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PrefaceThe radiation oncology community has received the three previous editions of *Clinical Radiation Oncology* very well, and it has become the standard radiation oncology textbook for many physicians. For the fourth edition of *Clinical Radiation Oncology*, the intent is to maintain the many excellent features of the previous editions while adding some new features, new chapters and chapter authors, and one new Associate Editor. The most exciting new feature is that the chapters in the online version of the fourth edition of *Clinical Radiation Oncology* (www.expertconsult.com) will be periodically updated.The fourth edition again has separate sections on Scientific Foundations of Radiation Oncology, Techniques and Modalities, and Disease Sites. Within the section on Scientific Foundations of Radiation Oncology, a new chapter has been added, “Radiation Physics: Stereotactic.” In the section on Techniques and Modalities, the chapter on stereotactic irradiation has been divided into separate chapters, “Stereotactic Irradiation: CNS Tumors” and “Stereotactic Body Irradiation: Extracranial Tumors,” in view of the expanded interest in and outcomes data for stereotactic body irradiation.The associate editors for Disease Sites chapters were an important component of the success of the three previous editions and have been maintained. One new associate editor has been selected for the fourth edition—Dr. Andrea Ng for Lymphoma and Hematologic Malignancies. Associate editors are involved in the selection of chapter authors and in editing the chapters for scientific content and accuracy. For most Disease Sites, the associate editors also wrote an Overview that allowed them to discuss issues common to various Disease Sites within the section and to give their unique perspective on important issues.Features that are retained within Disease Sites chapters include an opening page format summarizing the most important issues, a full-color format throughout each chapter, liberal use of tables and figures, and a closing section with a discussion of controversies and problems and a treatment algorithm that reflects the treatment approach of the authors. Chapters have been edited not only for scientific accuracy, but also for organization, format, and adequacy of outcome data (disease control, survival, and treatment tolerance).We are again indebted to the many national and international experts who contributed to the fourth edition of *Clinical Radiation Oncology* as associate editors, senior authors, or co-authors. Their outstanding efforts combined with ours will hopefully allow this new edition to be a valuable contribution and resource in the coming years.
Leonard L. Gunderson
Joel E. Tepper AcknowledgmentsWe wish to thank our wives, Katheryn and Laurie, and Dr. Tepper's secretary, Betty Bush, for their patience and assistance during the many months we were involved in the preparation of the fourth edition of *Clinical Radiation Oncology*.We also thank the associate editors and the many chapter senior authors and co-authors for their time, efforts, and outstanding contributions to this edition.We acknowledge the editors and production staff at Elsevier, especially Kate Dimock, Deidre Simpson, and Rachel McMullen, who have done an outstanding job in collating and producing the fourth edition of *Clinical Radiation Oncology*. Video Contents
Video 1: Intraoperative Irradiation
Video 2: Prostate Brachytherapy
Video 3: Ocular Melanoma
Video 4: Penile Brachytherapy **Section I**Scientific Foundations of Radiation Oncology**Outline**Part A RadiobiologyPart B PhysicsPart C Related Cancer Disciplines **Part A**Radiobiology**Outline**Chapter 1 The Biological Basis of Radiation OncologyChapter 2 Molecular and Cellular BiologyChapter 3 Dose-Response Modifiers in Radiation TherapyChapter 4 Interaction of Chemotherapy and RadiationChapter 5 Biologics and Their Interactions with Radiation **Chapter 1**The Biological Basis of Radiation Oncology*Elaine M. Zeman*What Is Radiation Biology?In the most general sense, *radiation biology is the study of the effects of electromagnetic radiation on biological systems*. Three aspects of this definition deserve special mention. First, effects may include everything from DNA damage to genetic mutations, chromosome aberrations, cell killing, disturbances in cell cycle

transit and cell proliferation, neoplastic transformation, early and late effects in normal tissues, teratogenesis, cataractogenesis, and carcinogenesis, to name but a few. Electromagnetic radiation refers to any type of radiant energy in motion with wave or particulate characteristics that has the capacity to impart some or all of its energy to the medium through which it passes. The amount of energy deposited can vary over some 25 orders of magnitude, depending on the type of electromagnetic radiation. For example, 1 kHz radio waves have energies in the range of 10−11 eV to 10−12 eV, whereas x- or γ-rays may have energies upwards of 10 MeV or more. The more energetic forms of electromagnetic radiation, the ionizing radiations, deposit energy as they traverse the medium by setting secondary particles in motion that can go on to produce further ionizations. Finally, biological systems may be, for example, simple cell-free extracts of biomolecules, or increasingly complex, from prokaryotes to single-celled eukaryotes, to mammalian cells in culture, to tissues and tumors in laboratory animals or humans, to entire ecosystems.Radiotherapy-oriented radiobiology focuses on that portion of the electromagnetic spectrum energetic enough to cause ionization of atoms. This ultimately results in the breaking of chemical bonds that can lead to damaging important biomolecules. The most significant effect of ionizing radiation in this context is cell killing, which directly or indirectly is at the root of nearly *all* of the normal tissue and tumor responses noted in patients.Cytotoxicity is not the only significant biological effect caused by radiation exposure, although it will be the main focus of this chapter. Other important radiation effects, carcinogenesis, for example, will also be discussed, although the reader should be aware that radiation carcinogenesis is a large discipline in and of itself, involving investigators from fields as diverse as biochemistry, toxicology, epidemiology, environmental sciences, molecular biology, tumor biology, health and medical physics, as well as radiobiology. Most radiation protection standards are based on minimizing the risks associated with mutagenic and carcinogenic events. Radiologic health professionals therefore are de facto educators of and advocates for the general public when it comes to ionizing radiation and need to be fully conversant in the potential risks and benefits of medical procedures involving radiation.Finally, the majority of this chapter will be devoted to so-called classical radiobiology, that is, studies that largely predate the revolution in molecular biology and biotechnology during the 1980s and 1990s. Although the reader might be tempted to view this body of knowledge as rather primitive by today's standards, relying too heavily on phenomenology, empiricism, and descriptive models and theories, the real challenge is to integrate the new biology into the already existing framework of classical radiobiology; this will be discussed in detail in Chapter 2.Radiotherapy-Oriented Radiobiology: A Conceptual FrameworkBefore examining any one aspect of radiobiology in depth, it is important to introduce several general concepts to put the information in its proper perspective.The Therapeutic RatioThe most fundamental of these concepts is what is termed the *therapeutic ratio*, which is in essence a risk-versus-benefit approach to planning a radiotherapy treatment regimen. Many of the radiobiological phenomena to be discussed in this chapter are thought to play important roles in optimizing, or at least fine-tuning the therapeutic ratio. In theory, it should be possible to eradicate any malignant tumor simply by delivering a sufficiently high dose of radiation. Of course in practice, the biological consequences for normal tissues that are necessarily irradiated along with the tumor limit the total dose that can be safely administered. As such, a balance must be struck between what is deemed an acceptable probability of a radiation-induced complication in a normal tissue and the probability of tumor control. Ideally, one would hope to achieve the maximum likelihood of tumor control that does not produce unacceptable normal tissue damage.The concept of therapeutic ratio is best illustrated graphically by comparing dose-response curves for both tumor control and normal tissue complication rates plotted as a function of dose. Examples of this approach are shown in Figure 1-1, for cases in which the therapeutic ratio is either “unfavorable,” “favorable,” or “optimal,” bearing in mind that these are theoretical curves. Actual dose-response curves derived from experimental or clinical data are much more variable, particularly for tumors, which tend to show much shallower dose responses.1 This serves to underscore how difficult it can be in practice to assign a single numerical value to the therapeutic ratio in any given situation.

Figure 1-1 Illustrating the concept of therapeutic ratio under conditions in which the relationship between the normal tissue tolerance and tumor control dose-response curves is unfavorable *(upper panel)*, favorable *(middle panel)*, and optimal *(lower panel)*.Many of the radiobiological properties of cells and tissues can have a favorable or adverse effect on the therapeutic ratio. Therefore, in planning a course of radiation therapy, the goal should be to optimize the therapeutic ratio as much as possible; in other words, using our graphical approach, increase the separation between the tumor control and normal tissue complication curves. This can be accomplished either by shifting the tumor control curve to the left with respect to the dose axis (toward lower doses, that is, radiosensitization), the normal tissue complication curve to the right (toward higher doses, that is, radioprotection), or perhaps, some combination of both. The key however is to shift these curves *differentially*, which is not necessarily an easy task given that there are not that many exploitable differences in the radiobiology of cells derived from tumors and those derived from normal tissues.The Radiation Biology “Continuum”There is a surprising continuity between the physical events that occur in the first few femtoseconds after ionizing radiation interacts with the atoms of a biomolecule and the ultimate consequences of that interaction on tissues. The consequences themselves may not become apparent until days, weeks, months, or even years after the radiation exposure. Some of the important steps in this radiobiology continuum are listed in Table 1-1. The orderly progression from one stage of the continuum to the next—from physical to physico-chemical to biochemical to biological—is particularly noteworthy not only because of the vastly different time scales over which the critical events occur, but also because of the increasing biological complexity associated with each of the endpoints or outcomes. Each stage of the continuum also offers a unique radiobiological window of opportunity: the potential to intervene in the process and thereby modify all the events and outcomes that follow.**TABLE 1-1Stages in the Radiobiology Continuum**Levels of Complexity in Radiobiological SystemsAnother important consideration in all radiobiological studies is the nature of the experimental system used to study a particular phenomenon, the assay(s) used, and the endpoint(s) assessed. For example, one investigator may be interested in studying DNA damage caused by ionizing radiation, and in particular, the frequency of DNA double-stranded breaks (DSBs) produced per-unit dose. As an experimental system, he or she might choose DNA extracted from mammalian cells. Then, using a DNA elution assay, the rate at which the irradiated DNA passes through a semipermeable membrane is measured as an endpoint and compared with the rate of elution of DNA extracted from cells that had not been previously irradiated. DNA containing more DSBs elutes faster than DNA containing fewer breaks, allowing a calibration curve to be generated that relates the dose received to the elution rate. A second investigator, on the other hand, may be interested in improving the control rate of head and neck cancers with radiation therapy by employing a nonstandard fractionation schedule. In this case, the type of experiment would be a clinical trial. The experimental system would be a cohort of patients, some of whom are randomized to receive nonstandard fractionation, and the rest, standard fractionation. The endpoints assessed could be one or more of the following: loco-regional control, long-term survival, disease-free survival, normal tissue complication frequency, and so on and evaluated at specific times after completion of the radiation therapy.In considering both the strengths and weaknesses of these two investigators' studies, any number of pertinent questions may be asked. Which is the more complex or heterogeneous system? Which is the more easily manipulated and controlled system? Which is more relevant for the day-to-day practice of radiation therapy? What kinds of results are gleaned from each, and can these results be obtained in a timely manner? In this example, it is clear that human patients with spontaneously arising tumors represent a far more heterogeneous and complex experimental system than an extract of mammalian DNA. On the other hand, the DNA system is much more easily manipulated, possible confounding factors can be more easily controlled, and the measurement of the desired endpoint (elution rate) plus the data analysis can be completed within a day or two. Obviously, this is not the case with the human studies, in which numerous confounding factors can and do influence results, manipulation of the system can be difficult if not impossible, and the experimental results typically take years to obtain.The issue of relevance is an

even thornier one. Arguably, both studies are relevant to the practice of radiation therapy insofar as the killing of cells is at the root of normal tissue and tumor toxicity caused by radiation, and that cell killing usually is, either directly or indirectly, a consequence of irreparable damage to DNA. As such, any laboratory findings that contribute to the knowledge base of radiation-induced DNA damage are relevant. Clearly however, clinical trials with human patients not only are a more familiar experimental system to radiation oncologists, but also efficacy in cancer patients is, ultimately, what leads to new standards of care in clinical practice, and becomes the gold standard against which all newer therapeutic strategies are judged.All things considered then, there is a time and place both for relatively simple systems and more complex ones. The relatively simple, homogeneous, and easily manipulated systems are best suited for the study of the mechanisms of radiation action, such as measuring DNA or chromosomal damage, changes in gene expression, perturbations of the cell cycle, or the survival of irradiated cells maintained in culture. The more complicated and heterogeneous systems, with their unique endpoints, are more clinically relevant, such as assays of tumor control or normal tissue complication rates. Both types of assay systems have inherent strengths and weaknesses, yet both are critically important if we hope to improve the practice of radiation therapy based on sound biological principles.Tissue HeterogeneityWhy is radiation therapy successful at controlling one patient's tumor but not another's, even when the two tumors seem identical? Why are we generally more successful at controlling certain types of cancers than others? The short answer to such questions is that, although the tumors may appear identical macroscopically, their component cells may be quite different phenotypically or genotypically. Also there may be important differences between the two patients' normal tissues.Normal tissues, being composed of more than one type of cell, are somewhat heterogeneous, and tumors, owing both to the genetic instability of individual tumor cells and to microenvironmental differences, are heterogeneous. Different subpopulations of cells have been isolated from many types of human and experimental cancers, and these may differ in antigenicity, metastatic potential, and sensitivity to radiation and chemotherapy (for reviews, see Heppner et al[2], Suit et al[3]). This heterogeneity is manifest both within a particular patient, and to a much greater extent, between patients with otherwise similar tumors. Both intrinsic and extrinsic factors contribute to this heterogeneity. Intrinsic factors may include the following: inherent radiosensitivity, gene expression, biochemical repair processes, modes of cell death (e.g., mitotic catastrophe versus apoptosis), genomic instability, cell cycle kinetics, and how the tissue is structurally and functionally arranged. Extrinsic factors, on the other hand, tend to be related to physiological differences between tissues, such as the degree of vascularity, availability of oxygen and nutrients, pH, energy charge, and the proximity of, and degree of contact between, normal host tissue and the tumor.What are the practical implications of normal tissue and tumor heterogeneity? First, if one assumes that normal tissues are the more uniform and predictable in behavior of the two, then tumor heterogeneity is responsible, either directly or indirectly, for most radiotherapy failures. If so, this suggests that a valid clinical strategy might be to identify the radioresistant subpopulation(s) of tumor cells, and then, tailor therapy specifically to cope with them. This approach is much easier said than done. Some clinical studies—both prospective and retrospective—now include one or more determinations of, for example, extent of tumor hypoxia,[4,5] or potential doubling time of tumor clonogens.[6] And although such measurements are not done routinely (because of their labor and intensiveness and longer turn-around times for results), the hope is that someday subsets of patients bearing tumors with different biological characteristics will be able to be assigned prospectively to different treatment groups.Another consequence of tissue heterogeneity is that any radiobiological endpoint measured in an intact tissue is necessarily related to the radiosensitivities of all the subsets of cells, plus all the other intrinsic and extrinsic factors contributing to the overall response of the tissue. And because data on normal tissue tolerances and tumor control probabilities are also averaged across large numbers of patients, heterogeneity is even more pronounced.Powers of TenTumor control is achieved only when all clonogenic cells are killed or otherwise rendered unable to sustain tumor growth indefinitely. To estimate the likelihood of cure, it is necessary to know, or at least have an appreciation for, approximately how many clonogenic cells the tumor contains, how

radiosensitive these cells are (i.e., some measure of killing efficiency per unit radiation dose), and what the relationship is between the number of clonogenic cells remaining after treatment and the probability of recurrence. The latter is perhaps the easiest to ascertain, given our knowledge of both the random and discrete nature of radiation damage and the general shape of dose-response curves for mammalian cells and tissues. For a given number of surviving cells per tumor, the probability of local control can be derived from Poisson statistics using the equation P = e−n, where *P* is the tumor control probability and *n* is the average number of surviving clonogenic tumor cells. For example, when, for a large number of tumors, an average of one clonogenic cell per tumor, remains at the end of radiation therapy, the tumor control rate will be about 37%, meaning that about 6 out of 10 tumors of the same size and relative radiosensitivity will recur. Should the treatment reduce clonogenic cell numbers to an average of 0.1 per tumor, the tumor control probability would increase to 90%, 0.05 per tumor, 95%, and 0.01 per tumor, 99%, respectively.The tumor control probability for a given fraction of surviving cells is not particularly helpful if the total number of cells at risk is unknown, however, and this is where an understanding of logarithmic relationships and exponential cell killing is useful. Based on the resolution of existing tools and technology for cancer detection, let us assume that a 1-cm3 (1-g) tumor mass can be identified reliably. A tumor of this size has been estimated to contain approximately 109 cells,[7] admittedly a theoretical value that assumes all cells are perfectly “packed” and uniformly sized and that the tumor contains no stroma. A further assumption, that all such cells are clonogenic (which is rarely, if ever, the case), suggests that at least 9 logs of cell killing would be necessary before any appreciable tumor control (about 37%) would be achieved, and 10 logs of cell killing would be required for a high degree of tumor control (i.e., 90%).After the first log or two of cell killing however, some tumors respond by shrinking, which is a partial response. After two to three logs of cell killing, the tumor may shrink to a size below the current limits of clinical detection, that is, a complete response. Although partial and complete responses are valid clinical endpoints, a complete response does not necessarily mean tumor cure. At least six more logs of cell killing would still be required before any significant probability of cure would be expected. This explains why radiation therapy is not halted if the tumor disappears during the course of treatment; this concept is illustrated graphically in Figure 1-2.

Figure 1-2 The relationship between radiation dose and tumor cell survival during fractionated radiotherapy of a hypothetical, 1-g tumor containing 109 clonogenic cells. Although a modest decrease in cell surviving fraction can cause the tumor to shrink (partial response) or disappear below the limits of clinical detection (complete response), few if any cures would be expected until at least 9 logs of clonogenic cells have been killed. In this example, a total dose of at least 60 Gy delivered daily, 2 Gy fractions would be required to produce a tumor control probability of 0.37, assuming each dose reduced the surviving fraction to 0.5. Adapted from Steel G, Adams G, Peckham M, editors: The biological basis of radiotherapy, New York, 1983, Elsevier.Finally, it should be noted that although the goal of curative radiation therapy is to reduce tumor cell survival by at least nine logs, even for the smallest tumor likely to be encountered, it is much less clear how many logs of cell killing a particular normal tissue can tolerate before it loses its structural or functional integrity. This would depend on how the tissue is organized structurally, functionally, and proliferatively; which constituent cells are the most and least radiosensitive; and which cells are the most important to the integrity of the tissue. It is unlikely however that many normal tissues could tolerate a depletion of two logs (99%) of their cells, let alone nine or more logs.Radiation Biology and Therapy: The First 50 YearsIn less than four years after the discovery of x-rays by Roentgen,[8] radioactivity by Becquerel,[9] and radium by the Curies,[10] the new modality of cancer treatment known as radiation therapy claimed its first apparent cure of skin cancer.[11] Today, nearly 120 years later, radiotherapy is most commonly given as a series of small daily dose fractions of approximately 1.8 to 2.0 Gy each, 5 days per week, over a period of 5 to 7 weeks to total doses of 50 to 70 Gy. Although it is true that the historical development of this conventional radiotherapy schedule was empirically based, there were a number of early radiobiological experiments that suggested this approach.In the earliest

days of radiotherapy, both x-rays and radium were used for cancer treatment. Because of the greater availability and convenience of using x-ray tubes and the higher intensities of radiation output achievable, it was fairly easy to deliver large single doses in short overall treatment times. Thus, from about 1900 to the 1920s, this “massive-dose technique”12 was a common way of administering radiation therapy. Unfortunately, normal tissue complications were often quite severe, and to make matters worse, the rate of local tumor recurrence was still unacceptably high.Radium therapy was used more extensively in France. Because of the low activities available, radium applications necessarily involved longer overall treatment times to reach comparable total doses. Although extended treatments were less convenient, clinical results were often superior. Perceiving that the change in overall time was the critical factor, physicians began to experiment with the use of multiple, smaller x-ray doses delivered over extended periods. By that time, there was already a radiobiological precedent for expecting improvement in tumor control when radiation treatments were protracted.As early as 1906, Bergonié and Tribondeau observed histologically that the immature, dividing cells of the rat testis showed evidence of damage at lower radiation doses than the mature, nondividing cells of the stroma.13 Based on these observations, they put forth some basic laws, stating that x-rays were more effective on cells that were actively dividing, likely to continue to divide indefinitely, and poorly differentiated.13 Because tumors were already known to contain cells that not only were less differentiated, but also exhibited greater mitotic activity, they reasoned that several radiation exposures might preferentially kill these tumor cells, but not their slowly proliferating, differentiated counterparts in the surrounding normal tissues.The end of common usage of the single-dose technique in favor of fractionated treatment came during the 1920s as a consequence of the pioneering experiments of Claude Regaud et al.14 Using the testes of the rabbit as a model tumor system (because the rapid and unlimited proliferation of spermatogenic cells simulated to some extent the pattern of cell proliferation in malignant tumors), Regaud showed that only through the use of multiple, smaller radiation doses could animals be completely sterilized without producing severe injury to the scrotum.15 Regaud suggested that the superior results afforded the multifraction irradiation scheme were related to alternating periods of relative radioresistance and sensitivity in the rapidly proliferating germ cells.16 These principles were soon tested in the clinic by Henri Coutard, who first used fractionated radiotherapy for the treatment of head and neck cancers, with, comparatively speaking, spectacularly improved results.17,18 Largely as a result of these and related experiments, fractionated treatment subsequently became the standard form of radiation therapy.Time-dose equivalents for skin erythema published by Reisner,19 Quimby and MacComb,20 and others21,22 formed the basis for the calculation of equivalents for other tissue and tumor responses. By plotting the total doses required for each of these “equivalents” for a given level of effect in a particular tissue (as a function of a treatment parameter such as overall treatment time, number of fractions, dose per fraction, etc.) an isoeffect curve could be derived. All time-dose combinations that fell along such a curve would, theoretically, produce tissue responses of equal magnitude. Isoeffect curves, relating the total dose to the overall treatment time, which were derived in later years from some of these data,23 are shown in Figure 1-3.

