

In addition to TGFβ, several other proteins have been studied in humans to evaluate their potential as biomarkers for radiation-induced injury. Most of this work has been carried out in the lung. Of these, the most promising include interleukins (IL) 1α, IL-6, IL-8, IL-10, Krebs von den Lungen protein (KL-6, which is expressed mainly on type II pneumocytes and bronchiolar epithelial cells), soluble intracellular adhesion molecule (sICAM)-1, and surfactant proteins A and D [26, 27, 43, 44, 49, 52, 58, 71, 76, 97, 102]. Of these, KL-6 is the most

extensively studied, and has been most consistently correlated with the risk of radiation-induced lung injury [37]. As with TGFβ, more prospective studies with larger patient numbers will be required to con ìrm its value as a predictive marker for lung injury.

11.7 Chronic Inflammation as a Mediator of Radiation Induced-Malignancy

Epidemiologic evidence has also suggested a correlation between chronic inflammation, such as that seen after exposure to radiation therapy, and the development of malignancy at the inflamed site. The underlying mechanism involves recruitment of inflammatory cells, as well as the expression of multiple mediators of inflammation, including cytokines, chemokines and enzymes. Proinflammatory cytokines, such as the interleukins and tumor necrosis factor α, cause an influx of in ìammatory cells and fibroblasts into the microenvironment [63, 94]. These cells, primarily macrophages [95, 107], become stimulated to produce ROS/RNS and additional proin ìammatory and pro ìbrotic cytokines [37] (Fig. 11.1). ROS/RNS functionally regulate transcription factors that also influence expression and activation of cytokines and growth factors [77, 101, 114, 115], and ROS/RNS also are important in intracellular signaling [14, 77, 98]. Recent evidence also suggests the importance of ROS/RNS generated by macrophages and tumor cells in the processes of initiation and progression of malignancy [41, 54, 88, 113]. Thus, it is likely that many, if not all, of the proteins involved in the development of radiation-induced normal tissue in ìammation and fibrosis might also be involved in the generation of radiation-induced malignancy.

In addition to creating a chronic inflammatory state, the microenvironmental stress resulting from hypoxia may also result in genomic instability [19, 23]. The ability of mammalian cells to detect and repair DNA damage has a critical impact on tumor response to ionizing radiation, but may also be important in normal tissue response. Mammalian cells have evolved a number of repair systems to deal with various types of DNA damage, and to maintain genomic integrity. The most harmful of all radiation-induced DNA damage is the double strand break (DSB). Errors in DSB repair can lead to deletions, insertions, chromosomal translocations and genomic instability that could lead to the development of malignancy [33, 103]. Specific surveillance proteins in ìuence the balance between cell cycle arrest, DNA repair and apoptosis [17, 29]. For example, ATM is an important cellular surveillance protein, and recent evidence suggests that TGFβ is important for a fully functioning ATM response [70, 116], the loss of which might contribute to the development of malignancy. DNA damage repair mechanisms involving ATM are extremely sensitive to ROS/RNS generated in response to hypoxia/reperfusion [19, 48], and inhibition of ATM results in increased cellular levels of ROS/RNS [16, 59, 60]. Thus, chronic overproduction of ROS/RNS, as demonstrated to occur after exposure to radiation, may lead to interference with DNA damage repair, inhibition of apoptosis and regulation of oncogenes or tumor suppressor genes in a manner that may predispose people to the development of malignancy [10].

11.8 Candidate Proteins for Predicting Radiation Injury

While many proteins have been implicated in the pathogenesis of radiation-induced injury, few have been evaluated as possible predictors of predisposition to such injury. At the present time, not every protein implicated in in ìammation, wound healing, fibrogenesis or radiation response can be detected in the blood, owing to the lack of availability of reliable antibodies to these proteins. Thus, it is not yet possible to screen for alterations in expression of every potential candidate protein. In addition, multiple proteins and signaling pathways are involved in these processes, and reliable antibodies are not available to target every individual protein involved in each pathway. Nevertheless, the list of proteins below represent components of all of the major mechanisms and pathways currently thought to be involved in the response of cells to radiation [99, 104]. This approach is likely to detect a pro ìle of protein expression associated with an increased risk of radiation injury, if in fact one exists. The role of each of these candidate proteins, relevant to radiation injury, is summarized in Table 11.1.

Table 11.1. Summary of the function of candidate proteins for profiling

Protein	Function
IL-1β	In ìammation, growth factor expression
IL-5	Proin ìammatory
IL-6	Proin ìammatory, decrease apoptosis of activated lung fibroblasts
IL-7	Proin ìammatory
IL-8	Angiogenesis, leukocyte chemotaxis, collagen synthesis
IL-10	Anti-in ìammatory (decrease TNFα production, decrease upregulation of endothelial cell adhesion molecules)
IL-13	Proin ìammatory
MCP-1	Inflammation, chemoattraction of monocytes
MIP-1alpha	Antiproliferative
PDGF BB	Angiogenesis, recruit smooth muscle cells
VEGF	Angiogenesis and increased vascular permeability
EGF	Epithelial cell motility, mitogenicity and differentiation
EGFR	Receptor for EGF, initial component of EGF signaling pathway
NFkappaB	Pleiotropic gene transcription responses
HIF-1	Transcription factor for genes regulating angiogenesis
TGF-alpha	Cell motility and proliferation
FGF 2	Angiogenesis and fibroblast proliferation
MMP-1	Degradation of collagen and extracellular matrix proteins
MMP-2	Matrix remodeling, growth factor release
MMP-3	Matrix remodeling, growth factor release
MMP-13	Matrix remodeling, growth factor release
SMAD 2/3	Signal transduction in the TGFß pathway
IGF-1R	Binding of IGF-1 (reepithialization and granulation tissue formation)
TNF-alpha	Growth factor expression, inflammation, matrix production and remodeling
TGFβ	Pro ìbrotic, immunosuppression, angiogenesis, metastasis
Beta-catenin	Epithelial-mesenchymal transition
Nitric oxide synthases	In ìammation
Superoxide dismutases	Endogenous anti-in ìammatory regulator

11.9 Strategies and Potential Targets for Intervention

There are 3 primary approaches to intervention in the injury process, depending upon the timing of intervention relative to radiation exposure, and whether or not injury has developed [81]. These approaches are: protection or prophylaxis, mitigation and treatment. Protection refers to treatments given before and/or during radiation. This is the most common strategy utilized in the clinic today and is illustrated by the use of the free radical scavenger amifostine in the prevention of injury following radiation to the head and neck [24]. Mitigation refers to therapies started after radiation exposure, but before overt injury is expressed, as exempli ìed by the use of angiotensin converting enzyme inhibi-

tors to prevent renal injury [82]. Treatment refers to interventions begun after overt injury develops, an example of which would be the use of vitamin and pentoxifylline to treat established radiation soft tissue fibrosis [32].

As we learn more about the specific molecular pathways involved in the process of radiation injury (Figs. 11.1–11.3), more targeted therapies are being studied as approaches to the prevention of radiation injury. Given the importance of the TGFβ pathway in the pathogenesis of radiation injury, several investigators have demonstrated the efficacy of blocking TGFβ in preventing radiation injury in animals [7, 36, 87]. These agents, to date, have not been utilized in humans for this purpose. TGFβ has also been demonstrated to work through Smad independent pathways [18], and targeting one or more of these pathways may also prove to be an effective approach to prevention of radiation induced injury. For example, one of these alternative pathways involves signaling via PI3-kinase and cAbl [68], and the use of imatinib, which targets cAbl, has been shown to reduce the severity of bleomycin-induced lung injury [28]. In addition to TGFβ, other pathways have been demonstrated to be viable targets to inhibit the development of radiation-induced in-

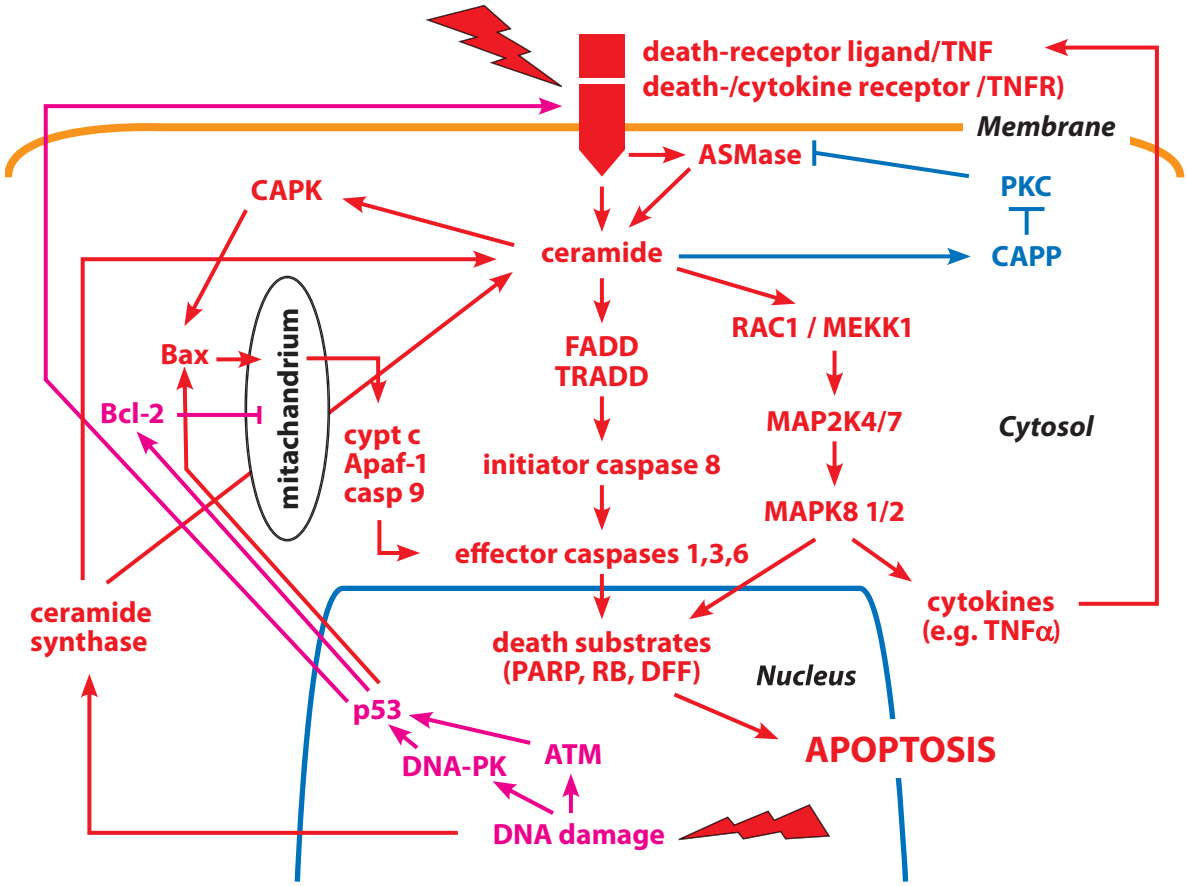

Fig. 11.2. DNA damage-independent and -dependent pathways of endothelial cell apoptosis. The primary apoptotic response to ionizing radiation in the endothelial cells is DNA damage independent and is mediated through radiation-induced activation of acid sphingomyelinase (*ASMase*) and the generation of ceramide. Ceramide mediates the activation of the MAPK8 pathway, the mitochondrial pathway or the death receptor pathway. The second source of ceramide occurs via production of DNA double-strand breaks and activation of ceramide synthase. *CAPK*, ceramide-activated protein kinase; *PKC*, protein kinase C; *TN* , tumor necrosis factor; *BAX*, bcl-2 associated protein X; *BAD*, bcl-2 antagonist of cell death; *cyt*, cytochrome; *casp*, caspase; *PARP*, poly(adenosine-5'-diphosphate-ribose) polymerase; *RB*, retinoblastoma protein; *FADD*, Fas-associated death domain; *TRADD*, TNF-receptor associated death domain; *RAC*, receptor for activated C-kinase; *MEK* , MAP/Erk kinase kinase; *MAPK*, mitogen activated protein kinase; *DFF*, DNA fragmentation factor; *Apaf*, apoptotic protease activating factor; *ATM*, ataxia-telangiectasis mutated. (Reproduced with permission from [93])

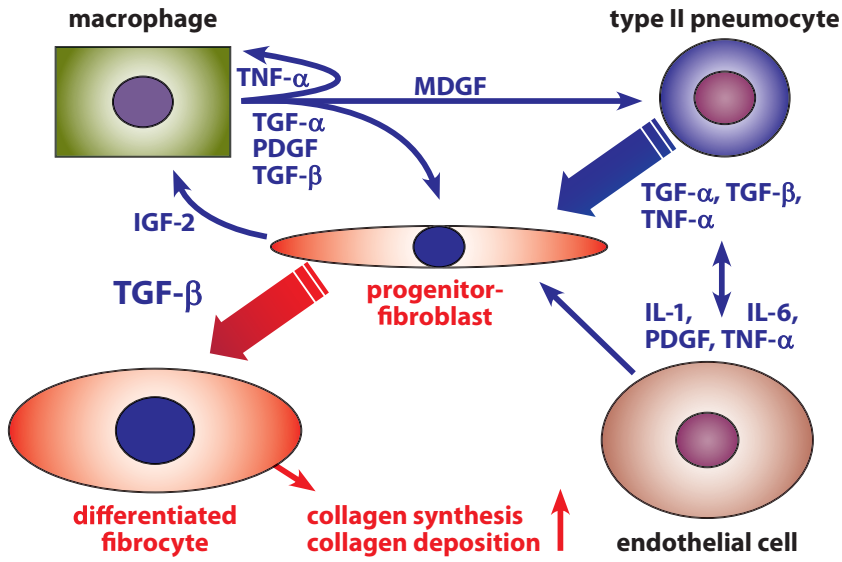

Fig. 11.3. The interaction between multiple cells, mediated via cytokines, in the process of connective tissue remodeling. *IL*, interleukin; *TGF*, transforming growth factor; *PDGF*, platelet derived growth factor; *IGF*, insulin-like growth factor; *TN* , tumor necrosis factor; *MDG* , macrophage derived growth factor. (Reproduced with permission from [93])

jury [1, 8, 31, 34, 46, 65, 109]. Given the redundancy and crosstalk between these multiple pathways, it is likely that strategies to prevent radiation injury may require agents that target multiple pathways simultaneously, or combinations of multiple agents with more specific targets.

An example of a class of drugs, which target multiple cellular pathways, and might prove beneficial in the struggle to prevent radiation induced normal tissue injury, are the statins. As noted above, vascular damage is an important component in the pathogenesis of radiation induced injury. Vascular damage is important in the phenotype of RT-induced rectal injury, where telangiectatic vessels are often responsible for the bleeding characteristic of this condition. The cholesterol lowering agents HMG coA reductase inhibitors (statins) have been demonstrated to reduce the risk of myocardial infarction, in part, through their vascular protective effects, which are not dependent on changes in serum cholesterol levels. In vitro, statins have been shown to protect human endothelial cells from ionizing radiation [21, 42, 84]. Multiple mechanisms appear to be involved, including attenuation of extracellular stress responses [80, 89], down-regulation of chemokines and chemokine receptors [111], and by exerting anti-in lammatory and anti-thrombotic effects [21, 86, 100, 105] on these cells. Of major interest recently has been the demonstration that statins induce the synthesis of nitric oxide synthase (NOS) in endothelial cells and may represent one mechanism for the beneficial effects of statins in chronic in lammation [61]. Elevated NOS activity is potentially a 2-edged sword and a number of other factors modulating nitric oxide activity may contribute to its beneficial, and also harmful, effect [39].