Figure 1-3 Isoeffect curves relating the log of the total dose to the log of the overall treatment time for various levels of skin reaction, and the cure of skin cancer. **A,** Isoeffect curves constructed by Cohen, based on a survey of previously published data on radiotherapy equivalents.19-22,189 See text for details. The slope of the curves for skin complications was 0.33, and that for tumor control, 0.22. **B,** Strandqvist's24 isoeffect curves, first published in 1944. All lines were drawn parallel, and had a common slope of 0.33. **A,** Adapted from Cohen L: Radiation response and recovery: Radiobiological principles and their relation to clinical practice. In Schwartz E, editor: The biological basis of radiation therapy, Philadelphia, 1966, JB Lippincott, p 208; **B,** adapted from Strandqvist M: Studien uber die kumulative Wirkung der Roentgenstrahlen bei Fraktionierung. Acta Radiol Suppl 55:1, 1944.The first published isoeffect curves were produced by Strandqvist in 194424 and are also shown in Figure 1-3. When transformed on log-log coordinates, isoeffect curves for a variety of skin reactions and the cure of skin cancer were drawn

as parallel lines, with common slopes of 0.33. These results implied that there would be no therapeutic advantage to using prolonged treatment times (i.e., multiple small fractions versus one, or a few, large doses) for the preferential eradication of tumors while simultaneously sparing normal tissues.25 It was somewhat ironic that the Strandqvist curves were so popular in the years that followed because it was already known that the therapeutic ratio *did* increase (at least to a point) with prolonged, as opposed to very short, overall treatment times. However, the overarching advantage was that these isoeffect curves were quite reliable at predicting skin reactions, which were the dose-limiting factors at that time.The Golden Age of Radiation Biology and Therapy: The Second 50 YearsPerhaps the defining event that ushered in the golden age of radiation biology was the publication of the first survival curve for mammalian cells exposed to graded doses of ionizing radiation.26 This first report of a quantitative measure of intrinsic radiosensitivity of a human cell line (HeLa, derived from a cervical carcinoma27), was published by Puck and Marcus in 1956.26 To put this seminal work in the proper perspective, however, it is first necessary to review the physico-chemical basis for why ionizing radiation is toxic to biological materials.The Interaction of Ionizing Radiation with Biological MaterialsAs mentioned in the introductory section of this chapter, ionizing radiation deposits energy as it traverses the absorbing medium through which it passes. The most important feature of the interaction of ionizing radiation with biological materials is the random and discrete nature of the energy deposition. Energy is deposited in increasingly energetic packets referred to as “spurs” (100 eV or less deposited), “blobs”(100 to 500 eV), or “short tracks” (500 to 5000 eV), each of which can leave from approximately three to several dozen ionized atoms in its wake. This is illustrated in Figure 1-4, along with a segment of (interphase) chromatin shown to scale. The frequency distribution and density of the different types of energy deposition events along the track of the incident photon or particle are measures of the radiation's linear energy transfer or LET (see also the “Relative Biological Effectiveness” section later in this chapter). Because these energy deposition events are discrete, it follows that although the average energy deposited in a macroscopic volume of biological material is rather modest, the distribution of this energy on a microscopic scale may be quite large. This explains why ionizing radiation is so efficient at producing biological damage; the total amount of energy deposited in a 70-kg human that will result in a 50% probability of death is only about 70 calories, which is about as much energy as is absorbed by drinking one sip of hot coffee.28 The key difference is that the energy contained in the sip of coffee is uniformly distributed, not random and discrete.

Figure 1-4 Hypothetical α-particle track through an absorbing medium, illustrating the random and discrete energy deposition events along the track. Each event can be classified according to the amount of energy deposited locally, which in turn determines how many ionized atoms will be produced. A segment of chromatin is also shown, approximately to scale. Adapted from Goodhead DT: Physics of radiation action: microscopic features that determine biological consequences. In Hagen U, Harder D, Jung H, et al, editors: Radiation research 1895-1995, Proceedings of the 10th International Congress of Radiation Research. Volume 2: Congress Lectures, Wurzburg, 1995, Universitatsdruckerei H. Sturtz AG, pp 43-48.Those biomolecules receiving a direct hit from a spur or blob receive, relatively speaking, a huge radiation dose, that is, a large energy deposition in a small volume. For photons and charged particles, this energy deposition results in the ejection of orbital electrons from atoms, causing the target molecule to be converted first into an ion pair and then into a free radical. Further, the ejected electrons—themselves energetic charged particles—can go on to produce additional ionizations. For uncharged particles such as neutrons, the interaction is between the incident particles and the nuclei of the atoms in the absorbing medium, causing the ejection of recoil protons (charged) and lower energy neutrons. The cycle of ionization, free radical production, and release of secondary charged particles continues until all the energy of the incident photon or particle is expended. These interactions are complete within a picosecond after the initial energy transfer. After that time, the chemical reactions of the resulting free radicals predominate the radiation response (see below).Any and all cellular molecules are potential

targets for the localized energy deposition events that occur in spurs, blobs, or short tracks. Whether the ionization of a particular biomolecule results in a measurable biological effect depends on a number of factors including how probable a target the molecule represents from the point of view of the ionizing particle, how important the molecule is to the continued health of the cell, how many copies of the molecule are normally present in the cell and to what extent the cell can react to the loss of working copies, how important the cell is to the structure or function of its corresponding tissue or organ, and so on. DNA for example is obviously an important cellular macromolecule and one that is present only as a single, double-stranded copy. On the other hand, other molecules in the cell may be less crucial to survival, yet are much more abundant than DNA, and therefore have a much higher probability of being hit and ionized. By far, the most abundant molecule in the cell is water, comprising some 70% to 80% of the cell on a per-weight basis. The highly reactive free radicals formed by the radiolysis of water are capable of augmenting the DNA damage resulting from direct energy absorption by migrating to the DNA and damaging it indirectly. This mechanism is referred to as indirect radiation action to distinguish it from the aforementioned direct radiation action.29 The direct and indirect action pathways for ionizing radiation are illustrated next.*Direct EffectIndirect Effect*The most highly reactive and damaging species produced by the radiolysis of water is the hydroxyl radical (•OH), although other free radical species are also produced in varying yields.30,31 Cell killing by indirect action constitutes some 70% of the total damage produced in DNA for low LET radiation.How do the free radicals produced by the direct and indirect action of ionizing radiation go on to cause the myriad lesions that have been identified in irradiated DNA? Because they contain unpaired electrons, free radicals are highly reactive chemically and will undergo multiple reactions in an attempt to either acquire new electrons, or rid themselves of remaining unpaired ones. These reactions are considered quite slow compared to the time scale of the initial ionization events but are still fast relative to normal enzymatic processes in a typical mammalian cell. For all intents and purposes, free radical reactions are complete in milliseconds after irradiation. The •OH radical is capable of both abstraction of hydrogen atoms from other molecules and addition across carbon-carbon or other double bonds. More complex macromolecules that have been converted to free radicals can undergo a series of transmutations in an attempt to rid themselves of unpaired electrons, many of which result in the breakage of nearby chemical bonds. In the case of DNA, these broken bonds may result in the loss of a base or an entire nucleotide, or a frank scission of the sugar phosphate backbone, involving either one or both DNA strands. In some cases, chemical bonds are broken initially but then are rearranged, exchanged, or rejoined in inappropriate ways. Bases in DNA may be modified by the addition of one or more hydroxyl groups (e.g., the base thymine converted to thymine glycol), pyrimidines may become dimerized, or the DNA may become cross-linked to itself or to associated protein components. And again, because the initial energy deposition events are discrete, the free radicals produced also are clustered, and therefore, undergo their multiple chemical reactions and produce multiple damages in a highly localized area. This has been termed the “multiply-damaged site”32 or “cluster”33 hypothesis. Examples of the types of damage found in irradiated DNA are shown in Figure 1-5.

Figure 1-5 Types of DNA damage produced by ionizing radiation. **A,** Segment of irradiated DNA containing single- and double-stranded breaks, cross-links, and base damage. **B,** Two types of modified bases observed in irradiated DNA include thymine glycol, which results from the addition of two hydroxyl (OH) groups across the carbon-carbon double bond of thymine, and 8-hydroxyguanine, produced by •OH radical addition to guanine.Biochemical Repair of DNA DamageDNA is unique insofar as it is the only cellular macromolecule with its own repair system. Until as recently as 30 years ago, little was known about DNA repair processes in mammalian cells, particularly because of the complexities involved and the relative lack of spontaneously occurring mutants defective in genes involved with DNA repair. As a result, most studies of DNA repair were carried out either in bacteria or yeasts and usually employed ultraviolet (UV) radiation as the tool for producing DNA damage. Although these were rather simple and relatively clean systems in which to study DNA repair, their relevance to mammalian repair systems, and to the broader

spectrum of DNA damage produced by ionizing radiation, ultimately limited their usefulness.The study of DNA repair in mammalian cells received a significant boost during the late 1960s with publications by Cleaver[34,35] that identified the molecular defect responsible for the human disease xeroderma pigmentosum (XP). Patients with XP are exquisitely sensitive to sunlight and highly prone to (skin) cancer. Cleaver showed that cells derived from such patients were likewise sensitive to UV radiation and defective in the nucleotide excision repair pathway (see discussion in this chapter). These cells were not especially sensitive to ionizing radiation however. Several years later, Taylor et al[36] reported that cells derived from patients with a second cancer-prone disorder, ataxia telangiectasia (AT), were extremely sensitive to ionizing radiation and radiation-mimetic drugs, however not UV radiation. In the years that followed, cell cultures derived from patients with these two conditions were used to help elucidate the complicated processes of DNA repair in mammalian cells. Today, dozens of other clinical syndromes associated with either radiosensitivity, cancer proneness, or both have been identified.[37,38]Today, many rodent and human genes involved in DNA repair have been cloned and extensively characterized.[39] Some 30 to 40 proteins participate in excision repair of base damage and about half that many are involved in the repair of strand breaks.[37] Many of these proteins function as component parts of larger repair complexes; some of these parts are interchangeable and participate in other DNA repair and replication pathways as well. It is also noteworthy that some are not involved with the repair process per se, but rather link DNA repair to other cellular functions, including transcription, cell cycle arrest, chromatin remodeling, and apoptosis.[40] This attests to the fact that the maintenance of genomic integrity results from a complex interplay between not only the repair proteins themselves, but also with others that serve as damage sensors, signaling mediators and transducers, and effectors. Collectively, this complex network of proteins that sense, initiate, and coordinate DNA damage signaling and repair with other cellular activities is termed the *DNA damage response* (DDR).[37,41] For example, the defect responsible for the disease AT is not in a gene that codes for a repair protein, but rather in a gene that acts as a damage sensor and also participates in the related pathway that normally prevents cells from entering S phase and beginning DNA synthesis while residual DNA damage is present. This is the *G1 cell cycle checkpoint response*.[42] Because of this genetic defect, AT cells do not experience the normal G1 arrest after irradiation, and they enter S phase with residual DNA damage. This accounts both for the exquisite radiosensitivity of AT cells and the resulting genomic instability that can lead to carcinogenesis.The molecular and biochemical intricacies of DNA repair in mammalian cells are described in detail in Chapter 2, however a brief overview is also presented here.**Base Excision Repair**The repair of base damage is initiated by DNA repair enzymes, *glycosylases*, which recognize specific types of damaged bases and excise them without otherwise disturbing the DNA strand.[43] The action of the glycosylase results in the formation of another type of damage observed in irradiated DNA—an apurinic or apyrimidinic (AP) site. The AP site is then recognized by another repair enzyme, an endonuclease that nicks the DNA adjacent to the lesion, in effect creating a DNA single-stranded break. This break then becomes the substrate for an exonuclease, which removes the abasic site along with a few additional bases. The small gap that results is patched by DNA polymerase using the opposite, and hopefully undamaged, DNA strand as a template. Finally, DNA ligase seals the patch in place.**Nucleotide Excision Repair**The DNA glycosylases that begin the process of base excision repair do not recognize all known forms of base damage however, particularly bulky or complex lesions.[43] In such cases, another group of enzymes, *structure-specific endonucleases*, initiate the excision repair process. These repair proteins do not recognize the specific lesion, but they are thought instead to recognize more generalized structural distortions in DNA, which necessarily accompany a complex base lesion. The structure-specific endonucleases incise the affected DNA strand on both sides of the lesion, releasing an oligonucleotide fragment made up of the damage site and several bases on either side of it. After this step, the remainder of the nucleotide excision repair process is similar to that of base excision repair; the gap is then filled by DNA polymerase and sealed by DNA ligase.For both types of excision repair, active genes in the process of transcription are repaired preferentially and more quickly. This has been termed *transcription-coupled repair*.[44]**Single-Stranded Break Repair**Single-stranded breaks in the DNA

backbone are common lesions, produced in the tens of thousands per day as part of normal cellular metabolism,45 as well as any additional breaks introduced by radiation exposure. These are repaired using the machinery of excision repair, that is, gap filling (if any) by DNA polymerase and sealing by DNA ligase.**Double-Stranded Break Repair**Despite the fact that unrepaired or mis-rejoined DSBs often have the most catastrophic consequences for the cell in terms of loss of reproductive integrity,46 how mammalian cells repair these lesions has been more difficult to elucidate than how they repair base damage. Much of what was originally discovered about these repair processes is derived from studies of rodent cells that were sensitive to x-rays and that were later discovered to harbor specific defects in strand break repair.47 Since then, dozens of other rodent and human cells characterized by DDR defects have been identified and are also used to help probe these fundamental processes.With respect to the repair of DSBs, the situation is more complicated in that the damage on each strand of DNA may be different, and therefore, no intact template would be available to guide the repair process. Under these circumstances, cells must rely on an error-prone process that rejoins the break(s) regardless of the loss of intervening base pairs for which there is no template (nonhomologous end joining [NHEJ]) or else depend on genetic recombination in which a template for presumably error-free repair is obtained from recently replicated DNA of a sister chromatid (homologous recombination [HR])48 to cope with the damage. NHEJ occurs throughout the cell cycle but predominates in cells that have not yet replicated their DNA in anticipation of a subsequent cell division, that is, cells in the G1 or G0 phases of the cell cycle. NHEJ involves a heterodimeric enzyme complex consisting of the proteins Ku-70 and Ku-80, the catalytic subunit of DNA protein kinase (DNA-PKCS), and DNA ligase IV. Cells that have already replicated most or all of their DNA—in the late S or G2 phases of the cell cycle—depend on HR to repair DSBs. HR involves the assembly of a nucleoprotein filament that contains, among others, the proteins Rad51 and Rad52. This filament then invades the homologous DNA sequence of a sister chromatid, which becomes the template for repair. The BRCA2 protein is also implicated in HR as it interacts with the Rad51 protein.38 Defects in either the *BRCA1* (which helps determine which DSB repair pathway will be used in a particular situation) or *BRCA2* genes are associated with hereditary breast and ovarian cancer.49**Mismatch Repair**The primary role of mismatch repair (MMR) is to eliminate from newly synthesized DNA errors such as base/base mismatches and insertion/deletion loops caused by DNA polymerase.50 Descriptively, this process consists of three steps: mismatch recognition and assembly of the repair complex, degradation of the error-containing strand, and repair synthesis. In humans, MMR involves at least five proteins, including hMSH2, hMSH3, hMSH6, hMLH1, and hPMS2, as well as other members of the DNA repair and replication machinery.Radiation-induced DNA lesions are not targets for MMR per se; however, one manifestation of a defect in MMR is germane to any study of oncogenesis: genomic instability,51 which renders affected cells hypermutable. This mutator phenotype is associated with several cancer predisposition syndromes, in particular, hereditary nonpolyposis colon cancer ([HNPCC], sometimes called Lynch syndrome).52,53 Genomic instability is considered one of the main enablers of nonmalignant cells to accumulate cancer-causing mutations and also drives tumor progression to more aggressive and potentially treatment-resistant phenotypes.The DDR as a Clinical TargetHistorically, attempts to inhibit the repair of radiation-induced DNA damage were of interest to researchers probing these fundamental processes; however, clinical translation was typically lacking, mostly out of concern that normal tissues would also be affected in an adverse way. More recently however, it has become clear that the cells of many tumors harbor one or more defects in the DDR, as a consequence of genomic instability, that are not present in normal cells and that this difference might be exploitable clinically.One approach along these lines currently in clinical trials is the use of inhibitors of the protein poly(ADP-ribose) polymerase (PARP).54,55 As of 2012, nearly a dozen trials, most in Phase I or II, were under way using PARP inhibitors.37,55 PARP is an enzyme involved in the repair of DNA damage by base excision repair and SSB repair; its inhibition leads to the persistence of existing SSBs as well as the creation of new ones. If left unrejoined, these breaks can cause the collapse of replication forks in DNA that then impede both DNA replication and HR repair,55 leading to radiosensitization and ultimately, cell death.56In normal cells, little or no toxicity caused by PARP inhibition would be

expected because all DDR pathways are intact and salvage repair pathways to bypass PARP inhibition are active. In tumor cells harboring defects in HR however, salvage pathways are typically dysregulated or absent, suggesting that PARP inhibition would be preferentially toxic to such repair-defective cells. One clinical example is the targeting of breast cancers harboring cellular defects in the BRCA1 and BRCA2 proteins—which either orchestrate or are directly involved in HR—for PARP inhibition. This overall approach of using the combined lethal effect of two genetic defects (one inherent HR defect, plus an additional synthetic one induced by PARP inhibition) that are otherwise nonlethal singly is termed *synthetic lethality*.54-56 Approaches using synthetic lethality targeting DDR proteins (including those other than PARP) likely will play increasingly important roles in the future.Cytogenetic Effects of Ionizing RadiationWhen cells divide following radiation exposure, chromosomes frequently contain visible structural aberrations, most of which are lethal to the cell. In some cases, these aberrations physically interfere with the processes of mitosis and cytokinesis and result in prompt cell death. In other cases, cell division can occur, but the loss or uneven distribution of genetic material between the cell's progeny is ultimately lethal as well, although the affected cells may linger for several days before they die (some may even be able to go through a few more cell divisions in the interim). It is clear that these aberrations are the result of any unrepaired or mis-rejoined DNA damage that persists from the time of irradiation until the time of the next cell division.Most chromosome aberrations result from an interaction between two damage sites, and therefore, can be grouped into three different types of “exchange” categories. A fourth category is reserved for those chromosome aberrations that are thought to result from a single damage site.57 These categories are described here, and representative types of aberrations from each category are also shown in Figure 1-6:1. *Intraarm Exchanges*: an interaction between lesions on the same arm of a single chromosome (e.g., interstitial deletion).2. *Interarm Exchanges*: an interaction between lesions on opposite arms of the same chromosome (e.g., centric ring).3. *Interchanges*: an interaction between lesions on different chromosomes, either homologous or nonhomologous (e.g., dicentric).4. *“Single-Hit” Breaks*: the complete severance of part of one arm of a single chromosome not obviously associated with any more than a single lesion (e.g., terminal deletion).

Figure 1-6 Types of radiation-induced chromosome aberrations that are the result of unrepaired or mis-rejoined DNA damage. Aberrations are classified according to whether they involve a single or multiple chromosomes, whether the damage is thought to be caused by the passage of a single charged particle track (one-hit aberration), or by the interaction of damages produced by two different tracks (two-hit aberration), and whether the irradiation occurred before or after the chromosomes had replicated (chromosome- vs. chromatid-type aberrations, respectively; only chromosome-type aberrations are shown). The aberrations can be further subdivided according to whether broken pieces of the chromosome rearrange themselves symmetrically (with no net loss of genetic material) or asymmetrically (acentric fragments produced).These four categories can be further subdivided according to whether the initial radiation damage occurred before or after the DNA is replicated during S phase of the cell cycle (a chromosome- vs. chromatid-type aberration, respectively), and, for the three exchange categories, whether the lesion interaction was symmetrical or asymmetrical. Asymmetrical exchanges always lead to the formation of acentric fragments, which are usually lost in subsequent cell divisions and therefore are nearly always fatal to the cell. These fragments may be retained transiently in the cell's progeny as extranuclear chromatin bodies, micronuclei. Symmetrical exchanges are more insidious in that they do not lead to the formation of acentric fragments and the accompanying loss of genetic material at the next cell division, they are sometimes difficult to detect cytologically, and they are not invariably lethal to the cell. As such, they will be transmitted to all the progeny of the original cell. Some types of symmetrical exchanges (e.g., a reciprocal translocation) have been implicated in radiation carcinogenesis, insofar as they have the net effect of either bringing new combinations of genes together or separating preexisting groups of genes.28 Depending on where in the genome the translocation takes place, genes normally on could be turned off or vice versa, possibly with adverse consequences.Quantitation of the types and frequencies of

chromosome aberrations in irradiated cells can be used to probe dose-response relationships for ionizing radiation, and to a first approximation, they also can serve as a radiation dosimeter. For example, the dose-response curve for the induction of exchange-type aberrations after exposure to low LET radiation tends to be linear-quadratic in shape, whereas that for single hit aberrations tends to be linear. In mathematical terms, the incidence, *I*, of a particular aberration as a function of radiation dose, *D*, can be expressed as:where α and β are proportionality constants related to the yields of the particular type of aberration, and *c* is the spontaneous frequency of that aberration in unirradiated cells. For fractionated doses or continuous low dose rates of low LET radiation, the yield of exchange type aberrations decreases relative to that for acute doses, and the dose-response curve tends to become more linear. For high LET radiations, dose-response curves for aberration induction become steeper (higher aberration yields) and more linear compared to those for low LET radiations.Cell Survival Curves and Survival Curve Theory**What Is Cell Death?**The traditional definition of death as a permanent, irreversible cessation of vital functions is not the same as what constitutes death to the radiation biologist or oncologist. For proliferating cells, including those maintained in vitro, the stem cells of normal tissues and tumor clonogens, cell death in the radiobiological sense refers to a loss of reproductive integrity, that is, an inability to sustain proliferation indefinitely. This type of reproductive or clonogenic death does not preclude the possibility that a cell may remain physically intact, metabolically active, and continue its tissue-specific functions for some time after irradiation. In fact, some reproductively dead cells can even complete a few additional mitoses before death in the more traditional sense.58Compared to nearly 60 years ago, when the term *clonogenic death* was first coined and used as an endpoint in assays of cellular radiosensitivity,26,59 it is now clearly an operationally defined term that encompasses several distinct mechanisms by which cells die, all of which result in a cell losing its ability to divide indefinitely. These modes of cell death include mitotic catastrophe, apoptosis, autophagy, necrosis, senescence, and strictly speaking, differentiation as well because differentiated cells lose their ability to divide.60,61Mitotic catastrophe is the major cause of radiation-induced death for most mammalian cells, and occurs secondary to chromosome aberrations or spindle defects that interfere with the cell-division process.62,63 Accordingly, this type of cell death occurs during or soon after an attempted cell division after irradiation (although not necessarily at the first division attempt) and leaves in its wake large, flattened, and multinucleated cells that are typically aneuploid. Apoptosis, or programmed cell death, is a type of nonmitotic or interphase death commonly associated with embryonic development and normal tissue remodeling and homeostasis.64 However, certain normal tissue and tumor cells also undergo apoptosis following irradiation, including normal cells of hematopoeitic or lymphoid origin, crypt cells of the small intestine, salivary gland cells, plus some experimental tumor cell lines of gynecological and hematological origin.65 Cells undergoing apoptosis exhibit a number of characteristic morphological (nuclear condensation and fragmentation, membrane blebbing, etc.) and biochemical (DNA degradation) changes that culminate in the fragmentation and lysis of the cell, often within 12 hours of irradiation and before the first postirradiation mitosis. Ultimately, the remains of apoptotic cells are phagocytized by neighboring cells, and therefore do not cause the type of inflammatory response, tissue destruction, and disorganization characteristic of necrosis. Apoptosis is an active and carefully regulated pathway that involves several genes and gene products and an appropriate stimulus that activates the pathway. The molecular biology of apoptosis, the apoptosis-resistant phenotype noted for many types of tumor cells, and the role that radiation may play in the process are discussed in detail in Chapter 2. Senescence refers to a type of genetically controlled cellular growth arrest that, although not eliminating damaged cells, does halt permanently their continued movement through the cell cycle, even in the presence of growth factors.66 Radiation can also induce senescence, presumably as a result of the permanent triggering of cell cycle checkpoints; however it might better be termed *radiation-induced permanent growth arrest*, to distinguish it from the normal process of cell-age–related senescence.67 The roles played by autophagy and necrosis, which are two additional modes of cell death that usually occur in response to diverse types of cellular stress, remain unclear with respect to radiation-induced cell death. Autophagy is defined as the controlled lysosomal degradation of

cytoplasmic organelles or other cytoplasmic components[68,69] in response to cellular stressors, including nutrient deprivation, hypoxia, DNA damage, or an excess of reactive oxygen species. Likewise, necrosis—characterized by cell swelling followed by membrane rupture and the release of cellular contents into the extracellular space—can occur as a somewhat passive response to nutrient deprivation, but it also can follow a molecular program initiated by immune cells or various toxins.[70,71]Most assays of radiosensitivity of cells and tissues, including those described in this chapter, use reproductive integrity, either directly or indirectly, as an endpoint. Although such assays have served the radiation oncology community well in terms of elucidating dose-response relationships for normal tissues and tumors, it is now clear that reproductive death is not necessarily the whole story. What remains unclear is whether and to what extent other modes of cell death, particularly apoptosis, contribute to our traditional measures of radiosensitivity based on clonogenic survival, and whether, for example, pretreatment assessment of apoptotic propensity in tumors or dose-limiting normal tissues is of prognostic significance. The interrelationships between these different pathways of cell death can be quite complex. Meyn[65] has suggested that a tumor with a high spontaneous apoptotic index may be inherently more radiosensitive because cell death might be triggered by lower doses than are usually required to cause reproductive death. Also, tumors that readily undergo apoptosis may have higher rates of cell loss, the net effect of which would be to partially offset cell production, thereby reducing the number of tumor clonogens. On the other hand,, recent studies suggest that the caspases that orchestrate the removal of radiation-damaged cells via apoptosis also may stimulate tumor cell repopulation during and after radiotherapy,[72] which is a decidedly undesirable outcome.**Cell Survival and Dose-Response Curve Models**Survival curve theory originated in a consideration of the physics of energy deposition in matter by ionizing radiation. Early experiments with macromolecules and prokaryotes established that dose-response relationships could be explained, in principle, by the random and discrete nature of energy absorption, if it was assumed that the response resulted from critical “targets” receiving random “hits.”[73] With an increasing number of shouldered survival and dose response curves being described for cells irradiated both in vitro and in vivo, various equations were developed to fit these data. Target theory pioneers studied a number of different endpoints in the context of target theory, including enzyme inactivation in cell-free systems,[29] cellular lethality, chromosomal damage, and radiation-induced cell cycle perturbations in microorganisms.[29,74] Survival curves, in which the log of the survival of a certain biological activity was plotted as a function of the radiation dose, were found to be either exponential or sigmoid in shape with the latter usually noted for the survival of more complex organisms.[29]Exponential survival curves were thought to result from the single-hit, “all-or-nothing,” inactivation of a single target, resulting in the loss of activity. A mathematical expression used to fit this type of dose-response relationship was S = . In this equation, *S* is the fraction of cells that survive a given dose; *D* and *D*0 are the dose increment that reduces the cell survival to 37% (1/e) of some initial value on the exponential portion of the curve (i.e., a measure of the reciprocal of the slope). The sigmoid curves, characterized by a shoulder at low doses, were consistent with target theory if one assumed that either multiple targets or multiple hits in a single target (or a combination of both) were necessary for radiation inactivation. A mathematical expression based on target theory that provided a fairly good fit to survival data was S = 1 − (1 −)n, with *n* being the back extrapolation of the exponential portion of the survival curve to zero dose. Implicit in this multitarget model was that damage had to accumulate before the overall effect was registered.It soon became apparent that some features of this model were inadequate.[75] The most obvious problem was that the single-hit, multitarget equation predicted that survival curves should have initial slopes of zero, that is, that for vanishingly small doses (e.g., repeated, small doses per fraction or continuous low-dose rate exposure), the probability of cell killing would approach zero. This was *not* what was observed in practice for either mammalian cell survival curves or as inferred from clinical studies in which highly fractionated or low-dose rate treatment schedules were compared to more conventional fractionation. There was no fractionation schedule that produced essentially no cell killing, that is, all other radiobiological factors being equal.A somewhat different interpretation of

cell survival was proposed by Kellerer and Rossi[76] in the late 1960s and early 1970s. The linear-quadratic or alpha-beta equation, S = , was shown to fit many survival data quite well, particularly in the low-dose region of the curve, and also provided for the negative initial slope investigators had described.[75] In this expression, *S* is again the fractional cell survival following a dose *D*, α is the rate of cell kill by a single-hit process, and β is the rate of cell kill by a two-hit mechanism. The theoretical derivation of the linear-quadratic equation is based on two different sets of observations. Based on microdosimetric considerations, Kellerer and Rossi[76] proposed that a radiation-induced lethal lesion resulted from the interaction of two sublesions. According to this interpretation, the α*D* term is the probability of these two sublesions being produced by a single event (the “intratrack” component), whereas β*D*2 is the probability of the two sublesions being produced by two separate events (the “intertrack” component). Chadwick and Leenhouts[77] derived the same equation based on a different set of assumptions, namely that a DSB in DNA was a lethal lesion and that such a lesion could be produced by either a single energy deposition involving both strands of DNA or by two separate events, each involving a single strand.A comparison of the features and parameters of the target theory and linear-quadratic survival-curve expressions is shown in Figure 1-7.

Figure 1-7 A comparison of two mathematical models commonly used to fit cell survival curve data. Left panel: The single-hit, multitarget model and its associated parameters, D0, n, and Dq. Although this model has since been invalidated, values for its parameters are still used for comparative purposes. Right panel: The linear-quadratic model and its associated parameters, α and β. This model forms the basis for current isoeffect formulae used in radiation therapy treatment planning.**Clonogenic Assays In Vitro**As mentioned previously, it was not until the mid-1950s that mammalian cell culture techniques were sufficiently refined to allow quantitation of the radiation responses of single cells.[59,78] Puck and Marcus'[26] acute-dose, x-ray survival curve for the human tumor cell line HeLa, is shown in Figure 1-8. Following graded x-ray doses, the reproductive integrity of single HeLa cells was measured by their ability to form macroscopic colonies of at least 50 cells (corresponding to approximately 6 successful postirradiation cell divisions) on petri dishes. A number of features of this survival curve were of particular interest. First, qualitatively at least, the curve was similar in shape to those previously determined for many microorganisms and was characterized by a shoulder at low doses and a roughly exponential region at high doses. Of note, however, was the finding that the D0 for HeLa cells was only 96 R, which was some 10- to 100-fold less than D0 determined for microorganisms, and 1,000- to 10,000-fold less than D0 for the inactivation of isolated macromolecules.[58] Thus, cellular reproductive integrity was found to be a much more radiosensitive endpoint for HeLa cells than for prokaryotes or primitive eukaryotes. The value of the extrapolation number, *n*, was approximately 2.0, indicating that the survival curve did have a small shoulder, but again, much smaller than typically observed for microorganisms. Puck and Marcus suggested that the *n* value was a reflection of the number of critical targets in the cell, each requiring a single hit before the cell would be killed, and they further postulated that the targets were, in fact, the chromosomes themselves.[26] However, the potential pitfalls of deducing mechanisms of radiation action from parameters of a descriptive survival curve model were soon realized.[79,80]

Figure 1-8 Clonogenic survival of HeLa cells in vitro as a function of x-ray dose. Like many mammalian cells of tumorigenic and nontumorigenic origin, the HeLa cell survival curve is characterized by a modest initial shoulder region (*n* = 2.0) followed by an approximately exponential final slope (D*0* ≈ 1.0 Gy). Adapted from Puck TT, Marcus PI: Action of x rays on mammalian cells. J Exp Med 103:653, 1956; copyright permission of The Rockefeller University Press.Survival curves for other types of mammalian cells, regardless of whether they were derived from humans or laboratory animals or from tumors or normal tissues, have been shown to be qualitatively similar to the original HeLa cell survival curve.**Clonogenic Assays In Vivo**To bridge the gap between the radiation responses of cells grown in culture and in an animal, Hewitt and Wilson developed an ingenious method to assay single cell survival in vivo.[81] Lymphocytic leukemia cells obtained from the livers of donor CBA mice were harvested, diluted,

and inoculated into disease-free recipient mice. By injecting different numbers of donor cells, a standard curve was constructed that allowed a determination of the average number of injected cells necessary to cause leukemia in 50% of the recipient mice. It was determined that the endpoint of this titration, the “50% take dose” or TD50, corresponded to an innoculum of a mere two leukemia cells. Using this value as a reference, Hewitt and Wilson then injected leukemia cells harvested from γ-irradiated donor mice into recipients and again determined the TD50 following different radiation exposures. In this way, the surviving fraction after a given radiation dose could be calculated from the ratio of the TD50 for unirradiated cells to that for the irradiated cells. Using this technique, a complete survival curve was constructed that had a D0 of 162 R, and an *n* value close to 2.0, which were values quite similar to those generated for cell lines irradiated in vitro. For the most part, in vivo survival curves for a variety of cell types were also similar to corresponding in vitro curves.A similar trend was apparent when in vivo survival curves for nontumorigenic cells were first produced. The first experiments by Till and McCulloch,[82,83] using normal bone marrow stem cells, were inspired by the knowledge that failure of the hematopoietic system was a major cause of death following total body irradiation and that lethally irradiated animals could be “rescued” by a bone marrow transplant. The transplanted, viable bone marrow cells were observed to form discrete nodules or colonies in the otherwise sterilized spleens of irradiated animals. Subsequently, these authors transplanted known quantities of irradiated donor bone marrow into lethally irradiated recipient mice, were able to count the resulting splenic nodules, and then calculate the surviving fraction of the injected cells in much the same way as was done for in vitro experiments. The D0 for mouse bone marrow was 0.95 Gy.[83] Other in vivo assay systems based on the counting of colonies or nodules included the skin epithelium assay of Withers,[84] the intestinal crypt assays of Withers and Elkind,[85,86] and the lung colony assay of Hill and Bush.[87] During the late 1960s and early 1970s, it also became possible to do excision assays, in which tumors irradiated in vivo were removed, enzymatically dissociated, and single cells plated for clonogenic survival in vitro, thereby allowing more quantitative measurement of survival and avoiding some of the pitfalls of in vivo assays (e.g., Rockwell et al[88]).**Nonclonogenic Assays In Vivo**Unfortunately some normal tissues and tumors are not amenable to clonogenic assays. Thus, new assays were needed that had clinical relevance, yet did not rely on reproductive integrity as an endpoint. Use of such assays required one leap of faith however, namely that the endpoints assessed would have to be a consequence of the killing of clonogenic cells, although not necessarily in a direct, one-to-one manner. Because nonclonogenic assays do not directly measure cell survival as an endpoint, data derived from them and plotted as a function of radiation dose are properly called dose-response curves rather than cell survival curves, although such data are often analyzed and interpreted similarly.Historically, among the first nonclonogenic assays was the mean lethal dose or LD50 assay, in which the (whole body) radiation dose to produce lethality in approximately 50% of the test subjects is determined, usually at a fixed time after irradiation, such as 30 (LD50/30) or 60 days (LD50/60). Clearly, the LD50 assay is not specific in that the cause of death can result from damage to a number of different tissues.Another widely used nonclonogenic method to assess normal tissue radioresponse is the skin reaction assay, which was originally developed by Fowler et al.[89] Pigs were often used because their skin is similar to that of humans in several key respects. An ordinate scoring system was used to compare and contrast different radiation schedules and was derived from the average severity of the skin reaction noted during a certain time period (specific to the species and whether the endpoint occurs early or late) following irradiation. For example, for early skin reactions, a skin score of “1” might correspond to mild erythema, whereas a score of “4” might correspond to confluent moist desquamation over more than half of the irradiated area.Finally, two common nonclonogenic assays for tumor response are the growth delay/regrowth delay assay[90] and the tumor control dose assay.[91] Both assays are simple and direct, are applicable to most solid tumors, and are clinically relevant. The growth delay assay involves the periodic measurement of a tumor's dimensions as a function of time after irradiation and a calculation of the tumor's approximate volume. For tumors that regress rapidly during and after radiotherapy, the endpoint

scored is typically the time in days it takes for the tumor to regrow to its original volume at the start of irradiation. For tumors that regress more slowly, a more appropriate endpoint might be the time it takes for the tumor to grow or regrow to a specified size, such as three times its original volume. Dose-response curves are generated by plotting the amount of growth delay as a function of radiation dose.The tumor control assay is a logical extension of the growth delay assay. The endpoint of this assay is the total radiation dose required to achieve a specified probability of local tumor control—usually 50% (TCD50)—in a specified period of time after irradiation. The TCD50 value is obtained from a plot of the percentage of tumors locally controlled as a function of total dose. The slope of the resulting dose-response curve may be used for comparative purposes as a measure of the tumor's inherent “radiosensitivity” or its degree of heterogeneity. More heterogeneous tumors tend to have shallower dose-response curves than more homogeneous ones, as do spontaneous tumors relative to experimental ones maintained in inbred strains of mice.Cellular Repair: Sublethal and Potentially Lethal Damage RecoveryTaking the cue from target theory that the shoulder region of the radiation survival curve indicated that hits had to accumulate before cell killing, Elkind and Sutton92,93 sought to better characterize the nature of the damage caused by these hits and how the cell processed this damage. Even in the absence of any detailed information about DNA damage and repair at that time, a few things seemed obvious. First, those hits or damages that were registered as part of the accumulation process, yet did not in and of themselves produce cell killing, were, by definition, sublethal. Second, sublethal damage (SLD) only became lethal when it interacted with additional SLD, that is, when the total amount of damage had accumulated to a sufficient level to cause cell lethality. But what would be the result of deliberately interfering with the damage accumulation process, by, for example, delivering part of the intended radiation dose, inserting a radiation-free interval, and then delivering the remainder of the dose? The results of such split-dose experiments turned out to be crucial to the understanding of why and how fractionated radiation therapy works as it does. The discovery and characterization of SLD, as low tech and operational the concept may be by today's standards, still stands as arguably the single-most important contribution radiation biology has made to the practice of radiation oncology.By varying the time interval between two doses of approximately 5.0 Gy and plotting the log of the surviving fraction of cells after both doses (i.e., 10 Gy total dose) as a function of the time between the doses, the resulting split-dose recovery curve was observed to rise to a maximum after about 2 hours and then level off. In other words, the overall surviving fraction of cells following 10 Gy was higher if the dose was split into two fractions with a time interval in between than delivered as a single dose. Elkind interpreted these results as indicating that the cells that survived the initial dose fraction had “repaired” some of the damage during the radiation-free interval, and as such, this damage was no longer available to interact with the damage inflicted by the second dose. At the time, Elkind referred to this phenomenon as *sublethal damage repair* (SLDR); however, in retrospect, it is perhaps preferable to call it *sublethal damage recovery*, because biochemical DNA repair processes were not actually measured, only changes in cell survival were.Of additional interest was the observation that the shape of the split-dose recovery curve varied with the temperature during the radiation-free interval (Figure 1-9). When the cells were maintained at room temperature between the split doses, the SLDR curve rose to a maximum after about 2 hours and then leveled off. When the cells were returned to an incubator at 37° C for the radiation-free interval, a different pattern emerged. Initially, the split-dose recovery curve rose to a maximum after 2 hours, but then, the curve exhibited a series of oscillations, dropping to a second minimum for a split of about 4 to 5 hours and then rising again to a higher maximum for split dose intervals of 10 hours or more. The interpretation of this pattern of SLDR was that other radiobiological phenomena operated simultaneously with cellular recovery. In this case, the fine structure of the split-dose recovery curve was not caused by an oscillating repair process, but rather, by a superimposed cell cycle effect: the so-called radiation age response through the cell cycle. This is discussed in detail in the “Ionizing Radiation and the Cell Cycle” section (see also Figure 1-14).

Figure 1-9 Split-dose or sublethal damage recovery demonstrated in cultured V79 hamster

cells that received a first x-ray dose at time = 0, followed by a second dose after a variable radiation-free interval. Cells were maintained at room temperature (24° C) or at 37° C during the split time. Adapted from Elkind M, Sutton-Gilbert H, Moses W, et al: Radiation response of mammalian cells grown in culture. V. Temperature dependence of the repair of x-ray damage in surviving cells (aerobic and hypoxic). Radiat Res 25:359, 1965.Since Elkind and Sutton's original work, SLDR kinetics have been described for many different types of mammalian cells in culture58 and for most normal and tumor tissues in vivo (e.g., Belli et al,94 Emery et al95). Pertinent findings include:1. The amount of SLD capable of being repaired for a given cell type varies both with the radiation quality (less for radiations of increasing LET) and the oxygenation status of the cells (recovery reduced or absent at extremely low-oxygen tensions).282. The half-time for SLDR in mammalian cells in culture is, on average, about 1 hour, although there is evidence that it may be somewhat longer for late responding normal tissues in vivo.283. The survival increase between split doses is a manifestation of the “regeneration” of the shoulder of the radiation survival curve. After an initial radiation dose and an adequate time interval for SLDR, the response of surviving cells to graded additional doses is nearly identical to that obtained from cells without previous radiation exposure. Thus, the width of the shoulder of the survival curve came to be associated with the capacity of the cells for recovery from sublethal damage. This concept is illustrated in Figure 1-10.

Figure 1-10 Sublethal damage recovery manifests as a return of the shoulder on the radiation survival curve when a total dose is delivered as two fractions separated by a time interval **(A)**. If the interfraction interval is shorter than the time it takes for this recovery to occur, the shoulder will only be partially regenerated (compare the shoulder regions of the survival curves for intervals of 1 vs. 3 hours). Regeneration of the shoulder **(B)** accounts for the observed survival increase in the corresponding split-dose assay (see Figure 1-9).4. Cells are able to undergo repeated cycles of damage and recovery without an apparent change in recovery capacity. As such, one would predict an equal effect per-dose fraction during the course of fractionated radiotherapy. In a more practical sense, this means that a multifraction survival curve can be generated using the formula $SF_n = (SF_1)^n$, where SF_1 is the surviving fraction of cells after a single-dose fraction (determined from a single-dose survival curve), and SF_n is the surviving fraction of cells after n dose fractions. Accordingly, multifraction survival curves are shoulderless and exponential (Figure 1-11).

Figure 1-11 Hypothetical multifraction survival curve *(dashed line)* for repeated 3.0-Gy fractions under conditions in which sufficient time between fractions is allowed for full sublethal damage recovery and cell cycle and proliferative effects are negligible. The multifraction survival curve is shallower than its corresponding single-dose curve *(solid lines)* and has no shoulder (i.e., the surviving fraction is an exponential function of total dose).5. Sublethal damage recovery is largely responsible for the dose rate effect for low LET radiation, which will be discussed in detail later in this chapter. As the dose per fraction (intermittent radiation) or dose rate (continuous irradiation) is decreased and the overall treatment time increased, the biological effectiveness of a given total dose is reduced. (Note that SLDR also occurs during continuous irradiation, that is, that a radiation-free interval is not required per se.)A second type of cellular recovery following irradiation is termed po*tentially lethal damage repair or recovery* (PLDR) and was first described for mammalian cells by Phillips and Tolmach96 in 1966. PLD is, by definition, a spectrum of radiation damage that may or may not result in cell lethality depending on the cells' postirradiation conditions. Conditions that favor PLDR include maintenance of cells in overcrowded conditions (plateau phase or contact-inhibited97,98) and incubation following irradiation at either reduced temperature,99 in the presence of certain metabolic inhibitors,96 or in balanced salt solutions rather than complete culture medium.99 What these treatment conditions have in common is that they are suboptimal for continued growth of cells. Presumably, resting cells (regardless of why they are resting) have more opportunity to repair DNA damage before cell division than cells that continue traversing the cell cycle immediately after irradiation. Phillips and Tolmach96 were the first to propose this repair-fixation or competition model to explain PLDR.Although admittedly, some of these postirradiation conditions are not likely to be encountered in vivo, slow growth of

cells in general, with or without a large fraction of resting cells, is a common characteristic of many tissues. As might be expected, tumors (and subsequently, select normal tissues amenable to clonogenic assay) were shown to repair PLD.98 Experiments using rodent tumors were modeled after comparable studies using plateau phase cells in culture, that is, a “delayed plating” assay was used. For such an experiment, irradiated cell cultures or animal tumors are left in a confluent state (either in the overcrowded cell culture or in the intact tumor in the animal) for varying lengths of time before removing them, dissociating them into single-cell suspensions and plating the cells for clonogenic survival at a low density. The longer the delay between irradiation and the clonogenic assay, the higher the resulting surviving fraction of individual cells, even though the radiation dose is the same. In general, survival rises to a maximum within 4-6 hours and levels off thereafter (Figure 1-12).