In vivo, lovastatin has been shown to protect mice from the effects of whole lung irradiation for up to 24 weeks [112]. Mice receiving lovastatin demonstrated improved survival, a decreased inflammatory response in the lung and reduced fibrosis. Thus statins may have the potential to protect against RT-induced late effects, and studies testing different statins as radioprotectors are currently underway.

Much work remains to be done, however, particularly in the areas of mitigation and treatment [109], and additional human studies will be required to identify the most effective agents and approaches to this complex problem.

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Late Toxicity from Hypofractionated Stereotactic Body Radiation

12

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CONTENTS

12.1 Introduction

Stereotactic body radiation therapy (SBRT) utilizes a three dimensional coordinate system to achieve more reproducible patient set-up [1, 2]. With SBRT, the margins for set-up uncertainty can be reduced, allowing greater volume sparing of the surrounding normal tissues. Since SBRT yields a reduced volume of normal tissue exposure, SBRT has been used to increase the fractional dose of radiation (hypofractionation) in an attempt to intensify the dose delivery without incrementally increasing the risk of normal tissue damage. This is becoming an important approach to treating discrete tumors, and has yielded impressive local control of treated tumors without signi icant toxicity. The benefit of SBRT is to achieve improved local control compared to conventional radiation, via improved target localization and more intense doles delivery, without the added toxicity. Arguably, SBRT can achieve similar or even improved outcome over surgical resection. One advantage that SBRT has over a limited resection (i.e. one that does not achieve wide margins) is that the penumbra dose around the target treats microscopic disease [3].

With three-dimensional planning, detailed dose-volume information can be obtained for critical organs at risk as well as gross and microscopic targets, providing more tools for the clinician to assess the acceptability of a plan. Ideally, well characterized dose-volume constraints should be used, though certainly more clinical data is needed [4], particularly with hypofractionated regimens.

While some authors assert that SBRT implies the use of hypofractionation in extracranial sites [1, 2, 5, 6], an alternative view is that SBRT is merely a tool, employing a three-dimensional coordinate system to more accurately target radiation delivery, which may allow the safe use of hypofractionation in certainly situations. Arguably, SBRT can be used with standard fractionation schemes with cranial or extracranial tumors. One example would be the treatment of benign tumors abutting the optic chiasm and nerves, where it is well known that hypofractionation increases the risk of toxicity [7]. With standard fractionation SBRT, the risk of late toxicity can be reduced solely by virtue of better patient set-up and lowering the volume of normal tissue exposure. Therefore when choosing the dose and fractionation with SBRT, several considerations are important, primarily: (1) the predicted risks of late toxicity and

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(2) the predicted efficacy of the radiation; but also: (3) patient convenience with respect to the number of treatments delivered, (4) the cost of treatment planning and delivery and (5) the number of time slots used on a given linear-accelerator.

In recent years, there has been a growing clinical experience treating patients with primary and metastatic tumors with hypofractionated SBRT. These patients certainly bene ït from reduced normal tissue exposure, but the effect of a greater fraction size, albeit to a smaller volume of normal tissue, is not well understood. As clinical experience with hypofractionated SBRT matures, we will obtain much needed patient outcome data. This review will focus on the radiobiology of hypofractionation and normal tissue tolerance to hypofractionated radiation, as well as the patient outcome in select trials using hypofractionated SBRT. Generally, SBRT implies the delivery of multiple (>1) fractions of radiation. When radiation is delivered in one fraction, it is termed stereotactic body radiosurgery (SBRS) or in the case of the treatment of cranial lesions, stereotactic radiosurgery (SRS). This review will apply to both SBRT and SBRS, but for simplicity we will only use "SBRT" in the text below (unless specifically referencing a study employing SBRS).

12.2 Technical Aspects of SBRT

SBRT requires a three-dimensional coordinate system for accurate and reproducible patient set-up. A system to detect and process this three-dimensional array is also needed. SBRT can be achieved through the use of internal fiducials or markers, external markers and/or image guidance. Systems which gate the delivery of radiation, such that radiation is only administered when the patient position falls within a predetermined set of positioning parameters, can also be used. Immobilization techniques such as relaxed patient breath-hold can allow further reduction in set-up uncertainty [8].

With SBRT, the clinician can choose the dose fraction, total dose as well as the isodose line prescribed to the periphery of the target. As a result, normal tissue adjoining the target can receive a lower dose than the target isocenter, simply by virtue of prescribing to a lower isodose line. Because the planning and delivery of SBRT often uses multiple non-coplanar fields and/or arcing ïelds, the dose gradient is steeper than with conventional radiation, though the low dose region can encompass a larger volume and be irregularly shaped.

Intensity modulated radiation therapy (IMRT) can used to further customize the dose delivery, allowing for greater relative sparing of normal tissues. With IMRT, the radiation is modulated spatially and/or temporally with inverse planning techniques [9]. The goal of IMRT is to shape the dose distribution so as to minimize the dose to normal tissues and/or escalate the dose delivery to the target. The physician decides upon dose constraints to the target and avoidance objects, and the optimal inverse plan yields a dose-volume distribution that best achieves these pre-determined dose constraints. Generally, with IMRT, there is an increase in monitor unit delivery, which corresponds to a greater integral dose-volume distribution. There has been much concern about the possibility of IMRT resulting in a greater risk of second cancers resulting from the greater volume of low dose exposure [10–14]. Because SBRT and IMRT are separate tools (which can be used together), this will not be explored in greater detail in this review.

12.3 Radiobiology of Hypofractionated Radiation

For a given fraction of radiation, larger fraction sizes are associated with fewer surviving cells as described by the classic linear-quadratic model. In this model, the log of cell survival is affected by two components: one proportional to the dose (linear), and the other proportional to the square of the dose (quadratic) [15]. Generally, with larger fractions, the cell survival becomes more greatly impacted by the quadratic component, and thus the radiation yields incrementally greater cell kill. Classically, late responding tissues have a smaller α/β ratio. The linear component (single cell killing events, characterized by the α) and the quadratic component (cell killing from the accumulation of sublethal events, characterized by the β) are equal at lower doses (in the vicinity of 2–3 Gy), beyond which the quadratic component dominates. With early responding tissues, such as many tumors and acutely reacting normal tissue, the linear and quadratic

components are equivalent in the vicinity of 10 Gy. Thus, late-effects are more greatly impacted by fraction size than early effects. Consequently, there is a reasonable concern about late toxicity with the use of hypofractionated regimens, even when stereotactic techniques are used to reduce the volume of normal tissue exposure.

Yet another model, the multi-target model, incorporates the effects from single event killing (more shallow slope at lower doses) and multi-target killing (steeper slope at higher doses) with a threshold value in which these effects transition. It should be appreciated that the linear-quadratic and the multi-target models are simply models which describe experimental findings, and do not necessarily describe the mechanism of cell killing.

The decades-long rationale for using standard smaller fractionation (with 1.8–2 Gy fractions) with conventional radiation is to allow for dose escalation while minimizing the risk of late toxicity [5]. If new technologies allow for hypofractionated radiation delivery without the added risk of toxicity, there is great potential for improving local control [5].

With hypofractionated schemes, the linear-quadratic model appears to predict a greater tumor effect than clinically observed, due to the quadratic term over-estimating cell kill [5, 16–19]. Possible reasons for this include: (1) inadequate tumor coverage due to the tight margins used with SBRT, (2) not providing time for tumor cell reassortment and reoxygenation [15], and (3) inadequacy of the linear quadratic model with hypofractionation. Pre-clinical data suggests that the margins we use should be adequate to account for tumor motion and microscopic extent of infiltration. Timmerman's group [19] from the University Texas Southwestern have suggested using a hybrid linear-quadratic model and multi-target model to better predict tumor control. Perhaps such a model would also be potentially useful to predict late effects, though as of yet, there are no data to test this. Though the linear-quadratic model does have its shortcomings, it is useful to gain insight into predicting late effects, and help in determining fractionation schemes that optimize cell kill while minimize normal tissue toxicity [20]. Researchers from Australia and Canada have performed intricate modeling of hypofractionated stereotactic radiation, and conclude that the lower absolute doses used with hypofractionated regimens, combined with the normal tissue exposure to lower isodose lines with stereotactic delivery, results in a biologically sound rationale to use such approaches [17].

While classically, radiation induced cell kill, as described by the linear-quadratic model, results from mitotic death following unrepaired DNA double strand breakage, other mechanisms can lead to cell death, particularly at higher doses. These mechanisms can be incorporated into established models [21]. Apoptosis is well characterized for lymphoma cell lines [15], and may play a role in other solid tumors at higher fractional doses. Perhaps more relevant is endothelial apoptosis, resulting in microvascular disruption and resultant death of the cells supplied by that vasculature [22]. The mechanism of apoptosis is initiated by radiation induced damage to the plasma membrane, and the resultant apoptotic pathway mediated by the ceramide pathway [23–26]. This mechanism appears to be most signi ìcant above a ~ 8–10 Gy threshold [27]. Radiation may also stimulate endothelial cells to express cell adhesion molecules, stimulating an influx of immunologic cells [28, 29]. Higher fractional doses may also result in a more potent immunologic effect, resulting from radiation induced triggering of cancer cell antigen presentation, and the resulting immunologic response [30–34], and/or the recruitment of an immunologic response resulting from cytokine or other signal release [35–37]. While it is unknown the extent to which these other mechanisms account for tumor and normal tissue response, and to what extent larger fractions may impact them, a better understanding of these mechanisms may lead to more rational design of radiation treatments with respect to fractional dose delivery, total dose delivery and dose-volume constraints.

Regardless of the mechanism accounting for radiation damage, it is accepted that eradication of the stem cells in the treated volume contribute to the observed toxicity. Arguably, hypofractionated SBRT can yields greater advantage in parallel functioning tissues, in which the functional subunits are discrete entities as opposed to serial functioning tissues, in which the functional subunits are arranged in a linear or branching fashion [1, 5, 15]. With parallel functioning tissues, SBRT reduces the number of subunits destroyed by radiation by virtue of reducing the treatment volume. Small, and perhaps even larger volumes of parallel functioning organs can tolerate supratheshold radiation dosing, because either there is enough reserve in the undamaged portion of the organ (such as lung, liver or kidney) and/or there is a capacity to regenerate (such as liver). With serial functioning tissues, there is recruitment of stem cells from neighboring tissues (though argu-

ably much less so with tissue such as spinal cord), and thus these tissues may achieve greater radiation tolerance with more standard fractionation [5]. Certainly SBRT techniques can be employed with lower fractional doses if there is concern about an adjoining serial functioning tissue. While small volumes of serially functioning tissues, such as the spinal cord, can safely tolerate suprathreshold doses [38, 39], it is not known in humans what constitutes a safe volume, what effect the lower dose penumbra may have on toxicity from suprathreshold doses [40, 41], nor what anatomical regions of serially functioning organs can tolerate these higher doses [42].

12.4 University of Rochester Experience with Hypofractionated SBRT

The University of Rochester has been using SBRT to treat primary and metastatic lesions since 2001 using the Novalis linear accelerator with the ExacTrac positioning platform and BrainLAB planning software [8, 43–45]. Table 12.1. describes the allowed dose and dose fractionation. These dose-fractionation schedules were selected on the basis of an expected 85% tumor control probability, using the linear-quadratic model as predicted by Okunieff et al. [46]. While many fractionation schemes have been employed in our patients, with the total and fractional dose depending on the tumor location and volume of disease, the preferred dose-fractionation schedule has been 50 Gy in ten daily fractions. The planning target volume is generated with a minimum gross target volume expansion of 10 mm in the craniocaudal direction, and 7–10 mm in other directions, which allows for coverage of between two and three standard deviations of motion [8]. Treatment is prescribed such that the 80% isodose line covers the planning target volume. SBRT is delivered using conformal shaped arcs or multiple fixed shaped coplanar beams.

Table 12.1. Select dose fractionation schemes employed at the University of Rochester

Fractional dose (Gy)	Number of fractions	Total Dose (Gy)[a]
3	9	
4	12–14	6
5		
6		8
8		8

[a] All treatments were delivered on a daily (Monday through Friday) basis. The preferred schedule was 5 Gy × 10

Over 160 patients were enrolled on two prospective studies investigating SBRT in the treatment of limited metastatic disease [43–45]. Though many patients experience no acute symptoms, acute grade 1–2 fatigue and grade 1 dermatitis occurred in some patients. Acute grade ≥3 toxicity was generally not observed. There were 57 patients who survived >2 years, with follow-up ranging from 24–77 months in these patients. Late toxicity was not commonly seen. The thoracic and liver toxicity are outlined in the following sections. Table 12.2. describes the dose-volume constraints used in our study. These guidelines have proven to be safe, though arguably, there is the potential to exceed these constraints, since we did not test toxicity in a Phase I approach. Additionally, these were maximum allowed constraints on study, though most patients were well below these constraints. For example, although a V20 (volume of lung receiving >20 Gy) of 40% was allowed on study, the V20 in actuality ranged from 1%–34% [45]. Table 12.2 outlines the allowed dose and dose fractionation.

12.5 Review of Select Clinical Trials Using Hypofractionated SBRT: Late Toxicity

The current literature mostly focuses on the acute and dose limiting toxicity of SBRT. The CTCAE version 3 grading system does not differentiate between early and late toxicity, and many authors (ourselves included) report overall toxicity. Arguably, more mature data is needed to more fully comprehend the risks of late toxicity with SBRT.

Extracranial SBRT is used in the treatment of many organ sites, including lung, liver, pancreas, spine, kidney, adrenal, and musculoskeletal sites [2, 18, 47, 48].Tissues such as lung and liver, with parallel functional units, are well suited for stereotactic approaches, since SBRT allows for minimizing the number of functional units exposed to suprath-

Table 12.2. Dose-volume constraints used at the University of Rochester

Lung (in patients with chronic lung disease)	1000 ml of tumor free lung 70% of normal lung <1.7 Gy per fraction and <17 Gy total 800 cc of normal lung <1.7 Gy per fraction and <17 Gy total
Lung (in patients with healthy lungs)	1000 ml of tumor free lung 60% of normal lung <2.0 Gy per fraction and <20 Gy total
Liver (in patients with chronic liver disease)	1000 ml of tumor free lung 70% of normal liver <30 Gy
Liver (in patients with healthy liver)	1000 ml of tumor free lung 60% of normal liver <30 Gy
Kidney (in patients with two functioning kidneys)	<50% of normal kidneys to receive >16 Gy
Kidney (in patients with one functioning kidney)	<10% of functioning kidney to receive >10% of prescribed dose and >1.5 Gy per fraction
Small bowel	Total dose <50 Gy
Spinal cord	Dose to center <2 Gy per fraction and <45 Gy total Surface dose <54 Gy total
Esophagus	Attempt to keep maximum dose <4 Gy per fraction

reshold doses [1, 5, 15]. Arguably, the treatment of abdominal and pelvic tumors is inherently riskier with large fractions due to the proximity of bowel, a parallel organ.