Figure 1-12 Potentially lethal damage recovery can be demonstrated using a delayed-plating assay in which a variable delay time is inserted between exposure to a large, single dose of radiation and the harvesting of the cells for a clonogenic assay. If cells are maintained in overcrowded or nutrient-deprived conditions during the delay period, the surviving fraction increases relative to that obtained when there is no delay. **A,** Potentially lethal damage recovery occurs in vitro in a nontumorigenic rodent fibroblast cell line and its transformed, tumorigenic counterpart. **B,** Potentially lethal damage recovery occurs in vivo in small and large mouse fibrosarcomas. **A,** Adapted from Zeman E, Bedford J: Dose-rate effects in mammalian cells. V. Dose fractionation effects in noncycling C3H 10T1/2 cells. Int J Radiat Oncol Biol Phys 10:2089, 1984; **B,** adapted from Little J, Hahn G, Frindel E, et al: Repair of potentially lethal radiation damage in vitro and in vivo. Radiology 106:689, 1973.The kinetics and extent of recovery from both SLD and PLD are correlated with the molecular repair of DNA and the rejoining of chromosome breaks.100,101 For the purposes of radiation therapy, however, the most important consideration is that both processes have the potential to increase the surviving fraction of cells between subsequent dose fractions. Such a survival increase could be manifest clinically as either increased normal tissue tolerance or decreased tumor control. It is also important to appreciate that small differences in recovery capacity between normal and tumor cells after a single-dose fraction are magnified into large differences after 30 or more dose fractions.**Repair in Tissues**

When considering repair phenomenon in intact tissues, it is important to remember that both the magnitude of the repair (related both to the shape of the shoulder region of the corresponding dose-response curve and the dose delivered) and the rate of the repair can influence how the tissue behaves during a course of radiation therapy. For example, a particular tissue—normal or tumor—may be quite capable of repairing most damage produced by each dose fraction, but if the interfraction interval is so short as to not allow all the damage to be repaired before the next dose, the tolerance of that tissue will be less than otherwise anticipated. Second, although the sparing effect of dose fractionation for both normal and tumor tissues can be explained largely by SLD recovery between fractions, at sufficiently small doses per fraction, the degree of sparing will reach a maximum below which no further sparing occurs, that is, all other radiobiological factors being equal. This is a reflection of the fact that some radiation damage is necessarily lethal and not modifiable by either further fractionation or changing postirradiation conditions.Ionizing Radiation and the Cell CycleAnother basic feature of the cellular response to ionizing radiation is perturbation of the cell cycle. Such effects can modify the radioresponsiveness of tissues either directly or indirectly, depending on the fraction of cycling cells present in the tissue, their proliferation rates, and the kinetic organization of the tissue or tumor as a whole.Advances in techniques for the study of cell cycle kinetics during the 1950s and 1960s paved the way for the generation of survival curves as a function of cell age. Using a technique known as autoradiography, Howard and Pelc102 were able to identify the S, or DNA synthesis, phase of the cell cycle. When combined with the other cytologically obvious cell cycle marker, mitosis, they were able to discern the four phases of the cell cycle for actively growing cells: G1, S, G2, and M.**Methodology**Several techniques were subsequently developed for the collection of synchronized cells in vitro. One of the most widely used was the mitotic harvest or shake-off technique first described by Terasima and Tolmach.103,104 By agitating cultures, mitotic

cells, which tend to round up and become loosely attached to the culture vessel's surface, can be dislodged, collected along with the overlying growth medium, and inoculated into new culture flasks. By incubating these flasks at 37° C, cells begin to proceed synchronously into G1 phase (and semisynchronously thereafter). Thus, by knowing the length of the various phase durations for the cell type being studied and then delivering a radiation dose at a time of interest after the initial synchronization, the survival response of cells in different phases of the cell cycle can be determined.A second synchronization method involved the use of DNA synthesis inhibitors such as fluorodeoxyuridine,105 and later, hydroxyurea,106 to selectively kill S phase cells, yet allow cells in other phases to continue cell cycle progression until they become blocked at the border of G1 and S phase. By incubating cells in the presence of these inhibitors for times sufficient to collect nearly all cells at the block point, large numbers of cells can be synchronized. In addition, the inhibitor technique has two other advantages, namely that some degree of synchronization is possible in vivo107 as well as in vitro, and that by inducing synchrony at the end of G1 phase, a higher degree of synchrony can be maintained for longer periods than if synchronization had been at the beginning of G1. On the other hand, the mitotic selection method does not rely on the use of drugs that themselves could perturb the normal cell cycle kinetics of the population.Developments in the early 1970s, however, provided what is now considered among the most valuable tools for the study of cytokinetic effects: the flow cytometer and its offshoot, the fluorescence-activated cell sorter.108 These have largely replaced the aforementioned longer and more labor-intensive cell cycle synchronization methods. Using this powerful technique, single cells are stained with a fluorescent probe that binds stoichiometrically to a specific cellular component, for example, DNA in the case of cell cycle distribution analysis. The stained cells are then introduced into a pressurized flow cell and forced to flow single file and at a high rate of speed through a focused laser beam that excites the fluorescent dye. The resulting light emission from each cell is collected by photomultiplier tubes, recorded, and output as a frequency histogram of cell number as a function of relative fluorescence, with the amount of fluorescence directly proportional to DNA content. Accordingly, cells with a “1X” DNA content would correspond to cells in G1 phase, cells with a “2X” DNA content in G2 or M phase, and cells with DNA contents between “1X” and “2X” in the S phase of the cell cycle. By performing a mathematical fit to the DNA histogram, the proportion of cells in each phase of the cell cycle can be determined, the phase durations can be derived, and differences in DNA ploidy can be identified. DNA flow cytometry is quite powerful in that not only can a static measure of cell cycle distribution be obtained for a cell population of interest, but also, dynamic studies of, for example, transit through the various cell cycle phases or treatment-induced kinetic perturbations, can be monitored over time (Figure 1-13). A flow cytometer can be outfitted to include a cell sorting feature; in this case, cells analyzed for a property of interest can be collected in separate “bins” after they pass through the laser beam, and if possible, used for other experiments.

Figure 1-13 The analytic technique of flow cytometry has revolutionized the study of cell cycle kinetics by allowing rapid determination of DNA content in cells stained with a fluorescent dye that binds stoichiometrically to cellular DNA. **A,** Frequency distribution for a population of exponentially growing cells. The large and small peaks correspond to cells with G1 (1×) and G2/M (2×) phase DNA content, respectively; the cells in S phase have an intermediate DNA content. *B* to *D*, DNA histograms for a cell population synchronized initially in mitosis and then allowed to progress into G1 **(B)**, S, and G2/M (**C** and **D**).**Age Response Through the Cell Cycle**

Results of an age response experiment of Terasima and Tolmach,104 using synchronized HeLa cells, are shown in the lower panel of Figure 1-14. Following a single dose of 5 Gy of x-rays, cells were found to be most radioresistant in late S phase. Cells in G1 were resistant at the beginning of the phase but became sensitive toward the end of the phase, and G2 cells were increasingly sensitive as they moved toward the most sensitive M phase. In subsequent experiments by Sinclair,109,110 age response curves for synchronized Chinese hamster V79 cells showed that the peak in resistance observed in G1 HeLa cells was largely absent for V79 cells. This is also illustrated in Figure 1-14 *(upper panel)*. Otherwise, the shapes of the age response curves for

the two cell lines were similar. The overall length of the G1 phase determines whether the resistant peak in early G1 will be present; in general, this peak of relative radioresistance is only observed for cells with long G1 phases. For cells with short G1 phases, the entire phase is often of intermediate radiosensitivity. An analysis of the complete survival curves for synchronized cells[110, 111] confirms that the most sensitive cells are those in M and late G2 phase, in which survival curves are steep and largely shoulderless, and the most resistant cells are those in late S phase. The resistance of these cells is conferred by the presence of a broad survival curve shoulder, rather than by a significant change in survival curve slope (Figure 1-15). When high LET radiations are used, the age response variation through the cell cycle is significantly reduced or eliminated because survival curve shoulders are either decreased or removed altogether by such exposures (see also “Relative Biological Effectiveness”). Similar age response patterns have been identified for cells synchronized in vivo.[107]

Figure 1-14 Cell cycle age response for sensitivity to radiation-induced cell killing in a representative rodent cell line (V79, *upper panel*) having a short G1 phase duration and in a representative human cell line (HeLa, *lower panel*) having a long G1 phase duration. Both cell lines exhibit a peak of radioresistance in late S phase and maximum radiosensitivity in late G2/M phase. A second trough of radiosensitivity can be discerned near the G1/S border for cells with long G1-phase durations. Adapted from Sinclair W: Dependence of radiosensitivity upon cell age. In Proceedings of the carmel conference on time and dose relationships in radiation biology as applied to radiotherapy. BNL Report 50203, Upton, NY, 1969, Brookhaven National Laboratory.

Figure 1-15 Cell survival curves for irradiated populations of Chinese hamster cells synchronized in different phases of the cell cycle *(left panel)*, illustrating how these radiosensitivity differences translate into the age-response patterns shown in the *right panel* (see Figure 1-14).The existence of a cell cycle age response for ionizing radiation provided an explanation for the unusual pattern of SLDR observed for cells maintained at 37° C during the recovery interval (see Figure 1-9). In Elkind and Sutton's experiments, exponentially growing cells were used, that is, cells that were asynchronously distributed across the different phases of the cell cycle. The cells that survived irradiation tended to be those most radioresistant. Thus, the remaining population became enriched with the more resistant cells. For low LET radiation, those cells that were most resistant were in S phase at the time of the first radiation dose. However, at 37° C, cells continued to progress through the cell cycle, so those surviving cells in S phase at the time of the first dose may have moved into G2 phase by the time the second dose was delivered. Thus, the observed survival nadir in the SLDR curve was not the result of a loss or reversal of repair, but rather, because the population of cells was now enriched in G2 phase cells, which are inherently more radiosensitive. For even longer radiation-free intervals, it is possible that the cells surviving the first dose would transit from G2 to M, and back into G1 phase, dividing and doubling their numbers. In this case, the SLDR curve again shows a surviving fraction increase because the number of cells has increased. None of these cell cycle-related phenomena occur when the cells are maintained at room temperature during the radiation-free interval because continued movement through the cell cycle is inhibited under such conditions; in that case, all that is noted is the initial survival increase resulting from SLDR.**Radiation-Induced Cell Cycle Blocks and Delays**Radiation is also capable of disrupting the normal proliferation kinetics of cell populations. This was recognized by Canti and Spear[112] in 1927 and studied in conjunction with radiation's ability to induce cellular lethality. With the advent of mammalian cell culture and synchronization techniques and time-lapse cinemicrography, it became possible for investigators to study mitotic and division delay phenomena in greater detail.Mitotic delay, defined as a delay in the entry of cells into mitosis, is a consequence of upstream blocks or delays in the movement of cells from one cell cycle phase to the next. Division delay, which is a delay in the time of appearance of new cells at the completion of mitosis, is caused by the combined effects of mitotic delay, and any further lengthening of the mitosis process itself. Division delay increases with dose and is, on average, about 1 to 2 hours per Gray[104] depending on the cell line.The cell cycle blocks and delays primarily responsible for mitotic and division delay are, respectively, a block in the G2-to-M phase transition and a block in the G1-to-S phase transition. The duration of the G2 delay,

like the overall division delay, varies with cell type, but for a given cell type is dependent on both dose and cell cycle age. In general, the length of the G2 delay increases linearly with dose. For a given dose, the G2 delay is longest for cells irradiated in S or early G2 phase and shortest for cells irradiated in G1 phase.113 Another factor contributing to mitotic and division delay is a block in the flow of cells from G1 into S phase. For x-ray doses of at least 6 Gy, there is a 50% decrease in the rate of tritiated thymidine uptake (indicative of entry into S phase) in exponentially growing cultures of mouse L cells. Little114 reached a similar conclusion from G1 delay studies using human liver LICH cells maintained as confluent cultures.A possible role for DNA damage and its repair in the etiology of division delay was bolstered by the finding that certain cells types that either did not exhibit the normal cell cycle delays associated with radiation exposure (such as AT cells42), or conversely, were treated with chemicals (such as caffeine) that ameliorated the radiation-induced delays,115 tended to contain higher amounts of residual DNA damage, and to show increased radiosensitivity.It is now known that the radiation-induced perturbations in cell cycle transit are under the control of cell cycle checkpoint genes, whose products normally govern the orderly (and unidirectional) flow of cells from one phase to the next. The checkpoint genes are responsive to feedback from the cell as to its general condition and readiness to transit to the next cell cycle phase. DNA integrity is one of the criteria used by these genes to help make the decision whether to continue traversing the cell cycle, or to pause, either temporarily, or in some cases, permanently. Cell cycle checkpoint genes are discussed in Chapter 2.**Redistribution in Tissues** Because of the age response through the cell cycle, an initially asynchronous population of cells surviving a dose of radiation becomes enriched with S phase cells. As a result of variations in the rate of further cell cycle progression, however, this partial synchrony decays rapidly. Such cells are said to have redistributed116 with the net effect of sensitizing the population as a whole to a subsequent dose fraction (relative to what would have been expected had the cells remained in their resistant phases). A second type of redistribution, in which cells accumulate in G2 phase (in the absence of cell division) during the course of multifraction or continuous irradiation because of a buildup of radiation-induced cell cycle blocks and delays, also has a net sensitizing effect. This has been observed during continuous irradiation by several investigators,117 and in some of these cases, a net *increase* in radiosensitivity is seen at certain dose rates. This so-called inverse dose rate effect, in which certain dose rates are more effective at cell killing than other, higher dose rates, was extensively studied by Bedford et al (for a review, see Bedford et al118). The magnitude of the sensitizing effect of redistribution varies with cell type depending on what dose rate is required to stop cell division. For dose rates below the critical range that causes redistribution, some cells can escape the G2 block and proceed on to cell division.Densely Ionizing Radiation**Linear Energy Transfer (LET)**The total amount of energy deposited in biological materials by ionizing radiation (usually expressed in units of keV, ergs or joules per g or kg) is in and of itself insufficient to describe the net biological consequences of those energy deposition events. For example, 1 Gy of x-rays, although physically equivalent in terms of total energy imparted per unit mass to 1 Gy of neutrons or α-particles, does not produce equivalent biological effects. It is the microdosimetric pattern of that energy deposition, that is, the spacing or density of the ionization events, that determines biological effectiveness. This quantity—the average energy deposited locally per unit length of the ionizing particle's track—is termed its LET.LET is a function both of the charge and mass of the ionizing particle. Photons set in motion fast electrons that have a net negative charge but a negligible mass. Neutrons on the other hand give rise to recoil protons or α-particles, that possess one or two positive charges, respectively, and are orders of magnitude more massive than electrons. Neutrons therefore have a higher LET than photons and are said to be densely ionizing, whereas the x- or γ-rays are considered sparsely ionizing. The LET concept is illustrated in Figure 1-16 for both densely and sparsely ionizing radiations. For a given ionizing particle, the rate of energy deposition in the absorbing medium increases as the particle slows down. Therefore, a beam of radiation can only be described as having an average value for LET.

Figure 1-16 Variation in the density of ionizing events along an incident particle's track for radiations of different values of linear energy transfer (LET). The more closely the ionizing events

are spaced, the more energy will be deposited in the target volume, and to a point, the more biologically effective per unit dose the type of radiation will be.Representative LET values for types of radiations that have been used for radiation therapy include 0.2 keV/µm for 60Co γ-rays; 2.0 keV/µm for 250 kVp x-rays; approximately 0.5 to 5.0 keV/µm for protons of different energies; approximately 50 to 150 keV/µm for neutrons of varying energy; 100 to 150 keV/µm for α-particles and anywhere from 100 to 2500 keV/µm for “heavy ions.”**Relative Biological Effectiveness (RBE)**Insofar as the “quality” (LET) of the type of radiation influences its biological effectiveness, two questions immediately come to mind. First, why do seemingly subtle differences in microdosimetric energy deposition patterns lead to vastly different biological consequences? Second, how is this differing biological effectiveness manifest in terms of the commonly used assays and model systems of classical radiobiology, and how can this difference be expressed in a quantitative way?Because high LET radiations are more densely ionizing than their low LET counterparts, it follows that energy deposition in a particular “micro” target volume will be greater, and therefore, more severe damage to biomolecules would be expected. In this case, the fraction of cell killing attributable to irreparable and unmodifiable DNA damage increases in relation to that caused by the accumulation of sublethal damage. Because of this, a number of radiobiological phenomena commonly associated with low LET radiation are decreased or eliminated when high LET radiation is used. For example, there is little, if any, sublethal[58] or potentially lethal damage recovery.[113] This is manifest as a reduction or loss of the shoulder of the acute-dose survival curve, little or no sparing effect of dose fractionation or dose rate, and a corresponding reduction in the tolerance doses for normal tissue complications, particularly for late-responding tissues.[119] Variations in the age response through the cell cycle also are reduced or eliminated for high LET radiation,[107] and the oxygen enhancement ratio (OER), which is a measure of the differential radiosensitivity of poorly- versus well-oxygenated cells (see discussion in this chapter), decreases with increasing LET.[120] The dependence of OER on LET is illustrated in Figure 1-17; at an LET of approximately 100 keV/µm, the relative radioresistance of hypoxic cells is eliminated.

Figure 1-17 Relative biological effectiveness (RBE) is demonstrated on the left *y*-axis as a function of linear energy transfer (LET) for a number of biological endpoints, including production of chromosomal aberrations, cell kill, and tissue reactions. The RBE rises to a maximum corresponding to an LET of approximately 100 KeV/µm and then decreases as the LET continues to rise. Shown below the *x*-axis are the ranges of LET for photons plus several different types of particulate radiations that have been used clinically. Dependence of the oxygen enhancement ratio (OER) on LET is shown on the *y*-axis.In light of these differences between high and low LET radiations, the term *relative biological effectiveness* (RBE) has been coined to compare and contrast two radiation beams of different LET. RBE is defined as the ratio of doses of a known type of low LET radiation (historically, 250 kVp x-rays were the standard, but 60Co γ-rays also can be used) to that of a higher LET radiation, to yield the same biological endpoint. RBE does not increase indefinitely with increasing LET however, but it reaches a maximum at approximately 100 keV/µm, and then decreases again, yielding an approximately bell-shaped curve.One interpretation as to why the RBE reaches a maximum at an LET of approximately 100 keV/µm is that at this ionization density, the average separation between ionizing events corresponds roughly to the diameter of the DNA double helix (approximately 2 nm). As such, radiations of this LET have the highest probability of producing DSBs in DNA, the putative lethal lesion, by the passage of a single charged particle. Lower LET radiations have a smaller likelihood of producing such “two-hit” lesions from a single particle track, and therefore are less biologically effective. Radiation beams of higher LET than the optimum are also less biologically effective because some of the energy is wasted because more ionization events than minimally necessary to kill a cell are deposited in the same local area. This phenomenon has been termed the *overkill effect*.**Factors That Influence RBE**RBE is highly variable and depends on several parameters including the type of radiation, total dose, dose rate, dose fractionation pattern, and the biological effect being assayed. Therefore, when

quoting an RBE value the exact experimental conditions used to measure it must be stated. Because increasing LET differentially reduces the shoulder region of the radiation survival curve compared to its exponential or near-exponential high-dose region, the single-dose RBE increases with decreasing dose (Figure 1-18). Second, the RBE determined by comparing two isoeffective acute doses is less than the RBE calculated from two isoeffective (total) doses given either as multiple fractions or at a low dose rate. This occurs because the sparing effect of fractionation magnifies differences in the initial slope or shoulder region of cell survival or tissue dose-response curves (Figure 1-19).

Figure 1-18 Theoretical cell survival curves for x-rays and neutrons, illustrating the increase in relative biological effectiveness (RBE) with decreasing dose. This occurs because higher linear energy transfer (LET) radiations preferentially decrease or eliminate the shoulder on cell survival curves. Adapted from Nias A: Clinical radiobiology, ed 2, New York, 1988, Churchill Livingstone.

Figure 1-19 Increase in the relative biological effectiveness (RBE) of neutrons relative to x-rays when comparing single radiation doses with fractionated treatment. For a given level of cell kill (or other approximately isoeffective endpoint), the more highly fractionated the treatment, the higher the RBE.The Oxygen EffectPerhaps the best known chemical modifier of radiation action is molecular oxygen. As early as 1909, Schwarz recognized that applying pressure to skin and thereby decreasing blood flow (and oxygen supply) caused a reduction in radiation-induced skin reactions.[121] For many decades thereafter, radiation oncologists and biologists continued to suspect that the presence or absence of oxygen was capable of modifying radiosensitivity. In 1955, however, Thomlinson and Gray[122] brought this idea to the forefront of radiation biology and therapy by proposing that tumors contain a fraction of still-clonogenic, hypoxic cells that, if persistent throughout treatment, would adversely affect clinical outcome. Although commonly considered a negative prognostic indicator for radiation therapy, hypoxia nevertheless has one particularly attractive feature: built-in specificity for tumors, to the extent that most normal tissues contain few, if any, hypoxic cells.By studying histological sections of a human bronchial carcinoma, Thomlinson and Gray[122] noted that necrosis was always seen in the centers of cylindrical tumor cords having a radius in excess of approximately 200 µm. Further, regardless of how large the central necrotic region was, the sheath of apparently viable cells around the periphery of this central region never had a radius greater than about 180 µm. The authors went on to calculate the expected maximum diffusion distance of oxygen from blood vessels and found that the value of 150 to 200 µm agreed quite well with the radius of the sheath of viable tumor cells observed histologically. With the advent of more sophisticated and quantitative methods for measuring oxygen utilization in tissues, the average diffusion distance of oxygen has since been revised downward to approximately 70 µm.[28] Thus, the inference was that the oxygenation status of tumor cells varied from fully oxic to completely anoxic depending on where the cells were located in relation to the nearest blood vessels. Accordingly, tumor cells at intermediate distances from the blood supply would be hypoxic and radioresistant, yet remain clonogenic.The first unambiguous demonstration that a solid rodent tumor did contain clonogenic, radioresistant hypoxic cells was by Powers and Tolmach[123] in 1963. These authors used the dilution assay to generate an in vivo survival curve for mouse lymphosarcoma cells. The survival curve for this solid tumor was biphasic, having an initial D0 of about 1.1 Gy, and a final D0 of 2.6 Gy (Figure 1-20). Because the survival curve for lymphoid cells is shoulderless, it was simple to back-extrapolate the shallower component of the curve to the surviving fraction axis and determine that the resistant fraction of cells constituted about 1.5% of the total population. This was considered compelling evidence (yet did not unambiguously prove) that this subpopulation of cells was both hypoxic and clonogenic.

Figure 1-20 Cell survival curve for a murine lymphosarcoma growing subcutaneously and irradiated in vivo. The biphasic curve suggests the presence of a small but relatively radioresistant subpopulation of cells, determined in accompanying experiments to represent the tumor's clonogenic hypoxic fraction. Adapted from Powers WE, Tolmach LJ: A multicomponent x-ray survival curve for mouse lymphosarcoma cells irradiated. Nature 197:710, 1963.The question then

became how to prove that this small fraction of tumor cells was radioresistant because of hypoxia, as opposed to being radioresistant for other reasons. An elegant if somewhat macabre method was developed to address this dilemma, called the *paired survival curve technique*.123,124 In this assay, laboratory animals bearing tumors were divided into three treatment groups, one group irradiated while breathing air, a second group irradiated while breathing 100% oxygen, and a third group killed first by cervical dislocation and then promptly irradiated. Within each group, animals received graded radiation doses so that complete survival curves were generated for each treatment condition. When completed, the paired survival curve method yielded three different tumor cell survival curves: a fully oxic curve (most radiosensitive), a fully hypoxic curve (most radioresistant), and the survival curve for air-breathing animals, which, if the tumor contained viable hypoxic cells, was biphasic and positioned between the other two curves. It was then possible to mathematically strip the fully aerobic and hypoxic curves from the curve for air-breathing animals and determine the radiobiologically hypoxic fraction.Across a variety of rodent tumors evaluated to date using the paired survival curve method, the percentage of hypoxic cells was found to vary between 0% and 50%, with an average of about 15%.124

Mechanistic Aspects of the Oxygen EffectA more rigorous analysis of the nature of the oxygen effect is possible with cells or bacteria grown in vitro. Historically, oxygen had been termed a dose-modifying agent, that is, that the ratio of doses to achieve a given survival level under hypoxic and aerobic conditions was constant, regardless of the survival level chosen. This dose ratio to produce the same biological endpoint is termed the *OER* and is used for comparative purposes (Figure 1-21). The OER typically has a value of between 2.5 and 3.0 for large single doses of x- or γ-rays, 1.5 to 2.0 for radiations of intermediate LET, and 1.0 (i.e., no oxygen effect) for high LET radiations.

Figure 1-21 Representative survival curves for cells irradiated with x-rays in the presence (aerobic) or virtual absence (hypoxic) of oxygen. The oxygen enhancement ratio (OER) is defined as the ratio of doses under hypoxic-to-aerobic conditions to yield the same biological effect, which in this case is a cell surviving fraction (SF) of 0.05.Increasingly, there is evidence that oxygen is not strictly dose modifying. Several studies have shown that the OER for sparsely ionizing radiation is lower at lower doses than at higher doses. Lower OERs for doses per fraction in the range commonly used in radiotherapy have been inferred indirectly from clinical and experimental tumor data and more directly in experiments with cells in culture.125,126 It has been suggested that the lower OERs result from an age response for the oxygen effect, not unlike the age responses for inherent radiosensitivity and cell cycle delay.28 Assuming cells in G1 phase of the cell cycle have a lower OER than those in S phase, and because G1 cells are also more radiosensitive, they would tend to dominate the low dose region of the cell survival curve.Although the exact mechanism(s) of the oxygen effect are obviously complex, a fairly simplistic model can be used to illustrate our current understanding of this phenomenon (Figure 1-22). The radical competition model holds that oxygen acts as a radiosensitizer by forming peroxides in important biomolecules (including but not necessarily limited to, DNA) already damaged by radiation exposure, thereby “fixing” the radiation damage. In the absence of oxygen, DNA can be restored to its preirradiated condition by hydrogen donation from endogenous reducing species in the cell, such as the free radical scavenger glutathione, a thiol compound. In essence, this can be considered a type of fast chemical restitution or repair. These two processes, fixation and restitution, are considered to be in a dynamic equilibrium, such that changes in the relative amounts of either the radiosensitizer, oxygen, or the radioprotector, glutathione, tip the scales in favor of either fixation (more damage, more cell killing, greater radiosensitivity) or restitution (less damage, less cell killing, greater radioresistance).

Figure 1-22 Schematic representation of the proposed mechanism of action for the oxygen effect. The radical competition model holds that oxygen acts as a radiosensitizer by forming peroxides in DNA already damaged by radiation, thereby fixing the damage. In the absence of oxygen, DNA can be restored to its preirradiated state by hydrogen donation from endogenous reducing species in the cell, such as the free radical scavenger glutathione. An oxygen-mimetic, hypoxic cell radiosensitizer may be used to substitute for oxygen in these fast, free radical

reactions, or an exogenously supplied thiol compound may be used to act as a radioprotector. Consistent with this free radical-based interpretation of the oxygen effect is the finding that, for all intents and purposes, oxygen need only be present *during* the irradiation (or no more than a few milliseconds after irradiation) to produce an aerobic radioresponse.127,128 The concentration of oxygen necessary to achieve maximum sensitization is quite small, evidence for the high efficiency of oxygen as a radiosensitizer. A sensitivity midway between a fully hypoxic and fully aerobic radioresponse is achieved at an oxygen tension of about 3 mm of mercury, corresponding to about 0.5% oxygen, which is much lower than partial pressures of oxygen usually encountered in normal tissues. This value of 0.5% has been termed *oxygen's k-value* and is obtained from an oxygen k-curve of relative radiosensitivity plotted as a function of oxygen tension129 (Figure 1-23). **Figure 1-23** An oxygen *k-curve* illustrates the dependence of radiosensitivity on oxygen concentration. If a fully anoxic cell culture is assigned a relative radiosensitivity of 1.0, introducing even 0.5% (3 mm Hg) oxygen into the system increases the radiosensitivity of cells to 2.0. By the time the oxygen concentration reaches about 2%, cells respond as if they are fully aerated (i.e., radiosensitivity ≈ 3.0). The *green-shaded area* represents the approximate range of oxygen concentrations encountered in human normal tissues.**Reoxygenation in Tumors**After the convincing demonstration of hypoxic cells in a mouse tumor,123 it was assumed that human tumors contained a viable hypoxic fraction as well. However, if human tumors contained even a tiny fraction of clonogenic hypoxic cells, simple calculations suggested that tumor control would be nearly impossible with radiation therapy.130 Because therapeutic successes obviously do occur, some form of reoxygenation must take place during the course of multifraction irradiation. This was not an unreasonable idea because the demand for oxygen by sterilized cells would gradually decrease as they were removed from the tumor, and a decrease in tumor size, a restructuring of tumor vasculature, or intermittent changes in blood flow could make oxygen available to these previously hypoxic cells.The reoxygenation process was extensively studied by van Putten and Kallman,131 who serially determined the fraction of hypoxic cells in a mouse sarcoma during the course of a clinically relevant multifraction irradiation protocol. The fact that the hypoxic fraction was about the same at the end of treatment as at the beginning of treatment was strong evidence for a reoxygenation process, because otherwise, the hypoxic fraction would be expected to increase over time as a result of repeated enrichment with resistant cells after each dose fraction. Reoxygenation of hypoxic, clonogenic tumor cells during an extended multifraction treatment would increase the therapeutic ratio, assuming that normal tissues remained well oxygenated. This is thought to be another major factor in the sparing of normal tissues relative to tumors during fractionated radiation therapy.What physiological characteristics would lead to tumor reoxygenation during a course of radiotherapy, and at what rate would this be expected to occur? One possible cause of tumor hypoxia, and by extension, a possible mechanism for reoxygenation, was suggested by Thomlinson and Gray's pioneering work.122 The type of hypoxia that they described is what is now called *chronic*, or *diffusion-limited hypoxia*. This results from the tendency of tumors both to outgrow their blood supply and have high oxygen consumption rates. It follows therefore that natural gradients of oxygen tension should develop as a function of distance from blood vessels. Cells situated beyond the diffusion distance of oxygen would be expected to be dead or dying of anoxia, yet in regions of chronically low-oxygen tension, clonogenic and radioresistant hypoxic cells could persist. Should the tumor shrink as a result of radiation therapy, or if the cells killed by radiation cause a decreased demand for oxygen, it is likely that this would allow some of the chronically hypoxic cells to reoxygenate. However, such a reoxygenation process could be quite slow—on the order of days or more—depending on how quickly tumors regress during treatment. The patterns of reoxygenation in some experimental rodent tumors are consistent with this mechanism of reoxygenation, but others are not.Other rodent tumors reoxygenate quickly, on a time scale of minutes to hours.132 This occurs in the absence of any measurable tumor shrinkage or change in oxygen utilization by tumor cells. In such cases, the model of chronic, diffusion-limited hypoxia and slow reoxygenation does not fit the experimental data. During the late 1970s, Brown133 proposed that a second type of hypoxia may exist in tumors, an acute, *perfusion-limited hypoxia*. Based on the growing understanding of the vascular

physiology of tumors, it was clear that tumor vasculature was often abnormal in both structure and function secondary to abnormal angiogenesis. If tumor vessels were to close transiently from temporary blockage, vascular spasm or high interstitial fluid pressure in the surrounding tissue, the tumor cells in the vicinity of those vessels would become acutely hypoxic almost immediately. Then, assuming blood flow resumed in minutes to hours, these cells would reoxygenate. However, this type of hypoxia can also occur in the absence of frank closure or blockage of tumor vessels (which is now considered a less common cause of acute hypoxia), from, for example, vascular shunting, longitudinal oxygen gradients, decreased red cell flux or overall blood flow rate, abnormal vascular geometry, and so on.134 Because of this, perfusion-*limited* hypoxia is perhaps misleading; a better moniker might be *fluctuant* or *intermittent* hypoxia. Although intermittent hypoxia would explain the rapid reoxygenation observed for some tumors, it does not preclude the simultaneous existence of chronic, diffusion-limited hypoxia.Intermittent hypoxia has since been demonstrated unambiguously for rodent tumors by Chaplin et al.135 and human tumors by Lin et al.134,136 It is still not clear how many human tumors contain regions of hypoxia (although many do—see discussion in this chapter), what type(s) of hypoxia is present, whether this varies with tumor type or site, and whether and how rapidly reoxygenation occurs. However, the knowledge that tumor hypoxia is a diverse and dynamic process opens up a number of possibilities for the development of novel interventions designed to cope with, or even exploit, hypoxia.**Measurement of Hypoxia in Human Tumors**Despite prodigious effort directed at understanding tumor hypoxia and developing strategies to combat the problem, it was not until the late 1980s that these issues could be addressed for human tumors because there was no way to measure hypoxia directly. Before that time, the only way to infer that a human tumor contained treatment-limiting hypoxic cells was by using indirect, nonquantitative methods. Some indirect evidence supporting the notion that human tumors contained clonogenic, radioresistant, hypoxic cells includes the following:1. An association between anemia and poor local control rates, which, in some cases, could be mitigated by preirradiation blood transfusions.1372. Success of some clinical trials in which hyperbaric oxygen breathing was used to better oxygenate tumors.138,139 3. Success of a few clinical trials of oxygen-mimetic hypoxic cell sensitizers combined with radiation therapy.140,141In 1988, one of the first studies showing a strong association between directly measured oxygenation status in tumors and clinical outcome was published by Gatenby.142 An oxygen-sensing electrode was inserted into the patient's tumor, and multiple readings were taken at different depths along the probe's track. The electrode was also repositioned in different regions of the tumor to assess intertrack variability in oxygen tension. Both the arithmetic mean pO2 value for a particular tumor, as well as the tumor volume-weighted pO2 value, directly correlated with local control rate. A high tumor oxygen tension was associated with a high complete response rate, and vice versa. In a similar, prospective study, Höckel et al4, 143 concluded that pretreatment tumor oxygenation was a strong predictor of outcome among patients with intermediate and advanced stage cervical carcinoma (Figure 1-24).
Figure 1-24 The disease-free survival probability of a small cohort of patients with cervical cancer stratified according to pretreatment tumor oxygenation measured using an oxygen electrode. Adapted from Höckel M, Knoop C, Schlenger K, et al: Intratumoral Po2 predicts survival in advanced cancer of the uterine cervix. Radiother Oncol 26:45, 1993.
Unfortunately, the use of oxygen electrodes has its limitations. One weakness is that relative to the size of individual tumor cells, the electrode is large, averaging an outer diameter of 300 µm, a tip recess of 120 µm, and a sampling volume of about 12 µm in diameter.144 Thus, not only is the oxygen tension measurement regional, but the insertions and removals of the probe also no doubt perturb the oxygenation status. Another problem is that there is no way to determine whether the tumor cells are clonogenic or not. If such cells were hypoxic yet not clonogenic, they would not be expected to impact on radiotherapy outcome.A second direct technique for measuring oxygenation status takes advantage of a serendipitous finding concerning how hypoxic cell radiosensitizers are metabolized. Certain classes of radiosensitizers, including the nitroimidazoles, undergo a bioreductive metabolism in the absence of oxygen that leads to their becoming covalently bound

to cellular macromolecules.145,146 Assuming that the bioreductively bound drug could be quantified by radioactive labeling,147 or tagged with an isotope amenable to detection using positron emission tomography148 or magnetic resonance spectroscopy,149 a direct measure of hypoxic fraction can be obtained. Another approach to detecting those cells containing bound drug was developed by Raleigh et al.150,151 This immunohistochemical method involved the development of antibodies specific for the already-metabolized, bound nitroimidazoles. After injecting the parent drug, allowing time for the reductive metabolism to occur, taking biopsies of the tumor and preparing histopathology slides, the specific antibody is then applied to the slides and regions containing the bound drug are visualized directly. This immunostaining method has the distinct advantages that hypoxia can be studied at the level of individual tumor cells,152 spatial relationships between regions of hypoxia and other tumor physiological parameters can be assessed,153 and the drug does not perturb the tumor microenvironment. However, the method remains an invasive procedure, is labor intensive, does not address the issue of the clonogenicity of stained cells (although such cells do have to be metabolically active), and requires that multiple samples be taken because of tumor heterogeneity.One hypoxia marker based on the immunohistological method is commercially available and called HypoxyProbe-1; it detects reductively bound pimonidazole and has been used in experimental and clinical studies around the world (e.g., Bussink et al,154 Nordsmark et al155). Applying HypoxyProbe-1 to human tumor specimens yields a range of hypoxic fractions similar to that noted for experimental rodent tumors and a mean value of approximately 15%.151,156 This marker can also be used to probe disease states other than cancer that may have the induction of tissue hypoxia as part of their etiology, such as cirrhosis of the liver157,158 and ischemia-reperfusion injury in the kidney.159There is also considerable interest in *endogenous* markers of tissue hypoxia160,161 that could reduce to some extent the procedural steps involved in, and the invasive aspects of, detecting hypoxia using exogenous agents. Among the endogenous cellular proteins being investigated in this regard are the hypoxia-inducible factor 1-alpha (HIF-1α, that acts as a transcription factor for hypoxia-regulated genes162), the enzyme carbonic anhydrase IX (CA-9 or CAIX, involved in respiration and maintenance of the proper acid-base balance in tissues163,164), glucose transporter-1 (GLUT-1, which facilitates glucose transport into cells165-167), lysyl oxidase (LOX, which oxidizes lysine residues in extracellular matrix proteins that can enhance the processes of tumor invasion and metastasis168,169) and osteopontin (OPN, a glycoprotein recently identified as a promoter of tumor angiogenesis, invasion, and metastasis170-172).Clearly, although aberrant expression of some individual hypoxia markers has been associated with poor clinical outcome, no one marker is likely to be sufficiently robust or reproducible to either be diagnostic for the presence of a malignancy (or at least, the presence of hypoxia in an already-diagnosed tumor) or be prognostic of treatment outcome. Thus, there has been increasing interest in the study of patterns of hypoxia-associated gene or protein expression for multiple markers simultaneously (e.g., Le,5 Erpolate et al,172 Toustrup et al173).Radiosensitizers, Radioprotectors, and Bioreductive DrugsThe perceived threat that tumor hypoxia posed spawned much research into ways of overcoming the hypoxia problem. One of the earliest proposed solutions was the use of high LET radiations,174 which were less dependent on oxygen for their biological effectiveness. Other agents enlisted to deal with the hypoxia problem included hyperbaric oxygen breathing,138 artificial blood substitutes with increased oxygen carrying capacity,175 oxygen-mimetic hypoxic cell radiosensitizers such as misonidazole or etanidazole,140 hyperthermia,176 normal tissue radioprotectors such as amifostine,177 vasoactive agents thought to modify tumor blood flow such as nicotinamide,178 agents that modify the oxygen-hemoglobin dissociation curve such as pentoxifylline,179 and bioreductive drugs designed to be selectively toxic to hypoxic cells such as tirapazamine.180,181**Radiosensitizers**Radiosensitizers are loosely defined as chemical or pharmacological agents that increase the cytotoxicity of ionizing radiation. True radiosensitizers meet the stricter criterion of being relatively nontoxic in and of themselves, acting only as potentiators of radiation toxicity. Apparent radiosensitizers still produce the net effect of making the tumor more radioresponsive, yet the mechanism is not necessarily synergistic, nor is the agent necessarily nontoxic when given alone. Ideally, a radiosensitizer is only as good as it is selective for

tumors. Agents that show little or no differential effect between tumors and normal tissues do not improve the therapeutic ratio, and therefore may not be of much clinical utility. Table 1-2 summarizes some of the classes of radiosensitizers (and radioprotectors, see discussion) that have been used in the clinic.**TABLE 1-2Selected Chemical Modifiers of Radiation TherapyHypoxic Cell Radiosensitizers**The increased radiosensitivity of cells in the presence of oxygen is believed to be as a result of oxygen's affinity for the electrons produced by the ionization of biomolecules. Molecules other than oxygen also have this chemical property known as electron affinity,182 including some agents that are not otherwise consumed by the cell. Assuming such an electron-affinic compound is not used by the cell, it should diffuse further from capillaries and reach hypoxic regions of a tumor, and acting in an oxygen-mimetic fashion, sensitize hypoxic cells to radiation.One class of compounds that represented a realistic trade-off between sensitizer efficiency and diffusion effectiveness was the nitroimidazoles, which include such drugs as metronidazole, misonidazole, etanidazole, pimonidazole, and nimorazole. The nitroimidazoles consist of a nitroaromatic imidazole ring, a hydrocarbon side chain that determines the drug's lipophilicity, and a nitro group that determines the drug's electron affinity. Misonidazole was extensively characterized in cellular and animal model systems, culminating in its use in clinical trials. Clinical experiences with misonidazole and some of its successor compounds are discussed in Chapter 7.The relative efficacy of a particular hypoxic cell radiosensitizer is most often described in terms of its sensitizer enhancement ratio (SER), which is a parameter similar in concept to the OER. Whereas the OER is the ratio of doses to produce the same biological endpoint under hypoxic versus aerobic conditions, the SER is the dose ratio for an isoeffect under hypoxic conditions alone versus under hypoxic conditions in the presence of the hypoxic cell sensitizer. Accordingly, if a dose of a sensitizer produces an SER of 2.5 to 3.0 for large single doses of low LET radiation, it can be considered, to a first approximation, as effective as oxygen. This statement can be misleading however, in that the dose of the sensitizer required to produce the SER of 3.0 would be higher than the comparable dose of oxygen and high enough in some cases to preclude its use clinically. Finally, because the primary mechanism of action of the nitroimidazoles is substitution for oxygen in radiation-induced free radical reactions, these drugs need only be present in hypoxic regions of the tumor at the time of irradiation.Unfortunately, the nitroimidazoles also have characteristics that decrease their clinical usefulness. The hydrocarbon side chain of the molecule determines its lipophilicity, and this chemical property affects the drug's pharmacokinetics, which is a primary determinant of drug-induced side effects.183 The dose-limiting toxicity of the fairly lipophilic agent misonidazole is peripheral neuropathy, an unanticipated and serious side effect.140,184 Etanidazole was specifically designed to be less lipophilic185 in the hopes of decreasing the neurological toxicity. Although this goal was accomplished as a proof of concept, clinical results with etanidazole were otherwise disappointing186 (see also Chapter 7).Finally, in considering the prodigious amount of research and clinical effort that has gone into the investigation of hypoxic cell radiosensitizers over the past 50 years, it is difficult not to be discouraged by the predominantly negative results of the clinical trials. However, these negative results have prompted a rethinking of the hypoxia problem and novel approaches to dealing with it, as well as consideration of other factors that may have contributed to the lack of success of the nitroimidazole radiosensitizers.184 Among the more obvious questions raised include:1. Did the patients entered on the various clinical trials actually have tumors that contained clonogenic hypoxic cells? At the time of most of these studies, hypoxia markers were not yet available, so it was not possible to triage patients into subgroups in advance of treatment.2. Do hypoxic cells really matter to the outcome of radiotherapy? If reoxygenation is fairly rapid and complete during radiotherapy, the presence of hypoxic cells before the start of treatment may be of little consequence.3. Given that the OER is lower for small than for large doses, it follows that the SER would be reduced as well. If so, a benefit in a subgroup of patients might not be readily observed, at least not at a level of statistical significance.**Bioreductive Drugs**In the wake of the failure of most hypoxic cell radiosensitizers to live up to their clinical potential, a new approach to combating hypoxia emerged: the use of bioreductive drugs that are selectively toxic to hypoxic cells. Although these agents kill rather than sensitize hypoxic cells, the net effect of combining them with radiation