12.5.1 Lung

The standard treatment Stage I non-small cell lung cancer remains resection. In patients with inoperable disease, or in those who refuse resection, radiation therapy is a curative option, with poorer outcome compared to resection, which is arguably a re lection of selection bias. In patients with limited lung metastases, resection is an option, particularly in the setting of isolated lung metastases. Radiofrequency ablation is another option. External radiation is the best option for those unwilling to undergo or medically unfit for more invasive procedures. Radiation can be used for tumors abutting large vessels and central structures, which are generally not safely treated with more invasive techniques.

Our group has previously reviewed the literature on the outcome and toxicity of SBRT for lung metastases [49]. Acute toxicity generally includes constitutional symptoms of fatigue and malaise, cough and mild dermatitis. Acute esophagitis is commonly seen in the treatment of central tumors [50]. Nearly all patients treated with SBRT to the lung experience acute radiographic pneumonitis, while grade ≥3 pneumonitis is uncommon. In a study from Hokkaido University in which 156 patients received SBRT for Stage I lung cancer, radiation pneumonitis requiring steroid use was not correlated with pretreatment pulmonary function tests, dose-volume metrics, total dose or fractional dose [51].

Late toxicity is reported to be relatively uncommon, with late grade ≥3 toxicity ranging from 0%–7%. Examples of reported grade ≥2 late toxicity include pneumonitis [52, 53], chronic cough [54, 55], pulmonary bleeding/hemoptysis [56, 57], pulmonary function decline [52, 57], apnea [57], pneumonia [57], pleural effusion [52, 53, 57], airway narrowing or obstruction [55, 58], tracheal necrosis [59], chest pain [52, 60], rib fracture [53, 61], and esophageal ulceration [56]. While pulmonary function decline is generally uncommon [60, 62], when its is appreciated, it is often transient or asymptomatic [63, 64]. SBRT may in fact improve the diffusion capacity in some patients [62].

The constraints to the normal lung as well as other normal structures, such as spinal cord, esophagus, liver, stomach and bowel are critical in minimizing the risk of acute and late toxicity [49]. With greater clinical experience, these constraints will become better formalized. Certainly the dose, fractionation, volume and location of the target account for much of the reported toxicity, particularly toxicity related to necrosis and hemorrhage. However, host and tu-

mor variables, which admittedly are largely uncharacterized, are also likely to be relevant, and arguably more relevant for pulmonary symptoms. In a study from Aarhus University, late dyspneic changes were not correlated to dose-volume parameters, and no consistent temporal variations were appreciated in their analysis [65]. Chronic obstructive pulmonary disease apparently accounted for symptomatic dyspnea rather than treatment effects.

The University of Indiana has the largest published North American experience treating Stage I lung cancer with SBRT. They enrolled patients with medically inoperable Stage I non-small cell lung cancer in a prospective Phase I study of three fractions of SBRT; the dose per fraction was escalated in 2 Gy increments, starting at 8 Gy per fraction [59, 63]. The spinal cord was limited to <6 Gy per fraction. In their initial report of 37 patients, six patients received steroid treatment for acute radiation pneumonitis. The authors noted a trend towards a decline in pulmonary function in the weeks that followed SBRT, which returned to baseline by 3–6 months after SBRT. Most ($n = 25$) patients developed radiographic evidence of fibrotic lung changes [63]. With a median follow-up of 15 months, no toxicity was seen beyond 6 weeks. In a follow-up report, with more patients accrued, three of five patients treated at the 24-Gy fraction dose experienced grade 3–4 toxicity, including pneumonitis ($n = 2$) and tracheal necrosis ($n = 1$) [59]. The timing of these toxicities was not addressed. In a subsequent Phase II study, in which 70 patients received 60–66 Gy in three fractions, eight were identified as having grade 3–4 toxicity, developing 1–25 months after SBRT, included decline in pulmonary function, pneumonia, pleural effusion, apnea, and skin reaction. Six patients were reported to have grade 5 (death) toxicity at 0.6–20 months (median 12) after SBRT (arguably in a frail population, with a limited life expectancy, in which SBRT may or may not have contributed to death) [57]. Four patients died from pneumonia, one from a pericardial effusion and another from massive hemoptysis. Univariate and multivariate analyses showed that tumor location (hilar/pericentral versus peripheral, $p = 0.004$) and tumor size (GTV >10 ml versus smaller tumors, $p = 0.017$) were strong predictors of grade 3–5 toxicity ($p = 0.004$).

The University of Rochester analyzed 49 patients who received SBRT to metastases in the thorax (either lung, hilum or mediastinum). Patients were required to have 1000 ml of tumor-free lung. For patients with chronic lung disease, 70% of the lung or 800 ml (whichever was larger), was kept under 1.7 Gy per fraction. For patients with otherwise healthy lungs, 60% of the lung was kept under 2.0 Gy per fraction. Most patients received ten fractions of 5 Gy. Grade 1, 2 and 3 toxicity (acute and late) was seen in 35%, 6% and 2%, respectively; toxicity was not well correlated with the V10 or V20 [45]. No grade 4–5 toxicity was seen. The observed grade 3 toxicity was a non-malignant pleural effusion successfully managed with pleurocentesis and sclerosis; most grade 2 toxicity was a self limited cough.

Following SBRT, characteristic radiographic changes reflect the acute and late changes in the lung parenchyma. Several authors have systematically described these radiographic changes [66–68]. The acute changes (generally occurring several months after radiation) generally demonstrate consolidation and ground glass opacities. Table 12.3 summarizes the acute changes as described in the literature. In a study from Hiroshima University, patients with the diffuse consolidation pattern as well as those with no increased density experienced a greater risk of grade ≥2 radiation pneumonitis [67]. In that same study, 80% of lesions that demonstrated acute diffuse consolidation developed into late changes of consolidation, volume loss and bronchiectasis (termed modified conventional pattern); 59% of lesions that demonstrated no acute consolidation or densities developed into late changes characterized by linear opacities with associated volume loss (termed scar-like pattern). Overall, the late changes (>6 months after SBRT) of modified conventional pattern, mass-like pattern (focal consolidation around tumor site) and scar-like pattern were seen in 62%, 17% and 21%, respectively. In a study from Kyoto University, late changes (>6 months after SBRT) were characterized as patchy consolidation (within irradiated lung, though not conforming to SBRT field) in 8%, discrete consolidation (within SBRT field, though not outlining shape of field) in 27% and solid consolidation (outlining SBRT ield) in 65% [66]. The shape of the radiation changes were described as wedge (35%), round (35%) and irregular (29%) and the extent of fibrotic change was described as peripheral (48%), central (6%), both (39%) and skip lesion(s) isolated from the tumor (6%). Certainly, late pulmonary fibrosis can change in shape and extent over time. In a study from Tokyo, over the course of follow-up imaging, 6–11 months after radiation. the fibrotic changes shrunk in 44% of patients and moved (generally toward the hilum) in 38% [68]. This same group from Tokyo recently published a

Table 12.3. Acute radiographic changes a er SBRT to the lung

	Diffuse changes		Patchy changes		Discrete	No change
Hiroshima University	Consolidation 9%	GGO 12%	Consolidation + GGO 15%	GGO 2%		33%
Kyoto University	Homogeneous slight ↑ in opacities 26%		Consolidation 68%		Consolidation 6%	0%

GGO, ground glass opacities

study in which late radiation fibrosis radiographically appeared as abnormal opacities, mimicking recurrent tumors [69]. The authors urged caution in the interpretation of these scans, suggesting close follow-up to monitor changes, PET scanning and/or biopsy. While all of the late changes described above reflect fibrosis, the clinical significance of these different characteristics of radiation change are not known.

12.5.2 Liver

The standard treatment for limited liver metastases or primary liver malignancies is resection. Other modalities include radiofrequency ablation and radiation. Arguably, radiation is the least invasive approach, and well suited for many patients with inoperable disease, comorbid conditions and/or several lesions.

Our group previously reviewed the literature on the outcome and toxicity of SBRT for liver tumors [49]. As with SBRT to the lung, SBRT to the liver often results in mild constitutional symptoms. Grade ≥3 late toxicity generally does not appear to occur.

The University of Colorado and University of Indiana enrolled 18 patients with between one and three liver metastases in a prospective Phase I study of three fractions of SBRT; the dose per fraction was escalated in 2-Gy increments, starting at 12 Gy per fraction [70]. No patients experienced grade >2 toxicity. It was required that 700 ml of normal liver receive <15 Gy total dose. Most patients were noted to have well circumscribed hypodense lesions corresponding to the dose distribution corresponding to ~30 Gy in 10-Gy fractions. In a follow-up analysis, including an additional 18 patients treated on a phase II study, one instance of subcutaneous tissue breakdown occurred, while no liver toxicity attributable to SBRT occurred [71].

The University of Rochester analyzed 69 patients who received SBRT to metastases in the liver, mostly treated to 50 Gy in 5-Gy fractions. The volume of liver not involved by gross tumor was required to be ≥1000 ml. For patients with no history of macronodular sclerosis, liver failure or hepatitis, the dose to 60% of the liver volume was required to be <30 Gy, and, for patients with a history of hepatitis or cirrhosis, the dose to 70% of the liver volume was required to be <30 Gy. Grade 1–2 elevation of liver function tests occurred in 28%, and no grade ≥3 toxicity was observed [44]. Clinically insigni icant radiographic changes were seen in all patients with treated liver lesions.

In a study from Aarhus University, 44 patients with liver metastases from colorectal cancer were treated to a dose of 45 Gy in 15-Gy fractions. Dose constraints included <30% of the liver receiving a total of >10 Gy, and the spinal cord maximum was <18 Gy. The dose to kidney, intestine and stomach was kept as low as possible. Acute toxicity (within 6 months) included colonic ulceration (one patient) and duodenal ulceration (two patients) and one patient developed hepatic failure. Acute grade 3–4 ulceration of the skin, pain, nausea and diarrhea were also observed. Late toxicity was not explicitly discussed [72].

Princess Margaret Hospital recently published a Phase I study, in which 41 patients with primary hepatocellular or intrahepatic biliary cancer received 24–60 Gy in six fractions, in a dose escalation study, in which patients were strati ied into three different dose escalation groups based on the effective liver volume irradiated [73]. A normal tissue complication model was used to stratify patients into three separate groups of dose escalation. Generally, the mean liver dose was kept <22 Gy and the mean kidney dose was <12 Gy. The maximal doses (to <0.5 ml of tissue) were as follows: 27 Gy to the spinal cord; 30 Gy to the stomach, small bowel and large bowel and 40 Gy to the heart. Roughly 25% of patients ex-

perienced acute grade 3 liver enzymes and roughly 25% experienced acute (within 3 months) progression of their Child-Pugh classification, seemingly due to disease progression. Acute transient biliary obstruction was seen in two patients. There was one late death from gastrointestinal bleeding resulting from a duodenal-tumor fistula and one patient required surgery for a bowel obstruction; both of these late toxicities were exacerbated by (and perhaps primarily attributed to) recurrent disease.

12.5.3 Pancreas

Locally advanced pancreatic cancer has an unfortunately dismal prognosis, with distant metastases and local progression almost invariably leading to death. Local control from radiation can yield palliation and prophylactic palliation of biliary obstruction, bowel obstruction and pain from splanchnic nerve invasion. SBRT may afford an advantage in terms of improved local control as well as shorter treatment duration.

In a Phase II study from Aarhus University, 22 patients with unresectable pancreatic cancer received 45 Gy in 15-Gy fractions [74]. The PTV (1-cm margins around the CTV) was covered by the 67% isodose line. Acute grade 3–4 toxicity included pain, nausea, diarrhea, severe duodenal and gastric mucositis and ulceration and perforation. Poor survival precluded a late toxicity analysis. Whether toxicity was related to disease progression or radiation could not be determined.

In the Phase I study from Stanford, 15 patients were treated with single dose, escalated from 15 to 25 Gy [75]. The 50% isodose line covered only the duodenal wall closest to the tumor. At the 25-Gy dose, the mean dose to 5% of the duodenum was 22.5 Gy and the mean dose to 50% of the duodenum was 14.5 Gy. No acute grade ≥3 toxicity was observed; late toxicity and symptom control were not explicitly reported, presumably due to the limited follow-up (median 5 months) and poor survival (median 11 months). The results from Stanford University conflict with those from Aarhus University in that Stanford found SBRS to be relatively tolerable. This may be a result of different dose fractionation, different treatment design and/or differences in patient population and/or disease failure. The group from Stanford attributes the differences in toxicity to their use of respiratory tracking. Stanford University enrolled 16 patients in a subsequent Phase II study in which 45 Gy of conventional radiation was followed by a single 25-Gy SBRS fraction [76]. Radiation guidelines included: 70% of the liver <15 Gy, 70% of each kidney <15 Gy, 95% of bowel <45 Gy, spinal cord maximum <30 Gy. Acute grade 3 toxicity included gastroparesis in two patients (one prior to SBRS). Late toxicity included some patients (the number not explicitly reported) who developed grade 2 duodenal ulceration 4–6 months after SBRS. In a later report, the authors document late gastrointestinal bleeding from unknown cause and duodenal obstruction in the same patient [77].

These authors from Stanford examined four patients with pancreatic cancer at autopsy, 5–7 months after SBRS, in order to characterize the histopathologic findings [77]. The primary tumors were characterized by extensive fibrosis, varying amounts of necrosis (tumor necrosis and ischemic necrosis) and widespread vascular injury (ibrinous exudate of arterial wall, necrosis and luminal occlusion). Stromal changes included fibrosis, atypical fibroblasts and fibrin deposition. Sparse residual cancer cells were seen. In the adjoining colorectal tissue in one patient, mucosal exudate with possible pseudomembrane formation and submucosal vascular damage were seen. The colonic mucosa was estimated to have received 4–11.5 Gy. Lymph nodes exposed to radiation were depleted of lymphocytes.

12.5.4 Prostate

Prostate cancer may benefit from hypofractionation due to the low α/β ratio of prostate cancer [78], though obviously there is concern about late effects given the proximity of the rectum and bladder, and the expected lengthy survival of these patients [18]. Hypofractionation can be delivered using IGRT, with ultrasound and/or CT, to improve target localization, IMRT to reduce rectal and bladder dose and/or proton therapy which can also reduce normal tissue exposure. Large fractional doses are also delivered with high dose rate brachytherapy. There is only limited published experience with SBRT, which is generally delivered with the assistance of fiducials implanted in the prostate and/or IGRT [79, 80].
Virgina Mason treated 40 prostate cancer patients with 33.5 Gy in 6.7-Gy fractions, which was expected to provide equivalent biochemical control to 78 Gy in 2-Gy fractions (assuming an α/β ratio of 1.5) [80].

The equivalent dose to the rectum (using an α/β ratio of 3) was estimated to be 65 Gy in 2-Gy fractions, which was expected to be safe given the low volume of rectum that would be exposed. SBRT was used with implanted fiducials. Late grade 1–2 toxicity (defined as >1 month post treatment) included 45% with genitourinary toxicity and 37% with gastrointestinal toxicity [79]. No late Grade 3 or higher toxicity was reported. In all, 26 patients reported potency before therapy, of which six have developed impotence.

12.5.5 Spine

Metastases to the spine can result in pain as well as neurologic symptoms, which are often well palliated with radiation. The standard approach to the treatment of spinal metastases is surgical decompression followed by radiation or radiation alone in those patients not amenable to surgery. The radiation is generally delivered in a short course (1–2 weeks) with daily fractions of 2.5–4 Gy. The prescribed dose of 20–40 Gy with these larger fraction sizes is generally accepted to be at the spinal cord tolerance (though certainly below the TD 5/5) [4], and thus further radiation of the cord is thought to be riskier, though potentially feasible [81]. Thus, in patients with previously irradiated, symptomatic spinal metastases, SBRT and SBRS can allow for a means to treat spinal tumors while minimizing the dose to the cord. While hypofractionation in this situation is counterintuitive, given the association of late toxicity with fraction size, early clinical data has shown it to be tolerable, albeit with limited patient follow-up.

Single fraction SBRS [82–89], and hypofractionated SBRT [87, 90–92] have been used to treat metastases (as well as the much rarer primary spinal tumors). SBRS has also been used as a boost treatment immediately following a course of fractionated radiation in patients who have not been previously irradiated [87]. Techniques such as IMRT can be used to help conform the dose around the spinal cord [84, 86, 89–91, 93], while IGRT and/or SBRT can be used to accurately position the patient and target [84, 85, 89–91, 93]. With SBRS and SBRT to the spine, the spinal cord dose is minimized, resulting in a maximum dose on the order of 10 Gy [82–92]. Data from 177 patients with 233 lesions treated at Henry Ford Hospital suggests that a single fractional dose of 10 Gy to <10% of the contoured spinal cord (6 mm above and below the target) is safe, and that small volumes (<1% of the contoured cord) can safely receive higher maximal doses, perhaps up to 20 Gy. More rigid dose constraints have yet to be published.

Given the palliative nature of this treatment, long term follow-up is generally limited to the extent that late toxicity is not readily assessable. Certainly, spinal SBRS and SBRT have proven to be tolerable and have produced excellent palliation without observed neural toxicity. At least one report has suggested that the acute toxicity using these approaches is perhaps better than conventional radiation [94]. Myelopathy and radiculopathy rarely occur. Henry Ford Hospital reported 1 patient out of 177 who developed radiation related spinal cord injury, resulting in slight unilateral lower extremity weakness (4 out of 5 strength) that responded to steroids [95]. In a recent report from Memorial Sloan Kettering, in which 103 lesions in 93 patients were treated with a single dose (18–24 Gy prescribed to the PTV, with the spinal cord limited to 12–14 Gy), late toxicity included vertebral body fracture and tracheoesophageal fistula [89]. In the largest series to date from the University of Pittsburgh, in which 393 patients with 500 lesions received a single dose of SBRS (12.5–20 Gy around the periphery, with only a small volume of spinal cord exceeding 8 Gy), no patient developed a new neurologic defect [82, 83]. No late effects were reported with a follow-up of 3–53 (median 21) months.

12.6 Conclusions

SBRT uses a three-dimensional coordinate system to more accurately localize the treatment target, and therefore reduce the uncertainty in patient set-up and. Because SBRT reduces the volume of normal tissue exposed to therapeutic doses, large fractional doses can be used to achieve maximal local control of the treated tumor in certain situations. The classic radiobiology models do not appear to be adequate to predict the clinical outcome of hypofractionated radiation delivery. Mechanisms such as tumor apoptosis, endothelial apoptosis, and immunologic effect may play a more prominent role after large fractional doses.

A large body of literature supports the use of SBRT to tumors in the lung and liver, which are organ whose subunits are arranged in series. SBRT can

therefore reduce the number of functional subunits destroyed by radiation. Caution must be heeded with tumors close to the esophagus, large airways and spinal cord. SBRT to liver and lung tumors appears to be safe (when adhering to published dose constraints), with minimal symptomatic acute or late toxicity.

There is a large body of literature that supports the use of SBRT/SBRS in the treatment of spine tumors, even after receiving full dose conventional fractionated radiation. Toxicity appears minimal, admittedly in a patient population with limited long-term follow-up. Other sites that have been investigated include pancreas, prostate and kidney. The proximity of these structures to bowel make SBRT potentially riskier, though single institution experience has shown promise.

The primary goal of dose escalation with SBRT is to improve tumor control. If local control rates of >90% are attained, further dose escalation may not yield further improved local control, and may not be warranted due to the risk of greater toxicity. Because toxicity is uncommon with the doses that are presently used, a large number of patients will need to be analyzed to determine optimal normal tissue dose-volume constraints. Long-term follow-up is also necessary given that late effects can occur years after radiation. Further study is needed to better assess the late toxicity of SBRT in all locations.

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The Radiation Spectrum of Normal Tissue Toxicity and Tolerance – Multiorgan Domino Effect

13

Philip Rubin

CONTENTS

13.1 Introduction

Conceptually, normal tissue tolerance is often viewed and defined in terms of a single specific normal tissue/organ site with the clinical illusion that during radiation treatment the adverse effects are localized and limited to those normal tissues within the radiation field. However, with the technological advances of highly computerized treatment delivery and dynamic multileaf collimation, the widespread use of intensity modulated radiation therapy (IMRT) allows for administering higher doses to defined tumor volume contours but in the process delivers more radiation to all the surrounding normal tissues in the axial segments being treated (Fig. 13.1) [1, 2].

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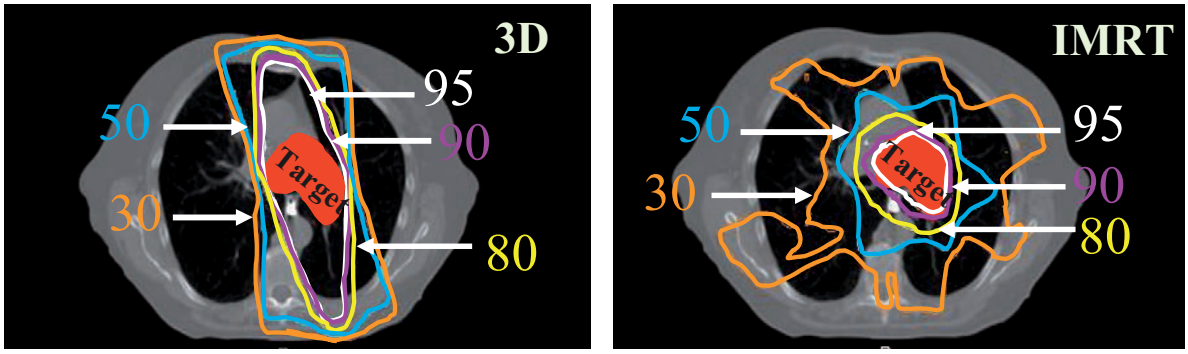
Thus, there is an increasing need to recognize the spectrum of normal tissues and organs which are exposed but often obscured in color wash isodose curves. Although each critical structure has radiation tolerance limits well de ined, it is important to have a more holistic view of the radiation effects in the large variety of normal tissues adjacent to the tumor, recognizing that all modalities leave the persistence of the memory of an untoward perturbation of their cellular, genetic, and molecular elements (Fig. 13.2) [3].

The four Rs of tumor radiobiology have dominated the concepts of the effectiveness of radiation cell kill and provide an understanding of cancer recurrence. The pioneering experiments of Hall, Elkind and Kallman [4–6] (Fig. 13.3) identified the processes of cell repair, reassortment, repopulation and reoxygenation. In an analogous fashion these radiobiologic concepts can be applied to the homeostatic mechanisms that apply to normal tissue preservation. That is, radiation cell kill within a normal organ or structure needs to be compensated for its continued function and to achieve a favorable therapeutic ratio. The Rs of tumor radiobiology are presented in this new context in five phases. This results in a progressive and unending biocontinuum for a lifetime (Fig. 13.4) [7].

13.2 Phase I: Release and Regeneration

The lymphocyte and macrophage are the irst tissue to react due to their inherent radiosensitivity. Their lysis and rapid apoptosis releases a cytokine and chemokine cascade which is perpetuated as the radiation effects other cells in the adjacent tissues. Our group's demonstration that the initial multicel-



a b

Fig. 13.1a,b. Percent relative isodose distributions for the 3DCRT plan (**a**) and IMRT-2 plan (**b**). The dose distributions show that the IMRT-2 plan is more conformal than the 3DCRT plan at the cost of slightly losing target coverage. Unlike the UTCP plan values, the $UTCP_{QALY}$ values, which incorporate clinically realistic quality of life data, suggest that the $UTCP_{QALY}$ formalism provides better differentiation between plans. (Reprinted with permission from [2])

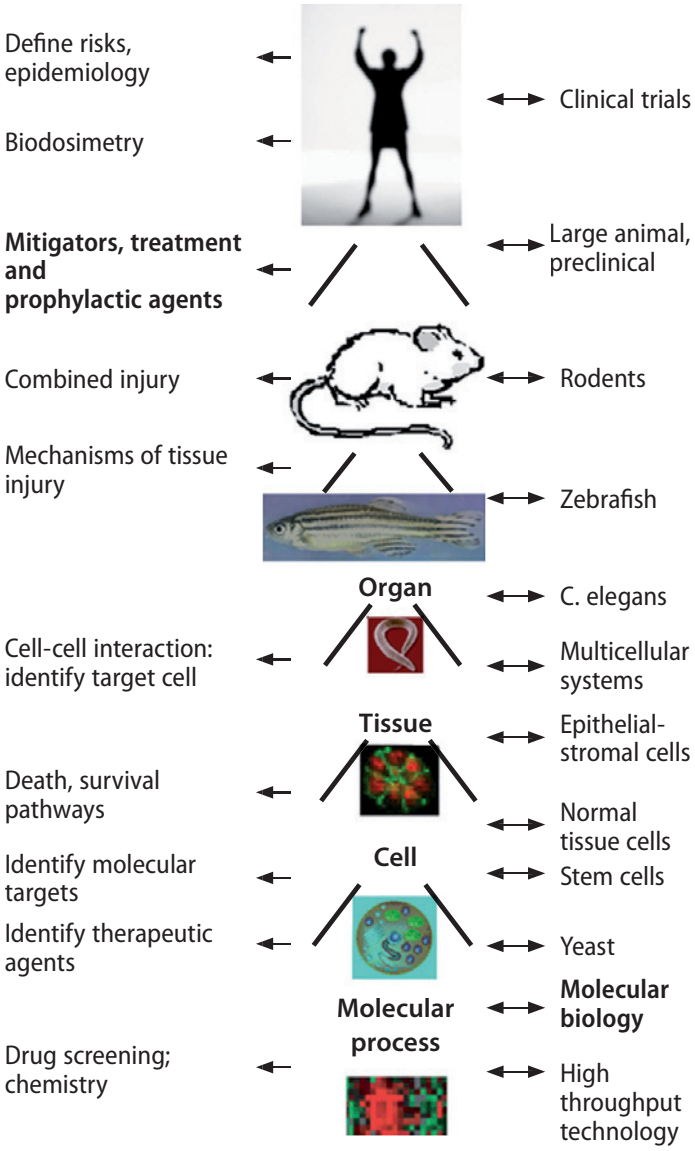


Fig. 13.2. Animal models for radiation countermeasures research. The overall goal of research is to go from underlying molecular mechanism to human application. This requires many model systems. (Adapted and reprinted with permission from [3]

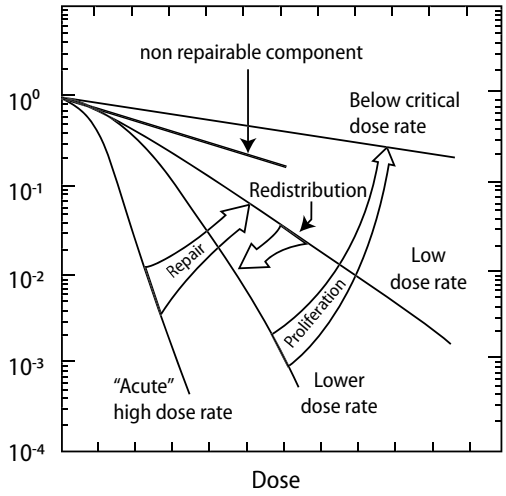


Fig. 13.3. The radiobiology of dose rate, Bedford and Hall style. (Reprinted with permission from [4])

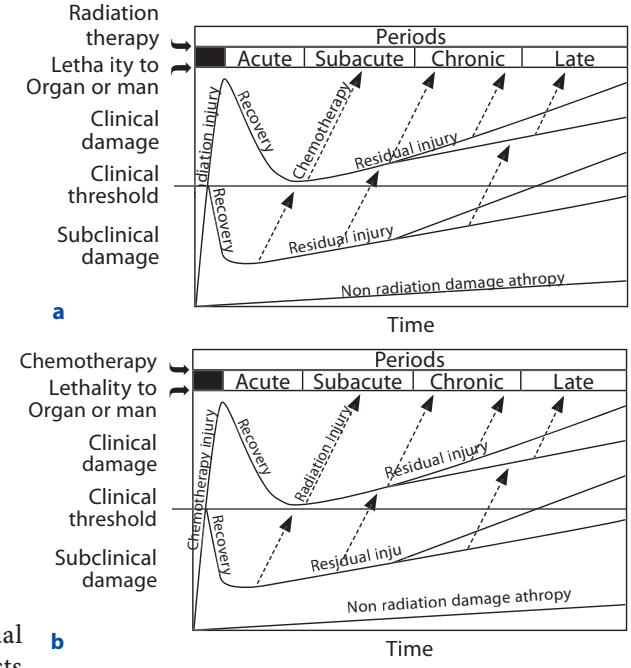


Fig. 13.4a,b. The clinicopathologic course of events following irradiation can be complicated by the addition of chemotherapy. Similarly, chemotherapy can result in parallel sets of events. **a** Classically, when radiation therapy precedes chemotherapy, the introduction of the second mode can lead to expression of subclinical damage or, when injury is present, to death. **b** The same is true if chemotherapy precedes radiation therapy. (Reprinted with permission from [7])

lular response to irradiation resulted in a perpetual cytokine cascade shifted the focus of radiobiologists from target cells to molecular messages released prior to their mitotic linked death by utilizing in vivo/in vitro set of experiments (Fig. 13.5) [8]. The ırst wave of early responding tissues includes the bone marrow in which alterations occur in the progenitor cells, i.e., erythroblasts, myeloblasts and thromboblasts as well as embedded lymphoid cells. This loss of bone marrow stem cells triggers the release of colony stimulating factors (CSFs) as a function of bone marrow volume as well as dose [9]. The delay in clinical expression of a falling blood count is a function of the mature blood cells' life cycle, i.e., platelets and leukocytes 2–3 weeks, red blood cells 3 months.