therapy is an apparent sensitization of the tumor, resulting from the elimination of an otherwise radioresistant subpopulation. Such drugs have been shown to outperform the nitroimidazole radiosensitizers in experimental studies with clinically relevant fractionated radiotherapy.187 To the extent that hypoxic cells are also resistant to chemotherapy because of tumor microenvironmental differences in drug delivery, pH or the cell's proliferative status, complementary tumor cell killing might be anticipated for combinations of bioreductive agents and anticancer drugs as well.188Most hypoxia-specific cytotoxic drugs fall into three categories: the nitroheterocyclics, the quinone antibiotics, and the organic nitroxides.189 All require bioreductive activation by nitroreductase enzymes, such as cytochrome P450, DT-diaphorase, and nitric oxide synthase, to reduce the parent compound to its cytotoxic intermediate, which is typically an oxidizing free radical capable of damaging DNA and other cellular macromolecules. The active species is either not formed or else immediately back-oxidized to the parent compound in the presence of oxygen, which accounts for its preferential toxicity under hypoxic conditions. Examples of nitroheterocyclic drugs with bioreductive activity include misonidazole and etanidazole190,191 and dual function agents such as RSU 1069.192 The latter drug is *dual function* because its bioreduction also activates a bifunctional alkylating moiety capable of introducing cross-links into DNA. Mitomycin C and several of its analogs (including porfiromycin and EO9) are quinones with bioreductive activity that have been tested in randomized clinical trials in head and neck tumors (e.g., Haffty et al193).The lead compound for the third class of bioreductive drugs, the organic nitroxides, is tirapazamine (SR 4233, see Table 1-2).180,181,187 The dose-limiting toxicity for single doses of tirapazamine is a reversible hearing loss; other effects observed include nausea and vomiting and muscle cramps.194Tirapazamine is particularly attractive because it both retains its hypoxia-selective toxicity over a broader range of (low) oxygen concentrations than the quinones and nitroheterocyclic compounds,195 and its hypoxic cytotoxicity ratio, which is the ratio of drug doses under hypoxic versus aerobic conditions to yield the same amount of cell killing, averages an order of magnitude higher than for the other classes of bioreductive drugs.188 Laboratory and clinical data also support a tumor sensitizing role for tirapazamine in combination with the chemotherapy agent cisplatinum.194To date, randomized Phase II and III clinical trials with tirapazamine combined with radiotherapy and/or chemotherapy (particularly, cisplatinum) have yielded mixed results194,196,197; however, it has improved outcomes for some standard treatments. Although it is disappointing that tirapazamine hasn't made a more significant impact on clinical practice, the search for more effective agents from the same or similar chemical class continues.**Proliferating Cell Radiosensitizers**Another source of apparent radioresistance is the presence of rapidly proliferating cells. Such cells may not be inherently radioresistant, but rather, have the effect of making the tumor seem refractory to treatment because the production of new cells outpaces the cytotoxic action of the therapy.Analogs of the DNA precursor thymidine, such as bromodeoxyuridine (BrdUdR) or iododeoxyuridine (IdUdR), can be incorporated into the DNA of actively proliferating cells in place of thymidine because of close structural similarities between the compounds. Cells containing DNA substituted with these halogenated pyrimidines are more radiosensitive than normal cells, with the amount of sensitization directly proportional to the fraction of thymidine replaced.198 In general, the radiosensitization takes the form of a decrease in or elimination of the shoulder region of the radiation survival curve. To be maximally effective, the drug must be present for at least several rounds of DNA replication before irradiation. Although the mechanism by which BrdUdR and IdUdR exert their radiosensitizing effect remains somewhat unclear, it is likely that both the formation of more complex radiation-induced lesions in the vicinity of the halogenated pyrimidine molecules and interference with DNA damage sensing or repair are involved.199The clinical use of halogenated pyrimidines began in the late 1960s, with a major clinical trial in head and neck cancer.200 In retrospect, the choice of head and neck tumors for this study was far from ideal because the oral mucosa is also a rapidly proliferating tissue and was similarly radiosensitized, causing severe mucositis and a poor therapeutic ratio overall. In later years, tumors selected for therapy with halogenated pyrimidines were chosen in the hopes of better exploiting the differential radiosensitization between tumors and normal tissues.201 Aggressively

growing tumors surrounded by slowly proliferating or nonproliferating normal tissues, such as high-grade brain tumors or some sarcomas, have been targeted for example.202,203 Later strategies for further improving radiosensitization by BrdUdR and IdUdR involved changing the schedule of drug delivery: giving the drug as a long, continuous infusion both before and during radiotherapy204 and administering the drug as a series of short, repeated exposures.205 Overall however, the use of halogenated pyrimidines in the clinic has remained experimental and has not become mainstream.**Chemotherapy Drugs as Radiosensitizers**Several chemotherapy agents have long been known to increase the effectiveness of radiotherapy, despite not being true radiosensitizers like the nitroimidazoles. This has driven the clinical practice of treating many more patients today than in the past with chemotherapy and radiation therapy given concurrently. Two drugs in particular used for chemoradiotherapy are 5-fluorouracil (5-FU, effective against GI malignancies206) and cisplatinum (effective against head and neck207 and cervical cancers208).Based on these clinical successes in combining radiation with concurrent chemotherapy and with ever-increasing numbers of molecularly targeted drugs and biologics available today, it is naturally of interest whether any of these novel compounds could also act as radiosensitizers. Two classes of such drugs already have entered the clinical mainstream as sensitizers: the anti-epidermal growth factor receptor (EGFR) inhibitors and the anti-vascular endothelial growth factor (VEGF) inhibitors. Cetuximab is a monoclonal antibody raised against the EGFR that has been shown to improve outcomes in advanced head and neck cancers when combined with radiation therapy,209 and bevacizumab, a humanized monoclonal antibody raised against VEGF, prolongs overall and progression-free survival in patients with advanced colorectal cancer when combined with standard chemotherapy.210 It is hoped that these and other targeted agents will play greater roles in cancer therapy in the future.**Normal Tissue Radioprotectors**Amifostine (WR 2721, see Table 1-2) is a phosphorothioate compound developed by the U.S. Army for use as a radiation protector. Modeled after naturally occurring radioprotective sulfhydryl compounds such as cysteine, cysteamine, and glutathione,211 amifostine's mechanism of action involves the scavenging of free radicals produced by ionizing radiation, which are radicals that otherwise could react with oxygen and “fix” the chemical damage. Amifostine can also detoxify other reactive species through the formation of thioether conjugates, and in part because of this, the drug can also be used as a chemoprotective agent.212 Amifostine is a pro-drug that is dephosphorylated by plasma membrane alkaline phosphatase to the free thiol WR 1065, the active metabolite. As is the case with the hypoxic cell radiosensitizers, amifostine need only be present at the time of irradiation to exert its radioprotective effect.In theory, if normal tissues could be made to tolerate higher total doses of radiation through the use of radioprotectors, then the relative radioresistance of hypoxic tumor cells would be less likely to limit radiation therapy. However, encouraging preclinical studies demonstrating radioprotection of a variety of cells and tissues notwithstanding,213,214 radioprotectors like amifostine would *not* be expected to increase the therapeutic ratio unless they could be introduced selectively into normal tissues but not tumors. The pioneering studies of Yuhas et al177,215,216 addressed this issue by showing that the drug's active metabolite reached a higher concentration in most normal tissues than in tumors and that this mirrored the extent of radio- or chemoprotection. The selective protection of normal tissues results from slower tumor uptake of the drug, and tumor cells being both less able to convert amifostine to WR 1065 (resulting from lower concentrations of the required phosphatases) and to transport this active metabolite throughout the cell.Dose reduction factors (DRFs, the ratio of radiation doses to produce an isoeffect in the presence versus the absence of the radioprotector) in the range of 1.5 to 3.5 are achieved for normal tissues, whereas the corresponding DRFs for tumors seldom exceed 1.2. Those normal tissues exhibiting the highest DRFs include bone marrow, gastrointestinal tract, liver, testes, and salivary glands.177 Brain and spinal cord are not protected by amifostine, and oral mucosa is only marginally protected.177 Comparable protection factors are obtained for some chemotherapy agents, including cyclophosphamide and cisplatin.217,218 The dose-limiting toxicities associated with the use of amifostine include hypotension, emesis, and generalized weakness or fatigue.219Amifostine is currently indicated for the reduction of renal toxicity associated with repeated cycles of cisplatin

chemotherapy in patients with advanced ovarian and non–small cell lung cancer. It is also approved for use in patients receiving radiotherapy for head and neck cancer, in the hopes of reducing xerostomia secondary to exposure of the parotid glands.Finally, just as there are apparent radiosensitizers, there are also apparent radioprotectors that have the net effect of allowing normal tissues to better tolerate higher doses of radiation and chemotherapy but through mechanisms of action not directly related to the scavenging of free radicals. Various biological response modifiers, including cytokines, prostaglandins (such as misoprostol220,221), anticoagulants (such as pentoxifylline222,223), and protease inhibitors are apparent radioprotectors because they can interfere with the chain of events that normally follows the killing of cells in tissues by, for example, stimulating compensatory repopulation or preventing the development of fibrosis. Finally, there is also growing interest in the use of biologics that inhibit apoptosis as normal tissue radioprotectors.224,225 Such agents should have little or no effect on tumor cells, most of which are already resistant to apoptosis. Clinical Radiobiology**Growth Kinetics of Normal Tissues and Tumors**In the simplest sense, normal tissues are normal because the net production of new cells, if it occurs at all, exactly balances the loss of old cells from the tissue. In tumors, the production of new cells exceeds cell loss, even if only by a small amount. Although the underlying radiobiology of cells in vitro applies equally to the radiobiology of tissues, the imposition by growth kinetics of this higher level of organizational behavior makes the latter far more complex systems.**Descriptive Classification Systems**Two qualitative classification systems based loosely on the proliferation kinetics of normal tissues are in use. Borrowing heavily from the pioneering work of Bergonié and Tribondeau,13 Rubin and Casarett's226 classification system for tissue radiosensitivity consists of four main categories:

Type I or vegetative intermitotic (VM) cells are considered the most radiosensitive and consist of regularly dividing, undifferentiated stem cells, such as are found in the bone marrow, intestinal crypts, and the basal layer of the epidermis of the skin.

Type II or differentiating intermitotic (DIM) cells are somewhat less radiosensitive and consist of transitional cells that are in the process of developing differentiated characteristics, yet are still capable of a limited number of cell divisions. Myelocytes of the bone marrow and spermatocytes of the testis are examples of Type II cells.

Type III or reverting postmitotic (RPM) cells are relatively radioresistant and consist of those few types of cells that are fully differentiated and do not divide regularly, yet under certain conditions can revert to a stem cell-like state and divide as needed. Examples of Type III cells include hepatocytes and lymphocytes, although the latter are unique in that they are a notable exception to the Rubin and Casarett classification system—an RPM cell type that is exquisitely radiosensitive.

Type IV or fixed postmitotic (FPM) cells are the most radioresistant and consist of the terminally differentiated, irreversibly postmitotic cells characteristic of most normal tissue parenchyma, such as neurons and muscle cells. Should such cells be killed by radiation, they typically cannot be replaced.A second, simpler classification system, based on anatomical and histological considerations, has been proposed by Michalowski.227 Using this system, tissues are categorized on the basis of whether the tissue stem cells, if any, and the functional cells are compartmentalized (so-called Type H or hierarchical tissues, such as skin, gut epithelium, testis, etc.), or intermixed (Type F or flexible tissues, such as lung, liver, kidney, and spinal cord).**Growth Kinetic Parameters and Methodologies**To predict the response of an intact tissue to radiation therapy in a more quantitative way, a number of kinetic parameters have been described that provide a better picture of the proliferative organization of tumors and normal tissues (Table 1-3).**TABLE 1-3 Estimated Cell Cycle Kinetic Parameters for Human Tumors**Data from Steel GG: Growth kinetics of tumours, Oxford, 1977, Clarendon Press; and Joiner M, van der Kogel A, editors: Basic clinical radiobiology, ed 4. London, 2009, Hodder Arnold.**Growth Fraction**Among the first kinetic characteristics described was the growth fraction (GF). The presence of a fraction of slowly or noncycling cells in experimental animal tumors was first noted by Mendelsohn et al228,229 and subsequently, in human tumors by others. Although normal tissues don not grow and therefore do not have a growth fraction per se, many are composed of noncycling cells that have

differentiated to carry out tissue-specific functions. Some normal tissues do contain a small fraction of actively proliferating stem cells, and others contain apparently dormant or resting cells that are temporarily out of the traditional four-phase cell cycle but are capable of renewed proliferation in response to appropriate stimuli. Lajtha gave these resting but recruitable cells of normal tissues the designation G0.[230] Although a tumor counterpart of the G0 cell may or may not exist, the majority of slowly or noncycling tumor cells are thought to be in such a state because of nutrient deprivation, not because of a normal cell cycle regulatory mechanism. Thus, Dethlefsen [231] has suggested that the term *Q cell* be reserved for quiescent cells in tumors to distinguish them from the G0 cell of normal tissues.Measurement of a tumor's growth fraction is problematic,[232,233] but an estimate can be obtained with a technique known as continuous thymidine labeling. Using this method, the tumor receives a continuous infusion of radio-labeled thymidine for a period of time long enough for all proliferating cells to have gone through at least one round of DNA synthesis and incorporated the radioactive label. Then, a biopsy of the tumor is obtained and tissue sections prepared for autoradiography. Once the slides are processed and scored, the continuous labeling index, which is that fraction of the total population of tumor cells containing tritiated thymidine, is calculated. This value is a rough estimate of the tumor's growth fraction.**Cell Cycle and Volume Doubling Times**The percent labeled mitosis (PLM) technique of Quastler and Sherman[234] was a key development in the study of the cell cycle in vivo because it provided a unique window into the behavior of that small fraction of cells within a tissue that was actively proliferating. By focusing on cells in mitosis, the assay allowed both the overall cell cycle time (Tc) and the durations of the individual cell cycle phases to be determined without the uncertainties introduced by the presence of noncycling cells in the population. Today, flow cytometric methods have largely replaced the arduous and time-consuming PLM assay.Briefly, the PLM technique involves tracking a cohort of proliferating cells over time that initially was in S phase (and exposed briefly to tritiated thymidine) and then proceeded through subsequent mitotic divisions. Serial biopsy samples from the tissue of interest are obtained at regular intervals following labeling, and the fraction of cells both in mitosis (identified cytologically) *and* carrying the radioactive label is determined. A first peak of labeled mitoses is observed within 24 hours after labeling, and as cells pass through their second division, a second wave of labeled mitoses is noted. The average Tc for the population of proliferating cells corresponds to the peak-to-peak interval of the resulting PLM curve, a plot of the fraction of labeled mitoses as a function of time following the radioactive pulse. With sufficiently robust data, the durations of the individual cell cycle phases can be obtained as well. The PLM technique is illustrated schematically in Figure 1-25.

Figure 1-25 The technique of percent labeled mitoses (PLM) for an idealized cell population with identical cell cycle times *(left panels)* and for a representative normal tissue or tumor with a dispersion in cell cycle times *(right panel)*. *Upper left panel*, After a brief exposure to tritiated thymidine or equivalent at time a, the labeled cohort of S-phase cells continues *(dark shading)* around the cell cycle and is sampled at times b, c, d, and e. *Lower left panel*, For each sample, the percentage of cells both in mitosis and containing the thymidine label is determined, and it is plotted as a function of time. From such a graph, individual cell cycle phase durations can be derived. *Right panel*, In this more practical example, a mathematical fit to the PLM data would be needed to calculate the (average) cell cycle phase durations.Historically, the interpretation of PLM curves was sometimes hampered by technical artifacts and by the fact that proliferating cell populations have distributed cell cycle times.[232,233,235] Despite these limitations, it is clear that most cells in vivo proliferate more slowly than their in vitro counterparts. Although the variation in intermitotic times is quite large, a median value for Tc of 2-3 days is a reasonable estimate.[232] Although the cycle times of proliferating cells in vivo are long by cell culture standards, they are quite short when compared to the corresponding population or volume doubling times (Td) for human tumors. Although highly variable from tumor type to tumor type and somewhat difficult to measure, the Td for human solid tumors averages about 3-4 months.[232] In many cases, sample calculations further suggest that the discrepancy between Tc for proliferating tumor cells and Td for the tumor as a whole cannot be accounted for solely by the tumor having a low growth fraction.**Cell Loss Factor**Cell kineticists initially adhered to the notion that the continued growth of

tumors over time reflected abnormalities in cell production. Pathologists and tumor biologists meanwhile had ample evidence that tumors routinely lost large numbers of cells, which was the result of cell death, maturation, or emigration.232,236,237 It is now clear that the overall rate of tumor growth, as reflected by its Td, is governed by the competing processes of cell production and cell loss. In fact, the cell loss factor, ϕ, which is the rate of cell loss expressed as a fraction of the cell production rate, is surprisingly high for both experimental and human tumors, as high as 0.9 or more for carcinomas, and lower, on average, for sarcomas.232 Cell loss is usually the most important factor governing the overall volume doubling time of solid tumors.The clinical implications of tumors having high rates of cell loss are obvious. First, any attempts at making long-term predictions of treatment outcome based on short-term regression rates of tumors during treatment are misleading. Second, although regression rate may not correlate well with eventual outcome, it may be a reasonable indicator of when best to schedule subsequent therapy, on the assumption that a smaller tumor will be more radio- and chemo-sensitive, as well as easier to remove surgically.**Potential Doubling Time and Effective Doubling Time**With the recognition that cell loss plays a major role in the overall growth rate of tumors and that it can mask a high cell production rate, a better measure of the potential repopulation rate of normal tissues and tumors was needed.238 One indicator of regenerative capacity is the potential doubling time, or Tpot.232, 239 By definition, Tpot is an estimate of the time that would be required to double the number of clonogenic cells in a tumor in the absence of cell loss. It follows therefore that Td will usually be much longer than Tpot because of cell loss, and Tc will usually be shorter than Tpot because of the presence of non-proliferating cells.235Tpot can be estimated from a comparison of the S phase pulse labeling index (LI) and the duration of S phase (TS) by using the following equation,where λ is a correction factor related to the nonuniform distribution of cell ages in a growing population (usually, λ ≈ 0.8). TS and LI can determined by the relative movement method.239,240 This technique involves an injection of a thymidine analog, usually BrdUrd, which is promptly incorporated into newly synthesized DNA and whose presence can be detected using flow cytometry. The labeled cohort of cells is then allowed to continue movement through the cell cycle, and a biopsy of the tumor is taken several hours later, at which point the majority of the cells containing BrdUrd have progressed into G2 phase or beyond. A value for LI is determined from the fraction of the total cell population that contains BrdUrd, and TS is calculated from the rate of movement of the labeled cohort during the interim between injection of the tracer and biopsy.Values for Tpot for human tumors have been measured, and although quite variable, typically range between 2 and 20 days.232,238,241 These findings lend support to the important idea that slowly growing tumors can contain subpopulations of rapidly proliferating cells. To the extent that these cells retain unlimited reproductive potential, they may be considered the tumor's stem cells (in a generic sense at least) capable of causing recurrences after treatment. These cells represent a serious threat to local control of the tumor by conventional therapies, especially protracted treatments (that provide them additional time to proliferate).The use of a cell kinetic parameter such as Tpot as either a predictor of a tumor's response to therapy or as a means of identifying subsets of patients particularly at risk for recurrence has been attempted, with some positive, but mostly negative, results.6,139,242 Lest these negative findings suggest that proliferation in tumors is *un*important, bear in mind that it is unlikely that a pretreatment estimate of Tpot—or any other single cell kinetic parameter (e.g., LI) for that matter—would be relevant once treatment commences and the growth kinetics of the tumor are perturbed.One approach to dealing with this problem is to measure proliferative activity *during* treatment. Although not without other limitations, the use of an effective clonogen doubling time (Teff or Tp) has been advocated.243-245 Estimates of Tp can be obtained from two types of experiments. In an experimental setting, Tp can be inferred from the additional dose necessary to keep a certain level of tissue reaction constant as the overall treatment time is increased. (When expressed in terms of dose rather than time, the proper term would be Deff, although the underlying concept is the same.) For example, acute skin reactions usually both develop and begin to resolve during the course of radiation therapy, suggesting that the production of new cells in response to injury gradually surpasses the killing of existing cells by each subsequent dose fraction. By intensifying

treatment once this repopulation begins, it should be theoretically possible to reach a steady state wherein the tissue reaction remains constant. In a clinical setting, Tp can be estimated from a comparison of tumor control rates for treatment schedules in which the dose per fraction and total dose used were held approximately constant, but the overall treatment time varied. In some cases, the rate of loss of local control with increasing overall treatment time provides an estimate not only of Tp but also of the delay time before the repopulation begins, sometimes referred to as Tk, which is the repopulation kickoff time.246-248**Repopulation in Tumors and Normal Tissues**As discussed previously, both normal tissues and tumors are capable of increasing their cell production rate in response to depopulation caused by radiation exposure, a process known as regeneration or repopulation. The time of onset of the regenerative response varies with the turnover rate of the tissue or tumor because cell death (and depopulation) following irradiation is usually linked to cell division. Generally, tissues that naturally turn over fairly rapidly repopulate earlier and more vigorously than tissues that turn over slowly. However, it has been shown that the repopulation patterns of normal tissues and tumors following the start of irradiation tend to be characterized by a delay (of at least several weeks in many cases; see Tk discussion) before the rapid proliferative response.246-248 Once this proliferative response begins, however, it can be quite vigorous. Although this is clearly desirable for early-responding normal tissues attempting to recover from radiation injury, rapid proliferation in tumors is certainly counterproductive.249 For example, clinical studies of local control of head and neck tumors indicate that an average of about 0.6 Gy per day is lost to repopulation.243 Unfortunately, attempts to counteract this accelerated proliferation by dose intensification during the latter part of a treatment course can be problematic because late-responding normal tissues do not benefit from accelerated repopulation during treatment and risk incurring complications.Increased cell production in response to injury is thought to occur by one or more of three basic mechanisms: a decrease in the cell loss rate, a shortening of the cell cycle time, or an increase in the growth fraction.57,232 Normal tissues are capable of using at least the latter two strategies to accelerate repopulation. It is still not clear how, when, and to what extent tumors accelerate their proliferation in response to cell killing. Laboratory and clinical findings suggest that a decrease in cell loss may be a major mechanism, possibly as a consequence of resistance to apoptosis, which is a hallmark of most types of cancer.244,250,251 This is a provocative finding in that it suggests that there may be a fundamental—and potentially exploitable—difference in the way normal and tumor tissues respond to ionizing radiation.Early and Late Effects in Normal Tissues**Early versus Late**
Normal tissue complications observed following radiation therapy are the result, either directly or indirectly, of the killing of critical target cells within the tissue that are crucial to the tissue's continued functional or structural integrity. The loss of these target cells can occur either as a direct consequence of the cytotoxic action of the radiation or indirectly as a result of the radiation injury or killing of other cells. In some cases, the tissue's response to the depletion of its component cells can exacerbate the injury, for example, when a hyperproliferation of fibroblasts and the resulting collagen deposition replace a tissue's parenchymal cells, resulting in fibrosis.It is important to realize that a particular tissue or organ may contain more than one type of target cell, each with its own radiosensitivity. One tissue may manifest more than one complication following radiation therapy, with the severity of each determined by the radiosensitivity of the particular target cell and the time-dose fractionation schedule employed. It follows from this that the severity of one complication does not necessarily predict for the severity of another complication, even within the same tissue (although consequential late effects secondary to severe early reactions are possible in some cases252). For example, dry or moist desquamation of the skin results from the depletion of the basal cells of the epidermis, fibrosis results from damage to dermal fibroblasts, and telangiectasia results from damage to small blood vessels in the dermis. For many tissues however, the target cell(s) whose death is (are) responsible for a particular normal tissue injury remain unclear.Although the radiosensitivity of the putative target cells determines the severity of an early or late effect in a normal tissue, the earliness or lateness of the clinical manifestation of that injury is related to the tissue's proliferative organization (discussed previously). The distinction between the radiosensitivity of a tissue's cells and the radioresponsiveness of the tissue as a whole