Since every segment of the anatomy has both lymphocytes embedded and bone marrow in its environment encased in its skeletal structure, these two systems, i.e., lymphoid and bone marrow, act as an alarm system that initiates an immediate response

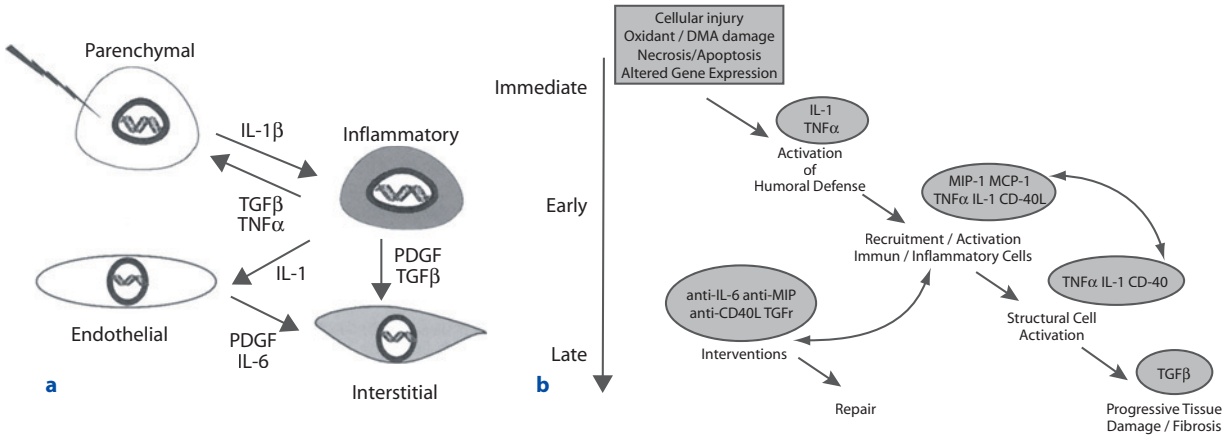


Fig. 13.5. a Suggested chain of events beginning with the initial injury to the primary target cell – the parenchymal cell – and culminating in activation of the interstitial cells (e.g., ıbroblasts) to lay down extracellular matrix. **b** Hypothetical pathway indicating the chain of events from initial injury to the final late effect (e.g., ıbrosis). (Reprinted with permission from [8])

mechanism so that released growth factors provoke a strong autocrine and paracrine stimulus to initiate the hematopoietic stem cell regeneration (Fig. 13.6, Table 13.1) [9, 10]. Lymphoid extranodal sites and major lymph node stations are noted for their ubiquity. The "dose volume tolerance" of the hematopoietic system was demonstrated both in the laboratory and clinic in which sterilization of defined bone marrow volumes resulted in dramatic functional compensatory alterations that persisted for years and decades [11, 12]. That is, although peripheral blood counts returned to normal, the bone marrow did not regenerate in the "irradiated local field", but resulted in either a hyperactive regeneration of shielded adjacent unexposed bone marrow or more remarkable was the ability of the bone marrow to be reactivated in the femurs, i.e., recapitulating its ontogeny [13]. These bone marrow radiation experiments demonstrated the robust systemic effects of local field treatment that once initiated continue over time for years and decades (Fig. 13.6, Table 13.2) [9].

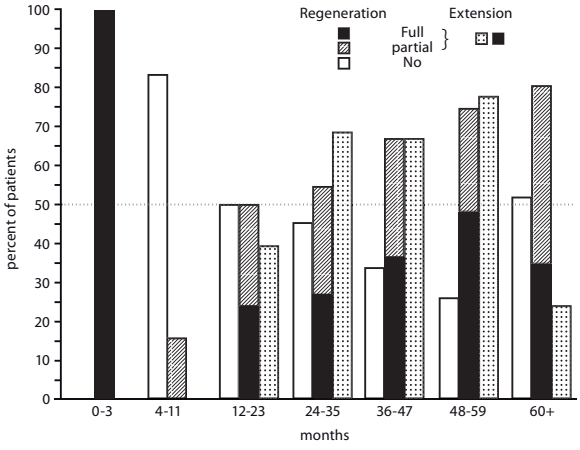


Fig. 13.6. Pattern of bone marrow regeneration and extension in Hodgkin's disease patients (after total nodal irradiation) as determined by ^{99m}Tc-S colloid. (Reprinted with permission from [9])

13.3 Phase II: Recruitment and Repopulation

The second wave of early responding tissues includes the epithelial cells of the gastrointestinal mucosal lining, from esophagus to anus, the basal germinating layer of upper aerodigestive systems and those of skin, all of which are rapidly proliferating and exist in some part in many body segments [7]. Their cell kinetic time for renewal determines the time of the expression of the inflammatory reaction, i.e., the gastrointestinal mucosa responds in a week, oral cavity, oropharynx, and esophageal mucositis starts at 2–3 weeks, dermatitis into 3–4 weeks [7]. The important observation is the inflammatory cells that provide the cytokines for epithelial cell repopulation are due to recruited unirradiated bone marrow derived white cells. That is, the acute reaction is due to the recruitment of bone marrow derived lymphocytes and macrophages interacting with dying epithelial cells. The most elegant studies are those of Krause et al. [14] who demonstrated in a chimera mouse model that the pluripotent bone marrow stem cell crossed germ lines and are capable of regenerating irradiated epithelial tissues. Although controversial, numerous independent studies support the probability of a subset of bone marrow stem cells is capable of regenerating epithelial linings including esophagus and lung [15]. Most investigators have demonstrated immunochemical and histochemical markers from bone marrow stem cell chromosomes which are reconstituted in repopulated mucosal cells, i.e., female bone marrow marker in male host epithelium. This has opened the door for bioengineering irradiated normal tissue by transfusing histocompatible pluripotent hematopoietic bone marrow stem cells (HBMSC). The inherent plasticity of HBMSC allows the bone marrow stem cells' genotypes to transdifferentiate into different cell phenotypes (Fig. 13.7) [15].

Table 13.1. Table of implicated inflammatory regulators [10]

Proposed inflammatory regulators			
PDGF	TGF-β	MIP-1α, -1β, and -2	VEGF
bFGF	L-selectin	Interferon inducible protein-10 (IP-10)	Lymphotactin
MCP-1	E-selectin	Prostacyclin	Eotaxin
IL-1α	RANTES	Plasminogen activator	
IL-6	Angiotensin converting enzyme (ACE)	TNF-α	

Table 13.2. Bone marrow regeneration (BMR) patterns and compensatory mechanisms [ ]

Techniques of irradiation	Regeneration			Doses (Gy)	
	Exposed bone marrow	Unexposed bone marrow	Extension	Daily	Total
Small field	N	Local-regional ↑ BMR	N	2	> 40
Large field	N	Generalized ↑ ↑ BMR	N	2	> 30
Subtotal body	Supressed BMR which then recovers	Generalized ↑ ↑ BMR	↑ ↑	2	40
Total body	Active	-	N	0.05–0.1	> 1

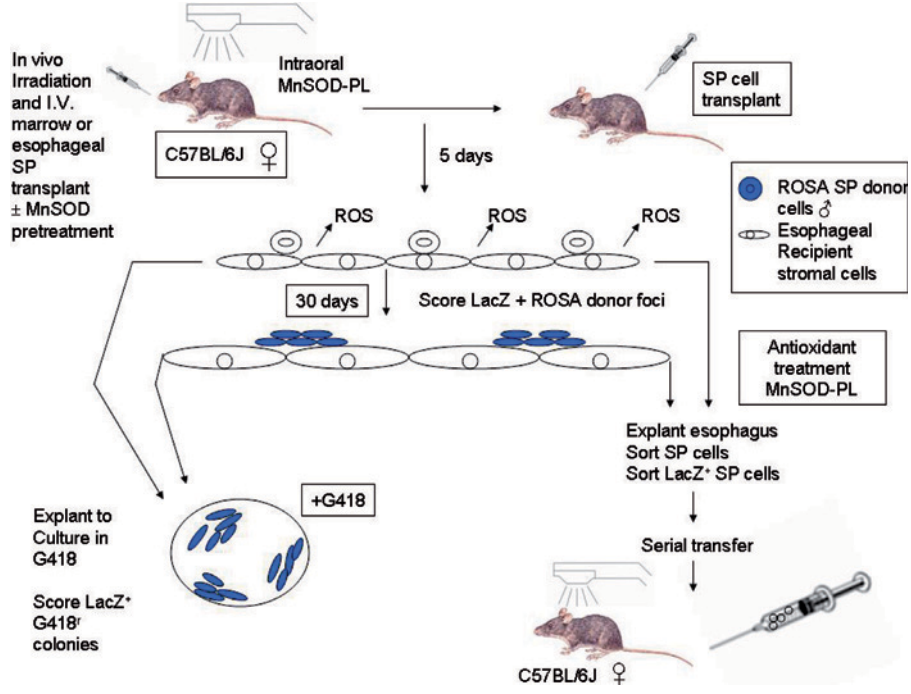


Fig. 13.7. Experimental model system to show donor marrow stem cell origin of esophageal stem cells. ROSA male donor mice have three markers (Y chromosome, G418 resistance, LAC-Z production). Recipient esophagus is irradiated 24 h after intraoral administration of MnSOD-PL, then donor marrow is given intravenously. Esophagus is removed. Cells shown to be donor origin in vivo and in vitro, and donor esophageal side population (stem cells) show self renewal by serial transfer to recipient second generation irradiated esophagus. (Reprinted with permission from [15])

13.4 Phase III: Replacement and Reoxygenation

The microvasculature and the interstitium of normal tissues are a key component of the radiation reaction. Rapidly dividing tissues in expressing their injury early interact with endothelial and fibroblast stroma which actively participate in the acute reactive phase. The importance of the capillary endothelium and stromal fibroblast is highlighted in slow responding tissues where irradiation results in the widespread damage of the microcirculation first [16]. Some of the most convincing evidence can be found in the meticulous studies by Fajardo and Stewart (Fig. 13.8) [16] on the irradiated rabbit heart where serial histopathologic analysis demonstrated a diffuse microthrombosis of the fine capillary network in the myocardium while sparing the cardiac myocyte. The control observation was the selective effect of myocardial cell vacuolation by adriamycin in contrast to radiation injury. Disruption of the capillary bed and leakage of cells, initiates a relative hypoxia that provokes a fibrogenic response (Fig. 13.9) [17]. The aftermath of radiation often results in extensive fibrosis when parenchyma fails to regenerate and atrophies. Casarett referred to this ongoing fibrogenic process as an increase of the histohematic barrier due to a failure of the normal tissue to fully reoxygenate. Some investigators have found bone marrow derived stromal and endothelial cells contributing to fibrosis and attempts at revascularization, respectively [15].

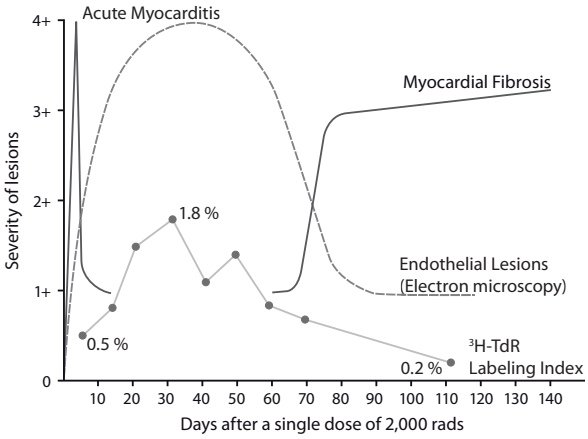


Fig. 13.8. Evolution of radiation-induced myocardial fibrosis in the rabbit heart following a single local dose of 20 Gy on day 0. The severity of the light microscopic lesions is indicated by the *solid line*, but the nature of such lesions varies: transient acute myocarditis initially and diffuse fibrosis after 70 days. The severity of the ultrastructural alterations is indicated by the *broken line*. The bottom line indicates the proportion of nuclei labeled by ^{3}HTdR (endothelial cells). (Reprinted with permission from [16])

There are some organs that respond in a delayed fashion dramatically due to decompensation of its entire microcirculation. The most striking illustrations were the development of radiation pneumonitis during half body and total body irradiation [18, 19]. This was also evident in whole liver irradiation following whole abdominal treatment for ovarian cancer [20]. Curiously, the liver failure was attributed to venous occlusive disease of central capillary veins of hepatic lobules. The triggering mechanism was platelet adhesion probably due to release of von Willebrand factors [21]. This global versus focal effect was presented conceptually by Byfield as the "volume effect" [22]. An extensive literature has recently mushroomed on "dose volume" as the critical determinant of normal tissue tolerance and toxicity. The lung and liver have been thoroughly investigated and elaborated [23, 24] (Fig. 13.10). It is possible to attribute loss of function with volume to loss of microcirculation volume as well as loss of functional units.

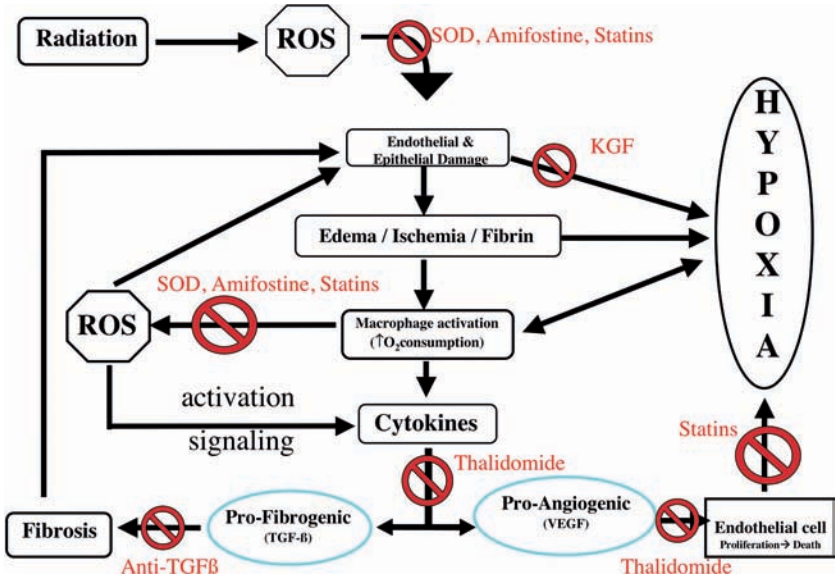
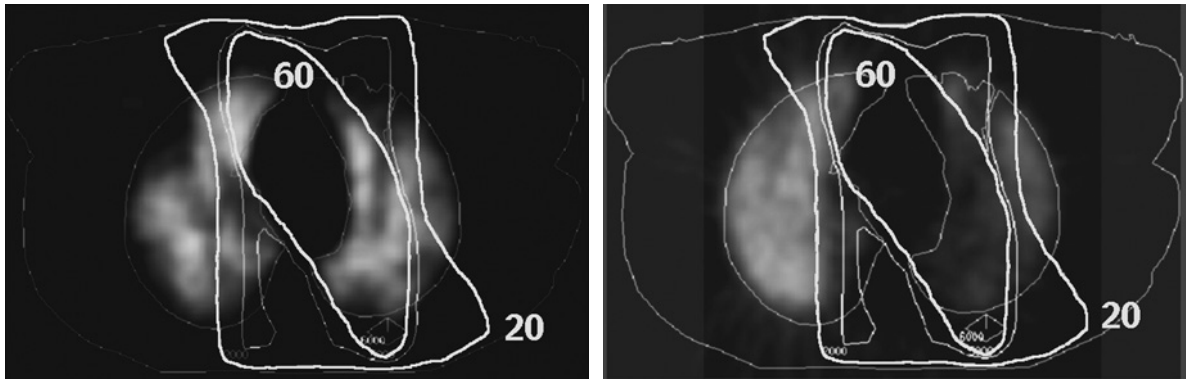


Fig. 13.9. Paradigm of hypoxia-mediated chronic lung injury. Initial tissue damage from radiation is generated by the direct action of reactive oxygen species (ROS) on DNA. This effect causes tissue injury including epithelial and endothelial cell damage, with an increase in vascular permeability, edema, and fibrin accumulation in the extracellular matrix. This tissue injury is followed by an in lammatory response including macrophage accumulation and activation. Macrophages, along with other inflammatory cells, are attracted to an area of injury or evolving in lammation. The majority of macrophages in lung are derived from circulating monocytes that enter the lung in response to inflammation. These macrophages are able to release a number of cytokines and ROS. Both vascular changes as well as an increase in oxygen consumption (due to macrophage activation) contribute to the development of hypoxia. Hypoxia further stimulates production of ROS, and pro ìbrogenic and proangiogenic cytokines. This response to hypoxia perpetuates tissue damage leading to fibrosis via TGFß production and stimulates angiogenesis through VEGF production. While attempting to respond to the proliferative stimulus of VEGF, endothelial cells die as a result of previously accumulated radiation damage. Thus, hypoxia continuously perpetuates a non-healing tissue response leading to chronic radiation injury. This injury pathway offers numerous potential targets for therapeutic intervention. (Reprinted with permission from [17])



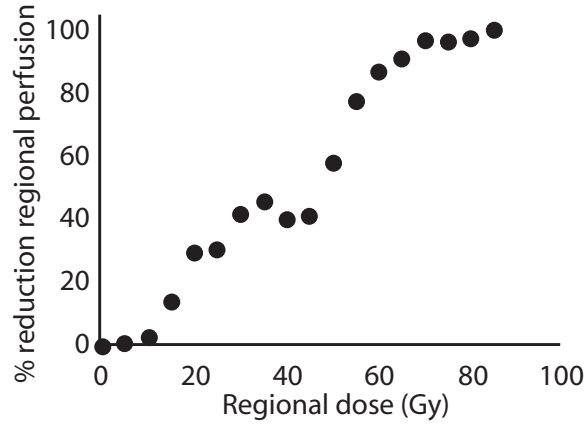


Fig. 13.10a–c. The pre- (**a**) and 6-month post-RT (**b**) transverse SPECT perfusion images from a patient irradiated for lung cancer are shown. The RT dose distribution is also shown. The post-RT perfusion defect is seen most prominently within regions of the lung receiving > 60 Gy. The dose–response curve for RT-induced reductions in regional perfusion, from this patient's SPECT scans, is shown. (Reprinted with permission from [23])

13.5 Phase IV: Reassortment and Remodeling

The late cell reassortment and remodeling phase is dominated by slow or non-renewal normal tissues that have little or no regenerative capacity. According to Rubin and Casarett [7], the normal tissues/organs that are dominated by parenchymal cells, which are reverting post mitotic cells have the capacity to either revert to an actively mitosing stem cell or enlarge by undergoing hypertrophy. The liver is the classic example of tissue loss being stored by reassortment and reversion of a resting cell into an actively dividing hyperplasia response to remodel the organ. Salivary glands and endocrine glands consisting of reverting post mitotic cells compensate by hyperplasia of spared volumes.

Alternatively, some normal tissues compensate by undergoing hypertrophy of the unirradiated portion of the organ system. The classic example are the kidneys when loss of one results in a compensatory hypertrophy of the other. The lung in a similar fashion compensates by hypertrophy of a lobe in response to loss of a lobe in the same lung. The hypertrophy or hyperplasia can be referred to as remodeling of an organ volume to compensate for a lost segment. The heart in an analogous fashion undergoes hypertrophy to compensate for a decrease in its ejection fraction due to myocardial cell loss either due to infarction or interstitial fibrosis (Fig. 13.11) [25, 26].

13.6 Phase V: Cell Repair and Resurgence

There are a number of normal vital parenchymal tissues that lack the mitotic potential to regenerate. These are fixed post mitotic cells. The classic example is the central nervous system, i.e., brain and spinal cord [7]. Although a temporary and transient demyelination may occur, injury to neurons are not replaced and in the spinal cord result in a permanent transection. The ganglioneurons tend to be fixed post mitotic cells and although controversy exists

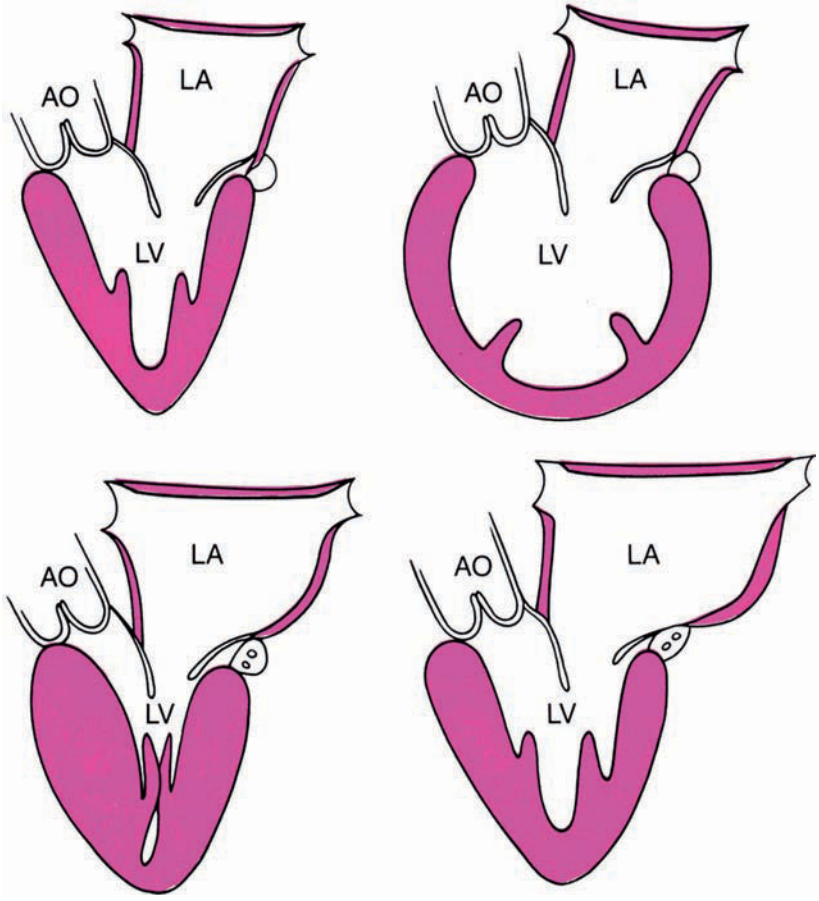


Fig. 13.11a–d. Characteristics of the normal heart and the three main types of cardiomyopathy. **a** Normal. **b** Dilated cardiomyopathy: Note the thin left ventricular (*LV*) walls and enlarged LV chamber, resulting in poor contraction of heart muscle (systolic dysfunction). **c** Hypertrophic cardiomyopathy: not related to the cardiotoxicity of cancer therapy. **d** Restrictive cardiomyopathy: note the normal to slightly thickened LV walls and slightly decreased LV chamber size. These changes are caused by ibrosis which stiffens the myocardium and results in poor chamber filling (diastolic dysfunction). (Reprinted with permission from [26])

as to the ability of periventricular stem cells to compensate, the resultant atrophy or loss of cerebral or spinal cord neurons is irreplaceable [27].

The musculoskeletal system is virtually nonreactive to large conventional radiation doses but can undergo atrophy or fail to hypertrophy with exercise as a stimulus [28]. Skeletal bone is tolerant of high doses and responds to a decrease in its vascularization by undergoing sclerosis and eventually necrosis or fracture. When a heavily irradiated long bone is fractured, the periosteal osteoblast cells fail to proliferate and are unable to form a stabilizing callous, and the bone requires pinning to seal itself [29].

Fortunately, the vital normal tissues with fixed post mitotic cells often are highly radioresistant, widely distributed and are non-responding clinically except under unusual circumstances, i.e., extremely high doses. These include the bulk of striated muscle including fascia, tendons and ligaments, peripheral and autonomic nerves, large arteries and veins. Articular cartilage and intervertebral discs allows for preservation of joint function and osteoarthritis is rarely if ever induced by radiation.

Most DNA damage is rapidly repaired [5]. However, radiation induces point mutations, gene deletions and overexpressions and in time radiation mutagenicity leads to radiation carcinogenicity and second malignant tumors (Fig. 13.12) [30–32].

13.7 Discussion

The holistic concept of the multiorgan domino effect is recapitulated by biocontinuum timelines [7]. The thorax in our illustration has been treated by IMRT and as a consequence all surrounding normal tissues have been irradiated. The Rs of radiobiology are applied to redefine reactions and interaction of the variety of normal tissues in the anatomic segments being treated.

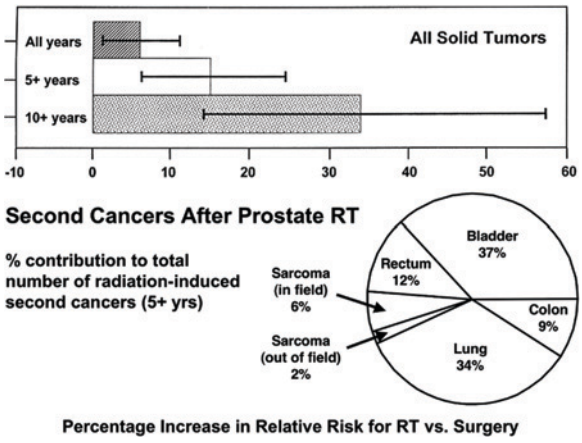


Fig. 13.12. The *upper panel* shows the percentage increase in relative risk for all solid tumors as a function of time after radiotherapy. The error bars represent 95% confidence limits. "*All years*" refer to all years post-treatment; the standard error is smaller in this case because of the larger number of patients; most did not survive to 5 or 10 years. The *lower panel* shows the distribution of the principal radiation-induced cancers, namely bladder, lung, rectum, and colon. There are also a small number of sarcomas that appear in heavily irradiated areas. (Reprinted with permission from [31])

Phase I: Release and Regeneration

The immediate lysis of lymphocytes occurs in the thymus, mediastinal lymph nodes, bronchial submucosal lymphoid tissue, and lung alveolar macrophages. The bone marrow in the sternum, thoracic vertebral bodies and ribs of their progenitor blast cells and release colony stimulating growth factors. Release of colony stimulating factors jump-starts regeneration of pluripotent hematopoietic bone marrow stem cells out of the ìeld, i.e., cervical/lumbar vertebrae and pelvis.

Phase II: Recruitment and Repopulation

The esophageal and bronchial mucositis is followed by in ìltration of bone marrow derived in ıammatory cells and HBMSC that over time are recruited to repopulate the epithelial loss by transdifferentiating into epithelial cells. The in ìltration of lymphocytes has been observed in unexposed ipsilateral lung segments and contralateral unirradiated lung. This abscopal "autoimmune" phenomenon attests to the systemic effects of local field irradiation (Fig. 13.13) [33]. This could be due to leakage of surfactant apoprotein, normally not present in blood, which is phagocytized by macrophages that we postulate return to the bone marrow and amplify, then home into lung bilaterally, i.e., into the contralateral unirradiated lung, due to type II pneumocyte and stored surfactant. [34].

Phase III: Replacement and Reoxygenation

The microvasculature and the interstitium respond more rapidly in early responding mucosal tissues (esophagus) and later in slow responding organs (lung and heart). In these latter normal tissues, especially when large doses and volumes of the organ are irradiated, there are a number of pathophysiologic microvascular events that lead to hypoxia and eventually drives a replacement fibrosis and the thinning of mucosal parenchymal cells that undergo gradual atrophy. In the esophagus, loss of smooth muscle leads to strictures, in contrast to interstitial ìbrosis in lung alveoli and myocardial interstitial ìbrosis.

Phase IV: Reassortment and Remodeling

The cell reassortment and remodeling phase can occur both within the lung and heart due to partial loss of functional volume. Compensatory hypertrophy allows partially spared lung lobes to recover breathing vital capacity. The reverting post mitotic cardiac muscle cells are capable of some regeneration if radiation doses are moderated and/or a significant volume of the heart is spared. The whole heart hypertrophies to increase its ejection fraction.

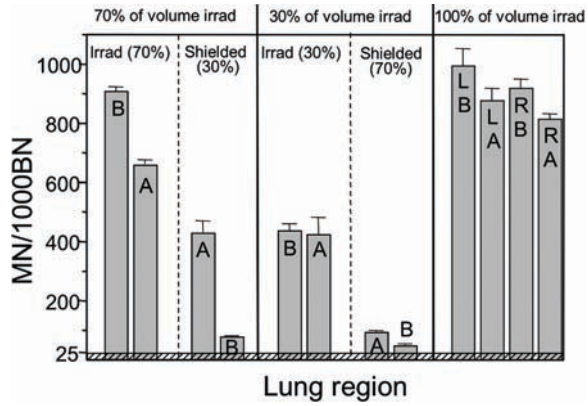


Fig. 13.13. DNA damage (micronuclei/1000 binucleate cells – MN/1000BN) observed in rat lung cells following a dose of 10 Gy given to different volumes of the lung base or lung apex. Cells from different regions of the lung were analysed (*B*, base; *A*, apex; *L*, left; *R*, right). The *bars* represent the mean (+/– SE) from groups of between four and seven rats. The *hatched region* at the bottom indicates the background level of micronuclei in non-irradiated rat lung; this does not vary for different regions of the lung. *irrad*, Irradiated. (Reprinted with permission from [33])

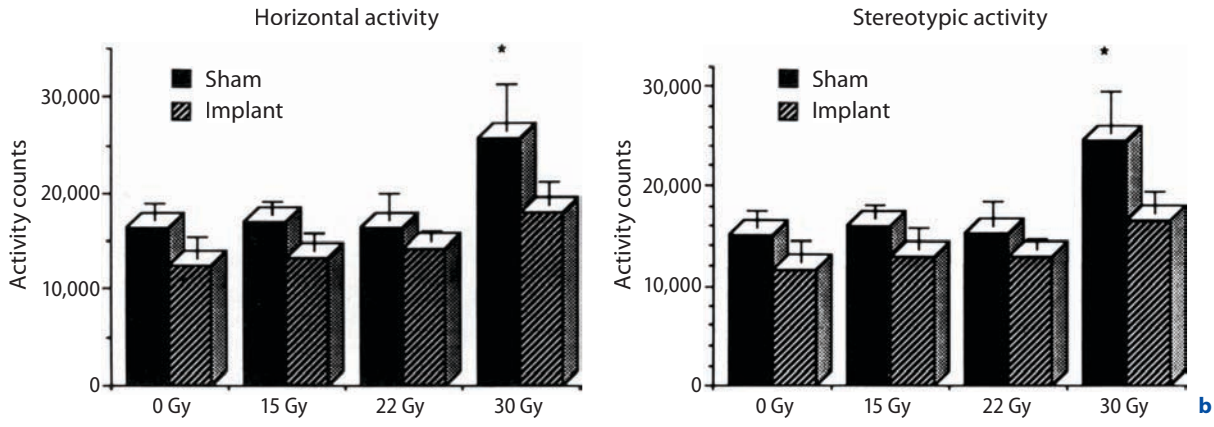
Phase V: Repair and Resurgence

The rapid repair of the cell's DNA was first noted in cell culture by Elkind in which the shoulder of the cell survival curve was recapitulated when radiation was fractionated over time. Most radiation induced DNA damage is rapidly repaired, but it is the occasional misrepair that can lead to the induction of point mutations, chromosomal translocations, gene fusions. This imperfect repair is initially unexpressed clinically but over time, perhaps decades later, leads to radiation induction of cancers and sarcomas. Thus, radiation can add to mutagenicity and eventually second cancers years later. This is well documented in long term Hodgkin's disease female survivors as breast cancers and more recently also lung cancer in the irradiated reactive segment of lung [35].