can be a source of confusion. Bergonié and Tribondeau's laws,13 for example, confused the concepts of radiosensitivity and radioresponsiveness to some extent, referring to tissues that responded to damage early as radiosensitive, when this was not necessarily the case.**Whole-Body Radiation Syndromes**Many human beings have been exposed to total body irradiation, including the survivors of Hiroshima and Nagasaki, Polynesian Islanders and military personnel present during aboveground nuclear tests during the 1950s, and victims of accidental exposures in the workplace (including Chernobyl, and possibly, Fukushima). Of the latter, about 100 fatalities resulting from radiation accidents have been documented since the mid-1940s.253-255The whole-body radiation syndromes are considered early effects of radiation exposure because, for sufficiently high doses, death occurs within weeks after irradiation. The clinical syndromes described herein only occur when most or all of the body is irradiated. Also, although total body irradiation (TBI) is a prerequisite for the manifestation of these syndromes, neither the dose received nor its biological consequences are necessarily uniform. The radiosensitivities of the respective target cells determine the effective threshold dose below which the syndrome does not occur, whereas the onset time of individual symptoms is governed more by the proliferative organization of the tissue.The mean lethal dose or LD50 is defined as the (whole body) dose that results in mortality for 50% of an irradiated population. The LD50 value is often expressed in terms of the time scale over which the deaths occur, such as at either 30 or 60 days after irradiation. For humans, the single-dose LD50/60 for x- or γ-rays is approximately 3.5 Gy in the absence of medical intervention and about twice that with careful medical management.28,255 The LD50 increases with decreasing dose rate of low LET radiation and decreases for radiations of higher LET.**The Prodromal Syndrome**The prodromal syndrome consists of one or more transient, neuromuscular and gastrointestinal symptoms that begin soon after irradiation and persist for up to several hours. The symptoms, which can include anorexia, nausea, vomiting, diarrhea, fatigue, disorientation, and hypotension, and their severity and duration, increase with increasing dose. Because in most radiation accident situations the dose victims received is unknown initially, careful attention to the prodromal syndrome can be used as a crude dosimeter.**The Cerebrovascular Syndrome**The cerebrovascular syndrome occurs for total body doses in excess of 50 Gy. The onset of signs and symptoms is almost immediate following exposure, consisting of severe gastrointestinal and neuromuscular disturbances including nausea and vomiting, disorientation, ataxia, and convulsions.28,255 The cerebrovascular syndrome is invariably fatal, and survival time is seldom longer than about 48 hours. Only a few instances of accidental exposure to such high doses have occurred, two of which (a nuclear criticality accident at Los Alamos National Laboratory in 1958, and a 235U reprocessing plant accident in Rhode Island in 1964) have been extensively documented in the medical literature.256,257The immediate cause of death for the cerebrovascular syndrome is likely vascular damage leading to progressive brain edema, hemorrhage, or cardiovascular shock.255 Following such high doses delivered acutely, even cells traditionally considered radioresistant, such as neurons and the parenchymal cells of other tissues and organs, will be killed, as well as the more radiosensitive vascular endothelial cells and the various glial cells of the central nervous system.**The Gastrointestinal Syndrome**For doses upward of about 8 Gy, the gastrointestinal syndrome predominates, characterized by lethargy, vomiting and diarrhea, dehydration, electrolyte imbalance, malabsorption, weight loss, and ultimately, sepsis. These symptoms begin to appear within a few days of irradiation and are progressive in nature, culminating in death after 5 to 10 days. The target cells for the gastrointestinal syndrome are principally the crypt stem cells of the gut epithelium. As mature cells of the villi are lost over a several-day period, no new cells are available to replace them, so the villi begin to shorten and eventually become completely denuded. This greatly increases the risk of bleeding and sepsis, both of which are aggravated by declining blood counts.Before the Chernobyl accident, in which approximately a dozen firefighters received total doses sufficient to succumb to the gastrointestinal syndrome, there was only one other documented case of a human dying of gastrointestinal injury.28,255 To date, no human has survived a documented whole-body dose of 10 Gy of low LET radiation.**The Hematopoietic Syndrome**Acute doses of approximately 2.5 Gy or more are sufficient to instigate the hematopoietic syndrome, which is a

consequence of the killing of bone marrow stem cells and lymphocytes. This syndrome is characterized by a precipitous (within a day or two) reduction in the peripheral blood lymphocyte count, followed by a more gradual reduction (over a period of 2 to 3 weeks) in the numbers of circulating leukocytes, platelets, and erythrocytes. The granulocytopenia and thrombocytopenia reach a maximum within 30 days after exposure, and death, if it is to occur, is usually a result of infection or hemorrhage.28,255 Theoretically, the use of antibiotics, blood transfusions, and bone marrow transplantation can save the lives of individuals who receive doses at or near the LD50. In practice however, the exact dose is seldom known and should it be high enough to reach the threshold for the gastrointestinal syndrome, such heroic measures would be in vain. Unfortunately, this was the case for all but 2 of the 13 Chernobyl accident victims who received bone marrow transplants.28 Of the two survivors, only one can rightfully be claimed as having had his life saved by the transplant; the other survivor showed autologous bone marrow repopulation.

TeratogenesisOne of the most anxiety-provoking risks of irradiation in the eyes of the general public is prenatal exposure of the developing embryo or fetus.254,255 In part, such concern is warranted because teratogenic effects are quite sensitive to induction by ionizing radiation, with readily measurable neurological abnormalities noted in individuals exposed prenatally to doses as low as approximately 0.06 Gy.28 The radiation-induced excess relative risk of teratogenesis during the most sensitive phase of gestation is approximately 40% per Gy.28 (By comparison, the spontaneous incidence of a congenital abnormality occurring during an otherwise normal pregnancy is about 5% to 10%.255)Information on the teratogenic effects of radiation in humans come from two major sources, the Japanese atomic bomb survivors and patients who received diagnostic or therapeutic irradiation either before the establishment of modern radiation protection standards or in clinical emergency situations. Although a range of abnormalities have been identified in individuals irradiated in utero (including anecdotal reports of miscarriages and stillbirths, cataracts and other ocular defects, gross malformations, sterility, etc.), the most commonly reported are microcephaly, mental retardation, other, more subtle neurological defects, and growth retardation.28,254,255 Each of these teratogenic effects has a temporal relationship to the stage of gestation at the time of irradiation, as well as a radiation dose and dose rate dependency. Lethality is the most common consequence of irradiation during the preimplantation stage (within 10 days of conception), growth retardation has been noted for irradiation during the implantation stage (10 to 14 days after conception), and during the organogenesis period (about 15 to 50 days after conception), the embryo is sensitive to both lethal, teratogenic, and growth retarding effects.255 Radiation-induced gross abnormalities of the major organ systems do not occur during the fetal period (more than 50 days after conception), although generalized growth retardation and some neurological defects have been noted for radiation doses in excess of 1 Gy.**Radiation-Induced Cataracts**Late effects resulting from irradiation of the eye were noted within a few years of the discovery of x-rays,226,255 with cataracts being the most frequent pathological finding. From a clinical perspective, the induction of a cataract following radiotherapy is a normal tissue complication that can be corrected surgically, and as such, is not considered quite as dire as other late effects. In addition, some radiation-induced lens opacities are subtle and do not interfere with vision. From a radiobiologic perspective, however, cataracts are unique among the somatic effects of radiation in several respects. First, although the lens of the eye is a self-renewing tissue complete with a stem cell compartment of epithelial cells that divide and gradually differentiate into mature lens fibers, there appears to be no clear mechanism of cell loss.28 As such, the primitive cells damaged by radiation (which manifest themselves as abnormal, opaque lens fibers) persist, eventually leading to a cataract. Second, radiation-induced cataracts are among the few lesions that *can* be distinguished pathologically from their spontaneously occurring counterparts; radiation-induced cataracts first appear in the posterior pole of the lens, whereas spontaneous cataracts usually begin in the anterior pole.258 Third, radiogenic cataracts exhibit a variable latency period (anywhere from about one year to several decades) that decreases with increasing radiation dose. Finally, cataract formation is a nonstochastic (deterministic) process; that is, there is a threshold dose below which no cataracts occur, but above the threshold, the severity of the cataract increases with increasing dose.258 For low LET

radiation, the single-dose threshold for cataractogenesis in humans is approximately 2 Gy, and this increases to about 4 Gy for fractionated exposures. Neutrons are also known to be quite effective at inducing cataracts, with RBEs of about 5 to 10 commonly observed in laboratory rodents.255**Radiation Carcinogenesis**Unrepaired or mis-rejoined DNA damage caused by radiation exposure is usually lethal to the cell, although this is not invariably the case, particularly when the genetic material is simply rearranged rather than deleted. Whether such changes have further implications for the cell bearing them depends on the location of the damage in the genome, the nature and extent of the mutational event, whether working copies of proteins can still be produced from the gene or genes involved, what function these proteins normally have, and the type of cell. There is compelling evidence that some of these radiation-induced genetic rearrangements—particularly ones that activate oncogenes or inactivate tumor suppressor genes—either alone or in combination with other such changes, predispose a cell to neoplastic transformation, a necessary early step in the process of tumor induction.254,259 **Laboratory Studies**Although ionizing radiation is one of the most studied and best understood carcinogens, it is not a particularly potent one. This fact hampers studies of radiation carcinogenesis in humans because the investigator must identify a modest radiogenic increment of excess risk with a long latency period against a high background spontaneous cancer rate and multiple confounding factors. Nevertheless, from a public health perspective, carcinogenesis is the most important somatic effect of radiation for doses of 1.5 Gy or less.28The use of cell cultures and laboratory rodents to study carcinogenesis avoid some of the pitfalls of human epidemiological studies but have their own inherent limitations. Cell culture systems employ neoplastic transformation as the endpoint, which is a prerequisite for, but by no means equal to, carcinogenesis in vivo. Neoplastic transformation is defined as the acquisition of one or more phenotypic traits in nontumorigenic cells that are usually associated with malignancy, such as immortalization, reduced contact inhibition of growth, increased anchorage-independent growth, reduced need for exogenously supplied nutrients and growth factors, various morphological and biochemical changes, and in nearly all cases, the ability to form tumors in histocompatible animals.235 Such systems can be used to study relatively early events in the carcinogenesis process, have much greater sensitivity and statistical resolution than in vivo assays, and can be used to measure dose-response relationships. Laboratory animal studies however are considered more relevant in that tumor formation is the endpoint, latency periods are shorter, statistical variability is reduced, and the carcinogen exposure conditions can be carefully controlled.Pertinent results from laboratory studies of radiation carcinogenesis include:1. Carcinogenesis is a stochastic effect, that is, a probabilistic function of the dose received, with no evidence of a dose threshold. Increasing the radiation dose increases the probability of the effect but not its severity.254,2592. The neoplastic transformation frequency increases with dose, at least over the low-dose range (about 1.5 Gy or less).3. There is a dose rate effect for transformation and carcinogenesis (for low LET radiations); protracted exposures carry a reduced risk relative to acute exposures.4. The processes of neoplastic transformation and carcinogenesis are necessarily in competition with the cell killing effects of ionizing radiation.28 As such, dose-response curves for tumor formation in vivo tend to be bell-shaped as a function of dose (e.g., Upton260). In vitro, where cytotoxicity can be assessed separately from transformation and appropriate corrections made, dose-response curves tend to be linear.**Epidemiological Studies in Humans**In humans, most of the information useful for risk estimation is derived from epidemiological studies, with the dose almost always exceeding 0.1 Gy, and often, exceeding 1.0 Gy. However, most of the controversy concerns doses less than 0.1 Gy, delivered over protracted, rather than acute, time periods. Therefore, to infer low-dose effects from high-dose data, epidemiologists make extrapolations and assumptions about dose-response relationships that may or may not be valid in all cases.Many sources of error can also plague epidemiological data, including selection bias, small sample size, heterogeneous population characteristics, and dose uncertainties.255 The human populations that have been, and continue to be, evaluated for radiation-induced excess cancers are Japanese atomic bomb survivors, persons exposed to fallout from nuclear tests or accidents, radiation workers receiving occupational exposure, populations living in areas characterized by

above-average natural background radiation, or in proximity to artificial sources of radiation, and patients exposed to repeated diagnostic or therapeutic radiation. Pertinent findings from these studies include:1. Within the limits of statistical resolution, the shape of the dose response curve is *not inconsistent* with a linear, no threshold model.254,2592. Different tissues have different sensitivities to radiation-induced carcinogenesis, with bone marrow (leukemias other than chronic lymphocytic), breast (female), salivary glands, and thyroid the most susceptible, followed by colon, stomach, lung, ovary, and skin.2553. The latency period between irradiation and the clinical presentation of a solid tumor averages 20 years or more and about half that for hematological malignancies. However, the latency period varies with the age of the individual, generally increasing with decreasing age at exposure.4. Two risk-projection models have been used to predict the risk of radiation carcinogenesis in the human population: the absolute risk model and the relative risk model. Using the absolute risk model, excess risk in an irradiated population begins after the latency period has passed and is *added* to the age-adjusted spontaneous cancer risk. After a period of time, the cancer risk returns to spontaneous levels. The relative risk model predicts that the excess cancer risk is a *multiple* of the spontaneous incidence. It remains debatable which model is the most appropriate for estimating the excess cancer risk.255 At present, the epidemiological data tend to support the relative risk model for most solid tumors and the absolute risk model for leukemia.5. The current recommendations of the International Commission on Radiological Protection (ICRP) state that the nominal probability of radiation-induced cancer death is approximately 4% per Sievert (Sv) for working adults and about 5% per Sv for the whole population, under conditions of chronic, low-dose exposure.261 These risk estimates double for acute, high-dose exposures. The Sv is a unit of dose equivalent used for radiation protection purposes and is equal to the radiation dose (in Gy) multiplied by a quality factor specific for the type of radiation (with the quality factor roughly equivalent to the radiation's RBE).**Carcinogenic Risk from Prenatal Irradiation**The risk of carcinogenesis as a result of prenatal radiation exposure is made even more controversial by conflicting results of epidemiological studies. One major study cohort consisted of several thousand children (plus a demographically similar population of unirradiated children) who received prenatal exposure from diagnostic procedures during the 1950s and 1960s. The Oxford Survey of Childhood Cancer262 reported nearly twice the incidence of leukemia in children who had received prenatal irradiation. Although other epidemiological studies lend credence to the Oxford Survey's findings,254,259 it is still unclear whether factors other than the x-ray exposure may have caused, or at least contributed to, the excess cancer risk. Other studies, particularly from the Japanese A-bomb survivors who were pregnant at the time of the bombing, did not support the Oxford Survey's findings of increased risk of childhood malignancy but did support an increased risk of malignancy later in life.28On the assumption that it is preferable to overestimate risk than underestimate it, it is prudent to assume that the carcinogenic risk of radiation exposure to the embryo or fetus is about twice that for postnatal exposure.**Carcinogenic Risk from Medical Imaging Procedures** Recent data gleaned from the Japanese A-bomb survivors indicate a small but statistically significant excess cancer risk even for doses as low as 35 to 150 mSv.263,264 That this is in the range of doses delivered during a CT scan—in particular, a pediatric CT scan265,266 —has made the headlines and both sparked controversy267 as well as increased awareness 268 of radiation's risks. Estimates are that an abdominal helical CT scan of a pediatric patient results in a risk of a fatal cancer later in life of approximately one in a thousand.264A small risk of radiation carcinogenesis from a CT scan may seem trivial, especially to the radiation oncologist who typically delivers more than ten times that dose to a patient each day (albeit not to the whole body). Nevertheless, the finding of a radiation-induced excess cancer risk associated with a medical imaging procedure whose use has skyrocketed over the past 35 years,264 certainly has the makings of a public health issue. Currently, more than 70 million CT scans are performed annually in the United States.264 That disproportionately, this increase in CT scanning has been in a pediatric population both inherently more sensitive to ionizing radiation *and* with the longest life span in which to express those radiation-induced malignancies, is all that much more concerning.Because of this, radiation oncologists, as de facto experts on the health and medical

effects of ionizing radiation, should be willing to serve as educators of the public as to both the benefits and risks associated with the common procedures they employ.**Early and Late Effects Following Radiotherapy**It is not the intent of this section to provide either an exhaustive review of the various histopathological changes observed in the irradiated normal tissues of radiation therapy patients, but the reader is referred to several textbooks and pertinent review articles on the subject (e.g., Rubin et al,226 Mettler et al,255 Fajardo269). This section will focus instead on recent developments that promise to increase our understanding of the etiology of normal tissue injury, and hopefully, provide clues as to how to decrease or even prevent their occurrence.**Molecular Cascades and Cytokines**As mentioned previously, the early and late effects that occur in irradiated normal tissues result directly or indirectly from the killing of critical target cells. Although this statement is true in a general sense, it is clearly an oversimplification of what is now known to be a highly complex and dynamic process of cellular signaling, radiation-induced gene expression, multiple modes of cell death, and compensatory proliferative responses. Cytokines and growth factors, inducible proteins released by irradiated tissues that stimulate other cells to produce a biological response, participate in some of these processes. Although produced locally within the irradiated volume and intended to influence the behavior of cells in the same tissue, some cytokines also enter the circulation and can stimulate cells distant from the irradiated site.For example, the radiation-inducible cytokines interleukin-1 (IL-1) and tumor necrosis factor (TNF), released by leukocytes and tumor cells, alter the growth of hematopoietic progenitor cells, stimulate the growth of vascular endothelial cells, and induce collagen deposition.270,271 These same molecules are involved in the acute inflammatory response often noted in irradiated tissues, and along with ICAM-1, an intracellular adhesion molecule, in the changes in vascular permeability that lead to edema. Lung irradiation results in the expression of TNF, several interleukins, transforming growth factor-β (TGF-β) and basic fibroblast growth factor (bFGF), all of which are involved in acute radiation pneumonitis or late fibrosis.272,273 Finally, TNF, bFGF, and platelet-derived growth factor (PDGF) are associated with aberrant growth of cells within blood vessels. These proteins are induced in the vascular endothelium to stimulate cell proliferation and minimize radiation-induced apoptosis.274 **Functional Subunits and Volume Effects**Radiation oncologists traditionally reduce the total dose when the irradiation field involves a large volume of normal tissue. Although this practice evolved empirically, the biological basis for decreasing normal tissue tolerance with increasing irradiation volume remains unclear. Withers238,275 proposed a descriptive model for the pathogenesis of radiation injury in normal tissues based on the structural and functional organization of the tissue at risk for a complication. Conceptually, tissues are considered to be organized into functional subunits (FSUs), which can be inactivated by radiation exposure secondary to the killing of their constituent target cell(s). These FSUs may be anatomically defined, such as the nephron of the kidney or the lobule of the liver, or anatomically undefined (skin, nervous system).28 The main difference between the two is that surviving target cells from surrounding FSUs have the potential to migrate and help repopulate anatomically undefined FSUs, but not anatomically defined ones, presumably because of the lack of any structural demarcation. This could have the net effect of making anatomically undefined FSUs able to tolerate higher radiation doses.Whether the inactivation of one or more FSUs impacts the overall tissue function (in the form of a radiation-induced complication) depends on how many of the tissue's FSUs are in the irradiation field and their spatial arrangement. The spinal cord responds to changing irradiation volume as if its corresponding FSUs are arranged in series. There is a steep reduction in the tolerance dose for white matter necrosis of the rat spinal cord with increasing treatment volume for small radiation fields (up to about 2 cm exposed cord length), but little or no volume dependence for larger treatment fields, presumably because inactivation of one FSU inactivates the entire cord.139 The lung on the other hand, seems to have a large functional reserve, and it is only when much larger volumes are irradiated, and correspondingly large numbers of FSUs inactivated, that a functional deficit develops. This is more in keeping with a tissue whose functional subunits operate relatively independently and are arranged in parallel. Some other organs are believed to behave as if they have both serial and parallel components.One immediate clinical implication for tissues with

parallel versus serially arranged FSUs is that a small dosimetric hotspot would be relatively innocuous for a parallel tissue but potentially catastrophic for a serial tissue.**Reirradiation Tolerance**A common problem that radiation oncologists face is whether or not to risk reirradiation of a previously treated site. If a decision is made to retreat, even in the most ideal case in which the previous treatment course is well-documented and the treatment fields still identifiable, the clinician is nevertheless left with the uncertainty of what time, dose, and fractionation pattern to use.Radiobiological research in this area has been slow in coming (given the nature of studies involving late effects), but some progress has been made and some of the factors thought to be important in normal tissue tolerance to retreatment have been identified. These include whether the initial treatment course was to “full tolerance” or not, the likelihood that residual damage from the first treatment course has persisted, the amount of time that has elapsed between the first course and the second, the target volume to be reirradiated compared to the original target volume, and the structural and function makeup of the tissue at risk.A few general concepts are beginning to emerge from studies with laboratory rodents (for reviews, see Joiner et al,139 Travis et al,276 Thames et al277):1. For rapidly proliferating tissues such as skin, bone marrow, or testis, recovery following the first course of treatment is rapid, such that the tissue can be reirradiated to near the full tolerance dose within about 2-3 months.2. Some slowly proliferating tissues such as spinal cord and lung are capable of more limited long-term recovery after the first course of treatment and can be retreated to a partial (50% to 75%) tolerance dose, with the dose generally increasing the longer the time between the two treatments (3 to 6 months minimum).3. Other slowly dividing tissues like bladder seem to show permanent, residual injury from the first treatment, such that the total dose for a second course must be reduced by at least half, regardless of how much time has elapsed between treatments. In addition, there is evidence that complications arising from retreatment tend to occur much earlier (relative to the second treatment) than they would have from a single treatment.4. One apparent exception to this type of classification system is the kidney, for which retreatment tolerance decreases with time between the first and second treatment courses.A model that is consistent with these observations suggests that target cells that survive the initial treatment course have three possible fates. Some may regenerate their numbers over time, making the tissue as a whole better able to tolerate a second treatment, with the rate of regeneration determining how much time should elapse between the two treatments and what total dose can be delivered safely during the second course. Other target cells may maintain a steady state number of survivors after the first treatment, and therefore, the tissue would appear to harbor residual damage and never be able to tolerate a full second course of radiation therapy. Finally, some target cells may undergo continued depletion after the first treatment, such that tolerance to a second treatment course will actually decrease the longer the time between treatments. This may be related to a progressive expression of otherwise subclinical residual damage from the initial treatment.**Radiation-Induced Second Malignancies**With increasing numbers of long-term cancer survivors, the risk of second malignancies arising as a consequence of prior treatment becomes significant. Leukemia is thought to account for about 20% of second malignancies, with the remainder presenting as solid tumors in and around the previously irradiated site.278,279 Certain subpopulations of patients previously treated are at an even higher risk than the majority and deserve special attention, including children and young adults, those with a known genetic predisposition to cancer, immunocompromised individuals, and those with known exposure to other carcinogens (such as chemotherapy). For example, large epidemiological studies have assessed the breast and lung cancer risk in Hodgkin's lymphoma survivors,280,281 leukemia and sarcomas in cervical cancer survivors,282 and sarcomas in long-term survivors of childhood retinoblastoma.283A final concern with respect to radiation-induced second malignancies is the growing use of highly conformal, intensity-modulated radiation therapy, designed specifically to deliver lower doses to normal tissues immediately surrounding the tumor but at the expense of leaked or scattered dose to larger volumes of the body.284 Such conditions—large volumes and low doses—tend to be those most associated with radiation carcinogenesis.Dose Rate and Dose Fractionation EffectsAlthough the sparing effects of

fractionated, external beam radiotherapy and brachytherapy are assumed to be a result of the repair of SLD, other factors may be involved as well, most notably, repopulation. In the isoeffect relationship derived by Strandqvist,24 however, the time factor included both the effects of dose fractionation (presumably, the result of SLDR) and overall treatment time (presumably, repopulation). It was not until 1963 that Fowler et al89,130,285 attempted to separate the contributions of these two factors by performing fractionation experiments with pig skin. In their experiments, five equal fractions were given in overall treatment times of either 4 or 28 days, and three different-sized doses per fraction were used to make sure that at least one of them would result in a measurable level of early skin reaction. In changing from an overall time of 4 days to 28 days, only an additional 6 Gy was required to reach the same level of skin response. This was thought to reflect the contribution of overall time (i.e., repopulation) to the isoeffect total dose because the size and number of fractions was kept constant. In a parallel series of experiments in which the overall time was kept constant (28 days), but the number of fractions was increased from 5 to 21, it was found that an additional 13 Gy was required to reach the skin isoeffect level. This increase was almost as great as the 16 Gy additional dose required when changing from a single-dose treatment to a treatment protocol of five fractions in 4 days, implying that the change in fraction number was more important than the change in the overall treatment time.During the 1960s and 1970s, dose rate effects were studied extensively. The clinical community was also becoming more attuned to the biological underpinnings of radiation therapy, especially the “Four R's of Radiotherapy”: repair, reoxygenation, redistribution, and repopulation.286 These are considered key radiobiological phenomena that influence the outcome of multifraction radiotherapy.Bedford and Hall287,288 generated in vitro survival curves for HeLa cells irradiated at various dose rates between about 0.1 Gy per hour and 7.3 Gy per minute. The killing effectiveness per unit dose decreased as the dose rate was reduced, however, a limit to this dose rate or dose fractionation effect was reached under conditions in which cell cycle and proliferative effects were eliminated by the use of lower temperatures289 or by growing cells to plateau phase before irradiation290-292 (Figure 1-26).