In summary, the multiorgan domino effect is due to a long list of Rs of normal tissue radiobiology: cell release, regeneration, recruitment, repopulation, replacement, reoxygenation, reassortment, remodeling, repair and resurgence. These new Rs provide a more holistic view of the biocontinuum of radiation as a perturbation of multiple normal tissues and organs for a lifetime. Recognizing and appreciating the systemic interactions offers new opportunities for novel interventions. The transfusion of pluripotent stem cells to regenerate parenchymal and endothelial cells is no longer the impossible dream. The new exciting advances in the bioengineering of adult skin cells by insertion of three genes into histocompatible stem cells opens the door for an improved therapeutic ratio, that is, the stabilization and reversal of the radiation biocontinuum [36].

An apocryphal study conducted with my neurobiologic research team, utilized rat embryonic grafts, implanted as a core of tissue in irradiated adult rat brain; appeared to reverse most the morphologic and functional aspects of neuronal damage. An elegant system of monitoring rat movements by interrupting laser beams documented the inevitable progression of radiation brain injury as hyperactivity in spontaneous movements standing and circling in their cages (Fig. 13.14) [37]. The fetal brain transplanted irradiated animals behaved similar to normal controls and upon sacrifice, the fetal hypothalamic graft was fully vascularized and integrated into the third ventricle of the irradiated brain which appeared free of hemorrhaging due to restoration of the blood brain barrier. There was absence of demyelination and neuronal loss in the fimbria and internal capsule in the irradiated brain suggesting migration of oligodendrolocytes, astrocytes and endothelial cells and/or release of neurotrophic cytokine factors to account for repair and regeneration of the radiation alterations (Fig. 13.15).

Upon presentation of these studies in 1989, the obvious question and dilemma was "Where would



a b

Fig. 13.14a,b. Spontaneous nocturnal locomotor activity was measured in an Omnitech Electronic automated Digiscan Analyser. Data represents total activity counts over the course of a 4-h testing period beginning at lights-off (6:00 pm). Rats in the 30-Gy sham implanted group showed significant hyperactivity in both horizontal (**a**) and stereotypic (**b**) behavioral measures as compared to non-irradiated sham implanted animals (*asterisk*s, $F = 2.66$, $p < 0.01$; $F = 2.94$, $p < 0.01$, respectively). Horizontal activity represents the total number of beam intersections during the 4-h testing period, and stereotypic activity is indicative of repeated intersections of a single beam. Elevated stereotypic activity is often seen in animals with basal ganglia damage. (Reprinted with permission from [37])

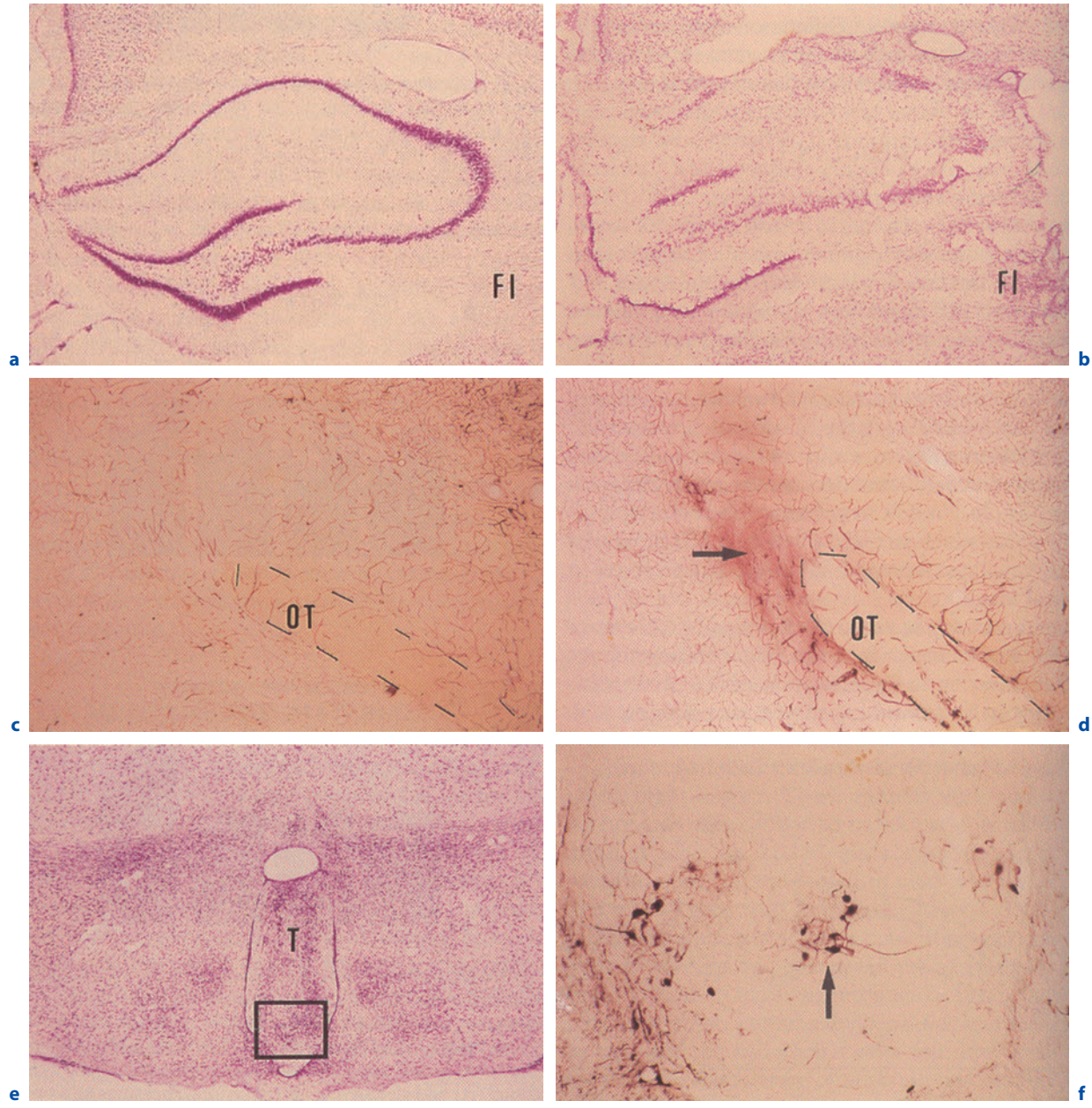


Fig. 13.15. a,**b** Coronal (30-µm) section through the anterior hippocampus, stained with cresyl violet. In comparison to normal controls (**a**), the 30-Gy irradiated sham animals (**b**) showed marked disruption of the cytoarchitecture of the hippocampal formation, large holes, and almost complete degeneration of the ìmbria (*FI*). **c**,**d** The brain parenchyma around the optic tract (*OT*) of a normal (**c**) animal shows an intact blood-brain barrier while the similar region from a 30-Gy irradiated (**d**) animal shows vascular leaks (*arrow*) after radiation treatment as demonstrated by HRP-tetramethylbenzidine reaction. **e** Cresyl violet stain of a transplant (*T*) in the third ventricle of a 30-Gy irradiated rat. The grafts survived well and often filled the ventricle. **f** Enlargement of an adjacent section to the boxed region in (**e**). The grafts contain TH positive neurons (*arrow*). (Reprinted with permission from [37])

you obtain histocompatible embryos?" The current answer lies in the rapid advance in cloning, creating stem cells from somatic cells by gene insertion and hoping these designer stem cells will transdifferentiate into different cell lines and unlock the DNA double helix to recapitulate its ontogeny.

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Risk Factors for Second Malignancies Following Stem Cell Transplant

14

Debra L. Friedman

CONTENTS

14.1 Introduction

Hematopoietic cell transplantation (HCT) offers potentially curative therapy for numerous malignancies, as well as immunologic, hematologic and metabolic disorders. While many patients are cured of their primary disease, a proportion develops post-transplant (secondary) malignant neoplasms (SMN) [2, 19, 29]. Individuals treated with HCT may have been exposed to pre-transplant chemotherapy or radiotherapy, then to additional cytotoxic therapy as part of the preparative regimen for transplantation, and eventually, to immune suppression. All of these factors may act alone or in concert to increase the risk for SMNs. Patients may also be innately cancer susceptible and have a genetic predisposition towards multiple primary malignancies. Potential risk factors for SMN following hematopoietic stem cell transplantation are listed in Tables 14.1 and 14.2.

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14.2 Risk Factors for SMN Following Autologous Versus Allogeneic Transplant

14.2.1 SMN Following Autologous HSCT

The incidence of hematologic malignancies and solid tumors following autologous transplantation varies widely across studies, with actuarial risks estimated from < 1% to 18% [14, 17, 19, 32]. Prior chemotherapy with large cumulative doses of alkylating agents as well as prior conventional radiotherapy are important risk factors for treatment-related or secondary MDS or leukemia (t-MDS, t-AML). In addition, patient age at transplant and the use of total body irradiation (TBI) in the preparative regimen, have been identi ied as risk factors. In some studies, patients transplanted with peripheral blood stem cells after chemotherapy priming showed a higher risk of t-MDS or t-AML than patients transplanted with cells isolated from the bone marrow without priming [35]. The incidence appears highest in patients treated for Hodgkin or non-Hodgkin lymphoma, an experience similar to that reported after conventional chemo- and radiotherapy for those diseases.

Table 14.1. General risk factors for post-transplant malignancies

Host	Disease	Treatment	Post-transplant complications	Exogenous exposures
Genetic predisposition Age at transplant Gender	Lymphoma > others	Pre-transplant therapy Total body irradiation Immunosuppressive agents Stem cell source	Graft vs. host disease Viral infections	Ultraviolet light Tobacco Alcohol Other carcinogens

Table 14.2. Post-transplant SMN specific risk factors

Type of SMN	Host	Primary disease	Treatment	Post-transplant complications
MDS/AML	Older patient age	Lymphoma	Alkylating agents Topoisomerase II inhibitors Nitrosoureas Peripheral blood stem cell source TBI Pre-transplant radiotherapy	GVHD
Hodgkin lymphoma	Unknown	Leukemia	Unknown	Acute GVHD Therapy for chronic GVHD
Skin and buccal mucosa	Male gender Older or younger patient age	Aplastic anemia	TBI	Acute or chronic GVHD
Solid tumors	Male gender Older or younger patient age Female donor	Aplastic anemia	TBI Azathioprine	Chronic GVHD

Metayer and colleagues [31] conducted a case-control study of 56 patients with t-MDS/AML and 168 matched controls within a cohort of 2,739 patients receiving autologous transplants for Hodgkin or non-Hodgkin lymphoma. In multivariate analyses, risks of t-MDS/AML significantly increased with the intensity of pre-transplantation chemotherapy with mechlorethamine or chlorambucil, compared with cyclophosphamide-based therapy. The use of TBI at doses of 12 Gy or less did not appear to increase the leukemia risk, but TBI doses of 13.2 Gy or higher increased the risk significantly. Peripheral blood stem cells were associated with a non-significantly increased risk of MDS/AML compared with bone marrow grafts [31].

In a series of 493 patients treated for NHL at The University of Texas M.D. Anderson Cancer Center, 22 patients developed tMDS or tAML. Multiple logistic regression analyses showed that TBI was independently associated with an increased risk of developing tMDS/tAML, and patients receiving TBI in combination with cyclophosphamide and etoposide were more likely to develop tMDS/tAML than patients who received TBI with cyclophosphamide or thiotepa [24].

In a series from the City of Hope National Medical Center, among 612 patients treated for lymphoma, 22 developed MDS or acute leukemia, with an estimated cumulative incidence of 8.6% ± 2.1% at 6 years. Multivariate analysis revealed stem cell priming with etoposide and pre-transplant radiotherapy to be significant risk factors [26].

Data related to 467 French patients treated with autologous transplantation for Hodgkin lymphoma was matched with 1179 conventionally treated patients listed in international databases. There were 18 secondary cancers, leading to a 5-year cumulative incidence of 8.9%. Risk factors for second cancer were age 40 years or older, the use of peripheral blood as a source of stem cells and treatment for re-

lapsed disease. Solid tumors were more frequent in patients treated with transplantation, although the incidence of t-MDS and AML was similar in the two groups [1].

14.2.2 SMN Following Allogeneic HSCT

Risks for specific types of SMN following allogeneic HSCT range from three- to 25-fold that of the general population, with cumulative incidences reaching 3%–11% at 10–15 years [7, 8, 11, 13, 15, 16, 19, 22, 29, 41]. In particular, the risks of melanoma and non-melanoma skin cancer and cancers of the oral cavity, liver, cervix, central nervous system, thyroid, bone and connective tissue are particularly elevated [7, 8, 11, 12, 41, 27]. Risk factors include the underlying diagnosis, pre-transplant therapy, transplantation for refractory or recurrent disease, the use of TBI and immunosuppressive agents and graft versus host disease (GVHD) [7, 8, 11, 13, 15, 16, 19, 29, 41]. Host factors include younger age at diagnosis in some series [11, 41] and, for squamous cell carcinoma of the buccal cavity and skin, male gender [11, 12, 15]. A recent series by Gallagher and Forrest [22] found older age at transplant and female donor to be a risk factor second solid tumors. Several large studies illustrate these risks.

In a review of 3,372 patients who underwent HSCT at the University of Minnesota between 1974 and 2001, 123 cases of SMN were reported, which represented an 8.1-fold increased risk over the that of the general population. This includes a significantly elevated risk for developing t-MDS or AML [standardized incidence ratio (SIR) = 300; 95% CI, 210 to 406], non-Hodgkin lymphoma including post-transplant lymphoproliferative disorder (PTLD; SIR = 54.3; 95% CI, 39.5–41.1), Hodgkin disease (SIR = 14.8; 95% CI, 3.9–32.9), or solid tumors overall (SIR = 2.8; CI, 2.0–3.7), and specifically for melanoma, brain, and oral cavity tumors. For t-MDS or AML, the cumulative incidence reached a plateau at 1.4% (95% CI, 0.9–1.9) by 10 years post-transplant. The cumulative incidence of developing a solid tumor did not plateau and was 3.8% (95% CI, 2.2–5.4) at 20 years post-transplant [2].

Among 2,129 patients who had undergone transplantation for hematologic malignancies at the City of Hope National Medical Center between 1976 and 1998, 29 developed solid cancers, which represented a two-fold increase in risk relative to a comparable normal population. The estimated cumulative incidence (± SE) for the development of a solid cancer was 6.1% ± 1.6% at 10 years. The risk was signi ìcantly elevated for liver cancer, cancer of the oral cavity, and cervical cancer. The risk was significantly higher for survivors who were younger than 34 years of age at time of transplant. Cancers of the thyroid gland, liver, and oral cavity occurred primarily among patients who received TBI. Again, there was no plateau noted in the incidence of solid tumors [8].

The risk of SMNs was reported by Curtis and colleagues [11] in 19,229 patients who received allogeneic (97.2%) or syngeneic transplants between 1964 and 1992 at one of 235 centers reporting to the International Bone Marrow Treatment Registry or the FHCRC. Among patients who survived 10 years or more, the risk of SMN was 8.3 times that of the general population. The cumulative incidence was 2.2% (95% CI 1.5, 3.0%) at 10 and 6.7% (95% CI 3.7, 9.6%) at 15 years. The risk was significantly elevated ($p < 0.05$) for malignant melanoma (SIR = 5.0), buccal cavity (SIR = 11.1), liver (SIR = 7.5), CNS (SIR = 7.6), thyroid (SIR = 6.6), bone (SIR = 13.4), and connective tissue cancers (SIR = 8.0). Younger age at time of transplant was associated with higher risk (p for trend < 0.001). In multivariate analyses, higher doses of TBI were associated with an increased risk. Chronic GVHD and male gender were strongly associated with an excess risk of squamous cell cancers of the buccal cavity and skin.

In a subsequent case-control study of 183 patients with subsequent solid cancers and 501 matched control patients within a cohort of 24,011 patients who underwent hematopoietic stem-cell transplantation (HSCT) at 215 centers, Curtis and colleagues [12] found that chronic GVHD and its therapy were strongly related to the risk for squamous cell carcinoma (SCC), but not for non-squamous cell carcinoma. Major risk factors for SCC were prolonged use of chronic GVHD therapy, use of azathioprine, particularly when combined with cyclosporine and steroids and severe chronic GVHD. Additional analyses determined that prolonged immunosuppressive therapy and azathioprine use were also significant risk factors for SCC of the skin and of the oral mucosa.

Among 18,531 patients receiving allogeneic transplant between 1964 and 1992 at the same centers, the risk of Hodgkin lymphoma was also signi ìcantly increased compared with the general population, even after excluding two human immunodeficiency virus-positive patients (observed

cases, $n = 6$; O/E = 4.7, 95% CI, 1.7–10.3). Mixed cellularity subtype predominated (five of eight cases, 63%). Five of six assessable cases contained Epstein-Barr virus (EBV) genome. Acute graft-versus-host disease (GVHD) or therapy for chronic GVHD were risk factors for post-transplant Hodgkin lymphoma [38].

Among 700 patients with severe aplastic anemia (AA) or Fanconi Anemia (FA), who received allogeneic HSCT at the Fred Hutchinson Cancer Research Center (FHCRC) or at Hôpital St. Louis in Paris, 23 developed malignancies at a median of 7.6 years after transplantation, with a cumulative incidence of 14% at 20 years. In univariate analysis, risk factors for solid tumors included the diagnosis of FA ($p = 0.0002$), use of azathioprine ($p < 0.0001$), radiotherapy ($p = 0.0002$), chronic GVHD ($p = 0.009$), acute GVHD ($p = 0.01$), and male gender ($p = 0.05$). In multivariate analysis, azathioprine therapy ($p < 0.0001$) and the diagnosis of FA ($p < 0.0001$) were statistically significant. Radiotherapy was statistically signi ìcant ($p = 0.004$) as a predictor only if the time-dependent variable azathioprine was not included in the analysis. Among non-FA patients, azathioprine ($p = 0.004$), age ($p = 0.03$), and radiotherapy ($p = 0.04$) were significant [15].

In an analysis of SMNs from the FHCRC, which included both allogeneic and autologous transplant, in a cohort of 5806 patients treated with transplant and who survived beyond 100 days, 381 SMNs were reported, excluding benign tumors, cancer in situ, or post-transplant lymphoproliferative disease. Using Cox proportion models, dose and fraction of TBI was analyzed, as well as the use of pre-transplant conventional radiotherapy. The analysis was adjusted for gender and age and found TBI to be a signi ìcant risk factor. As a simple yes/no factor, in adjusted analyses, the hazard of SMN among patients receiving TBI is 1.64 times (95% CI 1.3, 2.1) that of patients who did not receive TBI. Furthermore, use of TBI in a single dose increased the hazard by a factor of 2.3 (95% CI 1.6, 3.4) relative to patients receiving no TBI. Incorporation of the likelihood of pre-transplant radiation did not markedly increase the hazard ratio compared to TBI only. We also evaluated age at diagnosis. Using < 18 years as the reference group, hazard ratio for patients 18–39 years of age was 1.4 (95% CI 1.1, 1.9), and for those ≥ 40 years, 4.0 (95% CI 3.0, 5.5). The overall incidence of SMNs in our cohort was 17%, 6% and 13% at 10, 20 and 25 years, respectively. Patients with TBI exposure had a 25-year incidence of SMN of 21%, compared to 10% among patients not exposed to TBI. Risk continues to rise with elapsed time since transplant, without an obvious plateau. Of particular interest, for patients less than 10 years of age at HSCT, those with TBI exposure had a 25-year incidence of SMN of 19%, compared to 5% among patients not exposed to TBI. For patients ≥ 10 years of age at HSCT, those with TBI exposure had a 25-year incidence of SMN of 21%, compared to 11% among patients not exposed to TBI [19].

In a subsequent analysis of 8662 transplant recipients treated at the FHCRC, who survived at least 100 days post transplant, there were 1743 autologous and 6919 allogeneic HSCT recipients. Within this cohort, there were 56 SMNs amongst the autologous recipients and 224 amongst the allogeneic recipients. Cumulative incidence of SMN at 10 years post-HSCT was 2.6% in the allogeneic and 4.2% in the autologous HCT survivors. A multivariate Cox regression model adjusted for current age, TBI, gender and length of follow-up was fit and the hazard ratio (HR) for SMN for the allogeneic transplant survivors was 0.7 [95% confidence interval (CI) 0.5, 1.0] compared to the autologous transplant survivors (reference group), suggesting that the adjusted hazard of SMN is higher for autologous SCT recipients than for allogeneic. Risk factors appeared different between the two groups. For survivors of autologous transplantation, in multivariate Cox regression models, only age > 18 years at transplant was associated with decreased risk of SMN (< 18 years HR = 1.0; 18–39 years HR = 0.04; 40+ years HR = 0.004). Use of total body irradiation (TBI) was not significantly associated with risk among the autologous HCT recipients. For allogeneic transplant survivors, increased risk of SMN was associated with TBI and the effect of TBI was stronger for younger (< 18 years at HCT: HR = 4.6; 95% CI 1.6, 13.5) than for older (≥ 18 years; HR = 1.5; 95% CI 1.0, 2.3) HCT recipients (interaction $p = 0.04$) in multivariate Cox regression models. Risk was also increased after acute graft versus host disease (HR = 1.4; 95% CI 1.0, 1.9) and with ongoing follow-up time, with HRs of 1.7 (95% CI 1.1, 2.5) at 10–14 years, 2.2 (95% CI 1.3,3.7) at 15–19 years and 2.6 (95% CI 1.2, 5.4) at 20+ years of follow-up. Unrelated HSCT also increased risk of SMN (HR = 1.4; 95% CI 1.0, 2.0) [20].

The impact of patient-, disease-, treatment-, and toxicity-related factors on risk of basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) was determined in 4,810 patients who received allogeneic HCT at the FHCRC and who survived for at least 100 days. In this cohort, 237 developed at least one skin or mucosal cancer. The 20-year cumulative

incidences of BCC and SCC were 6.5% and 3.4%, respectively. Total-body irradiation was a significant risk factor for BCC, most strongly among patients younger than 18 years old at HCT. Light-skinned patients had an increased risk of BCC. Acute GVHD increased the risk of SCC, whereas chronic GVHD increased the risk of both BCC and SCC [27].

As risk for secondary breast cancer is elevated among cancer survivors treated with conventional therapy, a combined analysis at the FHCRC and the European Bone Marrow Transplant Registry evaluated the risk of breast cancer among 3337 female 5-year survivors who underwent an allogeneic hematopoietic cell transplantation. At total of 52 survivors developed breast cancer at a median of 12.5 (range: 5.7–24.8) years following HCT (SIR = 2.2). The 25-year cumulative incidence was 11.0%, higher among survivors who received total body irradiation (TBI) (17%) than those who did not receive TBI (3%). In multivariable analysis, increased risk was associated with longer time since transplantation (hazard ratio [HR] for 20+ years after transplantation = 10.8), use of TBI (HR = 4.0), and younger age at transplantation (HR = 9.5 for HCT <18 years). Hazard for death associated with breast cancer was 2.5 (95% CI: 1.1–5.8) [21].

14.3 Genetic Risk Factors for SMNs Following Transplant

Large numbers of patients are exposed to similar treatment exposures, yet only a small proportion develop second malignancies. Therefore, there may exist genetic risk factors that interact with such exposures to increase risk of SMN.

14.3.1 Radiation Exposure and Sensitivity

There is inter-individual variation in radiation sensitivity, evidenced by different degrees of skin erythema, fibrosis, and telangiectasia following radiotherapy [44]. It is therefore likely that a number of genetic elements work in concert to determine individual response to radiotherapy. Radiation sensitivity assays on ibroblasts and lymphocytes from apparently normal individuals have established at least a three-fold range in inter-individual sensitivity. Radiosensitivity is distributed normally in the population [33, 44]. There is growing evidence that variation in the degree of radiation sensitivity is genetically determined. Family members of radiosensitive individuals are more likely to be radiosensitive than unrelated individuals [37]. There are also data to suggest that individuals who develop malignancies are radiation-sensitive [3, 6, 9, 10, 23, 34, 39]. Furthermore, there is evidence of a correlation between radiation sensitivity and susceptibility to cancer [4, 5, 18]. What remains unclear is who is at risk for cancer following radiation therapy, what proportion of patients with SMNs have increased radiation sensitivity, what genetic elements contribute to sensitivity, and how sensitivity is most effectively characterized.

14.3.2 Genetic Biomarkers for Second Malignancies Following HCT and Radiation Therapy

In identifying genetic biomarkers of susceptibility for SMN, several classes of genes can be considered. There are genes that confer high individual, low population attributable risk, such as the tumor suppressor genes RB1 or TP53, where genetic susceptibility to cancer has been described in patients with germline mutations [25, 28, 30, 43, 42, 45]. However, these do not explain most of SMN occurrence. Other genes to consider are those that may have a high population attributable risk, because of the widespread occurrence of alleles with altered function. The cancer phenotype is generated in the presence both of specific environmental influences and the specific allelic variant. Examples are genes involved in DNA repair and in provision of nucleotides. Allelic variants may alter the way in which DNA damage from radiotherapy is repaired and thus may be markers for those susceptible to SMNs [36].

14.3.3 Genetic Susceptibility to Toxicity from Combined Cancer Therapy and Environmental Carcinogens: Common Pathways of Metabolism, DNA Damage and Repair

Patients treated with HCT are exposed to cytotoxic therapy. Some of them are also exposed to known carcinogens such as components of tobacco or UV

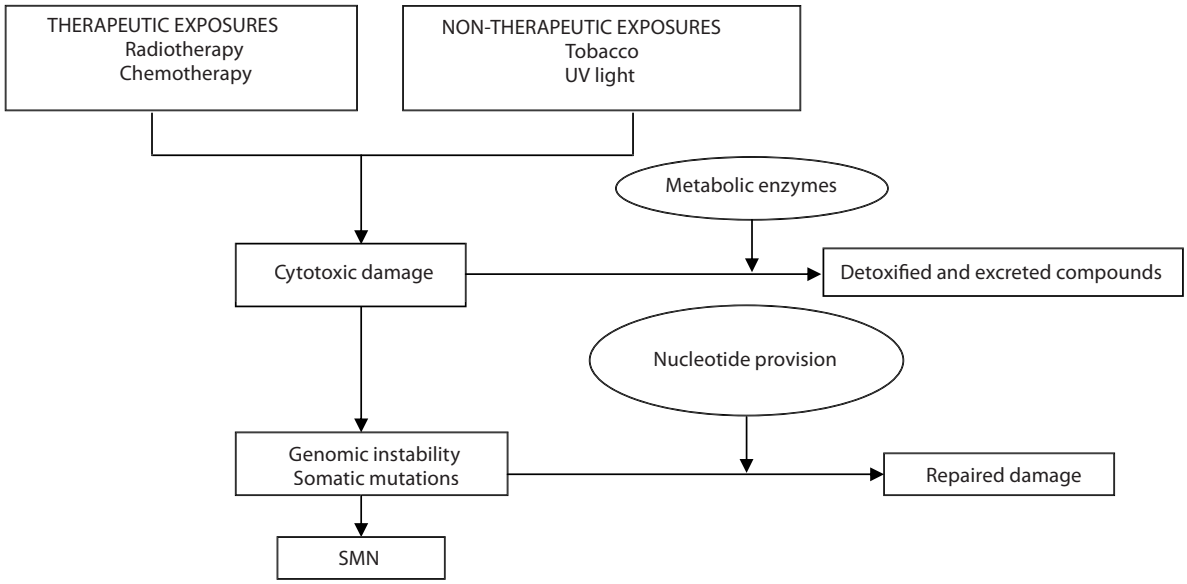

Fig. 14.1. Interaction of therapeutic and environmental exposures in SMN pathogenesis

light. This can lead to genomic instability, somatic mutations and, ultimately, malignancy. Opposing this likelihood are functional enzymes in xenobiotic metabolism, DNA repair and nucleotide provision. Specific allelic variants of these genes may result in enzymes with either increased or decreased activity, which in turn may modify the risk of SMN. This is outlined in Figure 14.1. Therefore, future research should include a simultaneous analysis of treatment, environmental and genetic risk factors, which, acting in concert may increase the risk of SMN post HCT.

14.4 Conclusions

The proportion of second cancers among all cancers in the United States has more than doubled in the past 20 years. With improving survivorship, this percentage is likely to increase. The role of genetic risk factors remains relatively unknown and is best evaluated in the context of treatment and disease-related risk factors, as well as health-related risk behaviors known to promote cancer, such as smoking, alcohol exposure, sun exposure and dietary risk factors. Identifying markers of cancer susceptibility after exposure to carcinogenic therapeutic agents can result in several important outcomes. Patients who harbor a genetic predisposition to subsequent cancers can be more closely monitored during their lifetime, and counseled regarding avoidance of potential co-carcinogens that may share similar metabolic pathways. Treatment for primary malignancies may be altered in those with identified inherited high-risk genotypes. Family members may be at a similarly increased risk of specific malignancies, when exposed to speci ic carcinogens and may require more targeted preventive strategies and lifetime monitoring for development of malignancy. Specific emphasis can be directed towards the interaction between environmental and therapeutic exposures and genetic susceptibility. The knowledge gained from these lines of investigation will, in part, be generalizable to the larger population of cancer survivors, treated with conventional radiotherapy and at risk for SMN, and at the very least will generate hypotheses.

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