Figure 1-26 The dose rate effect for nonproliferating C3H 10T1/2 mouse cells maintained in vitro. As the dose rate decreases from about 56 to 0.3 Gy/hr, survival curves become progressively shallower, reflecting the repair of radiation damage during the continuous irradiation interval. For dose rates less than about 0.3 Gy/hr, no further sparing effect of dose protraction is observed, suggesting that there is an effective limit to the repair-dependent dose rate effect. This is considered compelling evidence that cell survival curves have nonzero initial slopes. Adapted from Wells R, Bedford J: Dose-rate effects in mammalian cells. IV. Repairable and nonrepairable damage in non-cycling C3H 10T1/2 cells. Radiat Res 94:105, 1983.

Similar conclusions about the nature of dose rate and dose fractionation effects were reached from clinical studies. Dutreix et al293 studied dose fractionation effects in human skin under conditions where cell cycle and proliferative effects were minimized (i.e., short interfraction intervals and overall treatment times). Their data indicated that the incremental dose recovered as a result of SLDR when a single dose was replaced by two equal fractions became small when the size of the dose per fraction dropped below approximately 2 Gy (Table 1-4). This finding is consistent with the hypothesis that survival curves have negative (rather than zero) initial slopes, and therefore, that a limit to the repair-dependent dose fractionation effect should be reached for smaller and smaller-sized dose fractions or dose rates. Accordingly, these authors cautioned that isoeffect equations in common clinical use at the time (the NSD model—see discussion in this chapter) would be inaccurate for predicting tolerances when doses per fraction were quite small. Further, small differences in the initial slopes of survival curves for different cell types could be magnified into large differences in the limiting slopes for continuous or multifraction survival curves.

TABLE 1-4Recovered Dose as a Function of Dose per Fraction for Skin Reactions in Human Radiotherapy Patients

Single Dose (Ds)	Split Dose (2 Di)*	Recovered Dose (Dr = 2Di − Ds)
15 Gy	2 × 8.5 Gy	2 Gy
13 Gy	2 × 7.5 Gy	2 Gy

8 Gy2 × 5.5 Gy3 Gy
6 Gy2 × 4 Gy2 Gy
3.5 Gy2 × 2 Gy≤0.5 Gy*Interfraction interval (i) was 6 hours.Data from Dutreix J, Wambersie A, Bounik C: Cellular recovery in human skin reactions: application to dose fraction number overall time relationship in radiotherapy. Eur J Cancer 9:159-167, 1973.Time-Dose-Fractionation Relationships**The NSD Model**Based on Strandqvist's isoeffect curves,24 Fowler and Stern's pig skin experiments,89,285 and other laboratory and clinical findings,23 Ellis294,295 formulated the NSD concept in 1969. The NSD equation, D = (NSD) N0.24 T0.11, where *D* is the total dose delivered, *N* the number of fractions used, *T* the overall treatment time, and *NSD* the nominal standard dose (a proportionality constant thought to be related to the tolerance of the tissue being irradiated), became widely used for the design of biologically equivalent treatment schedules, particularly when its more mathematically convenient derivatives, such as the TDF296 or CRE297 equations became available.The introduction of the NSD equation theoretically allowed radiotherapy treatment practices worldwide to be compared and contrasted with respect to putative biological equivalence. It also permitted the calculation of dose equivalents for split-course treatments and brachytherapy and provided a means of revising treatment prescriptions in the event of unforeseen treatment interruptions. Because the NSD formula was based on observations of early-onset radiation effects, it was quite useful as a predictor of some tissue tolerances, as long as it was not used for treatments involving extremes of fraction number or overall time.On the other hand, the NSD formula was ill-equipped to deal with some clinical problems, particularly the prediction of late effects in normal tissues (especially at nonstandard doses per fraction), and the patterns of repopulation in normal tissues and tumors. 241 The use of a fixed exponent for the overall time component, T, gave the false impression that extra dose to counteract proliferation would be needed from the outset of treatment, rather than after a delay of several weeks, which is what is observed in practice (e.g., Joiner et al,139 Denekamp298).In light of the growing frustration with the NSD model, and research at the time focusing on the shape of the shoulder region of cell survival curves and the nature of dose rate and dose fractionation effects, new, radiobiologically based approaches to isoeffect modeling were developed during the late 1970s and early 1980s.**The Linear-Quadratic Isoeffect Model**In ambitious multifraction experiments using laboratory rodents in which a broad range of fraction sizes and interfraction intervals was used, Douglas and Fowler299 developed a novel method of data analysis in which they interpreted their resulting isoeffect curves in terms of the shape of the underlying dose-response curves for the putative target cells. Because overall treatment times were kept quite short, proliferative effects were assumed to be negligible, so repair was thought to be the only factor involved. The shapes of the underlying dose-response curves were deduced by plotting 1/D, where *D* was the total dose delivered (D = n × d), as a function of *d*, the dose per fraction. This was termed a reciprocal dose plot and is used to obtain values for the ratio α/β, parameters of the linear-quadratic survival curve formula,76,77 which, as discussed previously, provided good fits to experimental survival data, at least over the first two decades of cell killing. A representative reciprocal dose plot is shown in Figure 1-27. However, it should be noted that the curves obtained using this technique are not true cell survival curves, but rather, effective dose-response curves derived from isoeffect data for different time, dose, and fractionation combinations.

Figure 1-27 The reciprocal dose or Fe plot technique of Douglas and Fowler is used to determine a normal tissue or tumor's α/β ratio. Using this method, the reciprocal of the total dose necessary to reach a given isoeffect is plotted as a function of the dose per fraction. Assuming that the killing of target cells responsible for the tissue effect can be modeled using the linear-quadratic cell survival expression, , the α/β ratio can be obtained from the ratio of the isoeffect curve's intercept to slope. Adapted from Douglas B, Fowler J: The effect of multiple small doses of x rays on skin reactions in the mouse and a basic interpretation. Radiat Res 66:401, 1976.This new approach to isoeffect analysis, in which attention was focused on repair parameters and dose-response curve shapes, emphasized that the critical parameter in radiotherapy is the size of the dose per fraction, more so than the overall treatment time. During

the course of experimental and clinical fractionation studies, it became clear that there was a systematic difference between early- and late-responding tissues and tumors in their responses to different fractionation patterns. In other words, the capacity for repair of radiation damage was somehow related to the proliferative state of the tissues being irradiated (Figure 1-28).300,301 Isoeffect curves for the slowly or nonproliferating normal tissues, such as kidney and spinal cord for example, are steeper in general than those for more rapidly proliferating, early-responding tissues, such as skin and gut epithelium, and significantly, most tumors.300,301 A steep isoeffect curve implies that late effects tissues are more sensitive to changes in the size of the dose per fraction, experiencing greater sparing with decreasing fraction size than their early effects counterparts (Figure 1-29). This difference is also reflected in the α/β ratios derived for these tissues, which are usually low for late-responding tissues (on the order of 1 to 6 Gy, with an average of about 3 Gy), and high for early-responding tissues and tumors (usually 7 to 20 Gy, with an average of about 10 Gy) (Tables 1-5 and 1-6).

Figure 1-28 Isoeffect curves in which the total dose necessary to produce a certain normal tissue or tumor endpoint (indicated on the graph) is plotted as a function of the dose per fraction under conditions in which cell proliferation is negligible. Isoeffect curves for late-responding normal tissues *(solid lines)* tend to be steeper than those for early-responding normal tissues and tumors *(dashed lines)*. This suggests that for the same total dose, late reactions may be spared by decreasing the size of the dose per fraction used. It also follows that by using smaller dose fractions, a somewhat higher total dose could be given for the same probability of a late reaction but with the hope of higher tumor control probability. Adapted from Withers H, Thames H, Peters L, et al: Normal tissue radioresistance in clinical radiotherapy. In Withers H, Thames H, Peters L, editors: Biological basis and clinical implications of tumor radioresistance, New York, 1983, Masson, p 139.

Figure 1-29 Hypothetical survival curves for target cells whose deaths are responsible for an acute *(upper panel)* or late *(lower panel)* effect in an irradiated normal tissue, depending on whether the total dose C is delivered using dose fractions of size A or B. Because of the difference in the initial slopes of the corresponding single-dose survival curves for these cell types, reducing the fraction size from B to A preferentially spares late-responding normal tissues *(green-shaded areas).* Adapted from Withers H, Thames H, Peters L: Differences in the fractionation response of acutely responding and late-responding tissues. In Karcher K, Kogelnik H, Reinartz G, editors: Progress in radio-oncology II, New York, 1982, Raven Press, p 287.**TABLE 1-5Representative α/β Ratios for Human Normal Tissues and Tumors**

Tissue Type (and Endpoint)	α/β Ratio (± 95% Confidence Interval)
Early-Responding Normal Tissues	
Skin: erythema	10.6 (1.8; 22.8) Gy
Desquamation	11.2 (8.5; 17.6) Gy
Lung: pneumonitis ≤90 days after radiotherapy	>8.8 Gy
Oral mucosa: mucositis	8-15 Gy
Late-Responding Normal Tissues	
Skin: telangiectasia	~2.7 (−0.1; 8.1) Gy
Fibrosis	1.7 (0.6; 3.0) Gy
Breast: cosmesis	3.4 (2.3; 4.5) Gy
Fibrosis	3.1 (1.8; 4.4) Gy
Lung: pneumonitis >90 days after radiotherapy	4.0 (2.2; 5.8) Gy
Fibrosis	3.1 (−0.2; 8.5) Gy
Bowel: perforation/stricture	3.9 (2.5; 5.3) Gy
Various other	4.3 (2.2; 9.6) Gy
Spinal cord: myelopathy	<3.3 Gy
Muscle, vasculature, or cartilage: impaired movement	3.5 (0.7; 6.2) Gy
Nerve: brachial plexopathy	2.0-3.5 Gy
Optic neuropathy	1.6 (−7; 10) Gy
Head and neck: various	3.5-4 Gy

Tumors
Head and neck: nasopharynx16 (−11; 43) Gy
Vocal cord~13 Gy
Buccal mucoas~6.6 (2.9; ∞) Gy
Tonsil7.2 (3.6; ∞) Gy
Larynx14.5 (4.9; 24) Gy
Lung: squamous cell carcinoma~50-90 Gy
Cervix: squamous cell carcinoma>13.9 Gy
Skin: squamous cell carcinoma8.5 (4.5; 11.3) Gy
Melanoma0.6 (−1.1; 2.5) Gy
Prostate1.1 (−3.3; 5.6) Gy
Breast (early stage invasive ductal, lobular, and mixed)4.6 (1.1; 8.1) Gy
Esophagus4.9 (1.5; 17) Gy
Liposarcoma0.4 (−1.4 ; 5.4) GyData from Joiner M, van der Kogel A, editors: Basic clinical radiobiology, ed 4, London, 2009, Hodder Arnold.**TABLE 1-6Summary of the Linear-Quadratic Isoeffect Model Parameters and Concepts***Determined from the reciprocal dose plot technique of Douglas BG, Fowler JF: The effect of multiple small doses of x-rays on skin reactions in the mouse and a basic interpretation, Radiat Res 66:401-426, 1976.†Based on the assumption that differences in the calculated α/β ratio are usually caused by differences in α rather than β.‡Using the Thames and colleagues isoeffect curve plot. Thames HD, Withers HR, Peters LJ, et al: Changes in early and late radiation responses with altered dose fractionation: implications for dose-survival relationships, Int J Radiat Oncol Biol Phys 8:219-226, 1982. (See also Figure 1-28.)**Clinical Applications of the Linear Quadratic Isoeffect Model**The most important assumptions of the linear-quadratic model are300,301: an isoeffect in a tissue is a reflection of the isosurvival of appropriate target cells; turnover kinetics of target cells determine the time of expression of radiation injury; radiation damage is either repairable or irreparable, with the repairable damage accounting for the sparing effect of dose fractionation; sufficient time is allowed between dose fractions for the repairable damage to be completely repaired; repopulation during the course of treatment is negligible; and the damage caused by each successive dose fraction is the same as that produced by the prior dose fraction, that is, there is an equal effect per fraction.Within this conceptual framework then, the shapes of tissue and tumor isoeffect curves and their calculated α/β ratios have a number of clinical applications. One possible application is to design radiotherapy treatments for which the α/β ratios for the dose-limiting tissues are known reasonably well.246 It is also possible using α/β ratios to equate treatment schedules employing different-sized doses per fraction to match the probability of causing a tissue injury, assuming the overall treatment times are similar in both schedules or the tissue at risk of a complication is relatively insensitive to treatment duration.246 The equation, D2/D1 = (α/β + d1) / (α/β + d2) can be used for this purpose, where *D*1 and *d*1 are, respectively, the total dose and dose per fraction (in Gy) of one radiotherapy treatment plan, *D*2 and *d*2 are the total dose and dose per fraction for an alternate treatment plan designed to be biologically equivalent for a particular tissue effect, and with the fractionation sensitivity of that tissue defined by its unique α/β ratio. Of course, avoiding a normal tissue complication is not the sole criterion used in treatment planning; in considering a particular time, dose and fraction size combination, the responses of the tumor, and *all* incidentally irradiated normal tissues should be taken into account simultaneously.An important implication of the steeper isoeffect curves for late-responding tissues compared to those for tumors is that it might be possible to increase the therapeutic ratio by using larger numbers of smaller fractions to a somewhat higher total dose than traditionally used.246,247,302 Although such treatments might be expected to exacerbate acute effects in normal tissues and the tumor, late effects would be spared preferentially. The use of multiple fractions per day of smaller than conventional size (less than about 1.8 Gy) but to a somewhat higher total dose, with little or no change in overall treatment time, has been termed *hyperfractionation*. With particularly aggressive tumors that proliferate rapidly, it has been suggested that multiple treatments per day might also be useful to

decrease the overall treatment time, thereby allowing less time for repopulation of clonogenic tumor cells.303,304 Treatment with multiple daily fractions of approximately standard size and number (and to about the same total dose), but in shorter overall times, has been called *accelerated fractionation*. In practice, however, a combination of accelerated and hyperfractionated treatment is often used.277 Finally, *hypofractionation*, the use of one or a few large dose fractions delivered over short periods of time (e.g., stereotactic radiosurgery or radiotherapy, intraoperative radiation therapy, or, to some extent, high dose rate brachytherapy) is also an option. Indications for such would include cases where the frank ablation of small tumors is the goal, or in the relatively unusual circumstance in which the tumor is suspected of having a low, rather than high, α/β ratio. Prostate cancer appears to be a tumor type that meets this criteria, although this remains a controversial and sometimes contentious subject, as does the use of hypofractionation in general.305-307 Whatever the case, extra care must be taken to exclude late-responding normal tissues from the treatment field when hypofractionation is considered because the use of high doses per fraction for tissues with low α/β ratios is associated with higher complication frequencies.The decision to opt for one of these fractionation protocols would depend not only on the α/β ratios for the tissues being irradiated but also on their relative repair rates and proliferative responses before, during, and after exposure. At present, although data related to the former parameters continue to accumulate and become more reliable, data on proliferation characteristics, especially for tumors, are still lacking.241,303 With nonstandard fractionation now the standard, radiation oncologists find themselves confronted with the same problem faced by their counterparts in the 1930s, that is, how to compare and contrast different treatment schedules for presumptive isoeffectiveness? The biologically effective dose (BED) method,248 another derivative of the linear-quadratic model, attempts to address this issue. Although this method is a bit confusing to use in practice, conceptually, the ideas are fairly straightforward. Knowing that cell survival and dose-response curves have negative initial slopes, and that, for a sufficiently low dose per fraction or dose rate, a limit to the repair-dependent dose fractionation effect occurs that “traces” this initial slope, the question may be asked, “In the limit, for an infinite number of infinitely small dose fractions, what total radiation dose will correspond to normal tissue tolerance, tumor control, or any other endpoint of interest?” Clearly, this theoretical dose will be quite large for a tissue characterized by a dose-response or survival curve with a shallow initial slope (like many late-responding normal tissues) and appreciably smaller for a tissue characterized by a dose-response curve with a steep initial slope (like most tumors and early-responding normal tissues). *It is also important to bear in mind that BEDs are not real doses, but rather extrapolates based on the limiting slope of the multifraction dose response curves for the tissues at risk, which in turn depend on the* α/β *ratio*. For this reason, the units used to describe these extrapolated doses are, for example, Gy3 and Gy10, rather than Gy, where the subscripts 3 and 10 refer to the assumed α/β ratio of the tissue at risk. A second caveat is that, although two different radiotherapy treatment schedules can be compared qualitatively on the basis of their respective Gy3 *or* Gy10 doses, Gy3 *and* Gy10 cannot be intercompared.A mathematical rearrangement of the linear-quadratic survival expression S = e−(αD + βD2), yields:where *E* is the (iso)effect being measured (*E* is divided by α to obtain the BED value in units of dose), *n* is the number of fractions, *d* is the dose per fraction, and the α/β ratio is specific for the tissue at risk. The factor “(1 + d/α/β)” has been called the *relative effectiveness term* because, in essence, it is a correction factor for the fact that treatment is not really given as an infinite number of infinitely small dose fractions but rather as a finite number of fractions of a finite size.Perhaps the best way to illustrate the use of the BED equation is by example. Suppose that a radiation oncologist is developing a clinical protocol in head and neck cancer comparing standard fractionation (30 fractions of 2 Gy to a total dose of 60 Gy in an overall treatment time of about 6 weeks) to a schedule of 50 fractions of 1.4 Gy to a total dose of 70 Gy in approximately the same overall treatment time. The tissues of most concern for radiation injury are the tumor, the oral mucosa, and the spinal cord, that is, two early- and one late-responding tissues. Finally, assume an α/β ratio of 10 Gy is

appropriate for the tumor and oral mucosa, and an α/β ratio of 3 Gy is appropriate for the spinal cord. *For calculation purposes, an α/β ratio of 10* *Gy can be used for most early-responding normal tissues and tumors, and 3* *Gy for most late-responding normal tissues, unless more robust, better-vetted values are available.* For example, an α/β ratio of 4 Gy may be more appropriate for breast cancer; 20 Gy for non–small cell lung cancer; approximately 2 Gy for central nervous system, kidney and prostate cancer; and approximately 0.6 Gy for melanoma.245For the standard fractionation schedule therefore:For tumor and mucosa:For the spinal cord:And, for the more highly fractionated schedule (rounded off to the nearest whole number):For tumor and mucosa:For the spinal cord:Although little quantitative information can be gleaned from this exercise, a few qualitative statements can be made. First, a comparison of the Gy10 values for the two treatment schedules suggests that the more highly fractionated schedule should result in somewhat better tumor control, albeit at the expense of more vigorous mucosal reactions (i.e., 72 Gy10 compared to 80 Gy10, an 11% increase in “biodose”). However, the comparison of the Gy3 values for the two schedules suggests that the spinal cord tolerance would be essentially unchanged (i.e., 100 Gy3 compared to 103 Gy3, a 3% increase).Even with the BED concept being only semiquantitative at best, its use for treatment planning purposes over the past two decades has provided a wealth of clinical data that has allowed a better definition of what is or is not tolerable for a particular normal tissue, in terms of Gy3 or Gy10. Using head and neck cancer as an example, Fowler et al245,308,309 have suggested that the tolerance dose for acute mucosal reactions is in the range of 59 to 63 Gy10, and for late reactions, in the range of 110 to 117 Gy3.Finally, another way of using the BED equation would be to design schedules to match an acceptable probability of a complication, radiation myelitis for example, and then calculate the expected benefit (or lack thereof) with respect to tumor control.It would be remiss to conclude any discussion of the linear-quadratic isoeffect model, or any biologically based model with potential clinical application, without a few words of warning. First, this model, although certainly more robust than the NSD model and much better grounded in biological principles, is still a theoretical model. Some limitations of the basic model are obvious: an overly simplistic assumption that an isoeffect in a tissue directly corresponds to an isosurvival of a particular cell type; no provision for the influence of cell cycle, proliferative or microenvironmental effects in the overall dose-response relationship; no way to account for differences in repair rates between different tissues; no consideration of volume effects; uncertainty surrounding the model's applicability for extremes of fractionation; and a limited understanding of how to apply the model in patients receiving multimodality therapy.Various add-ons to the linear-quadratic model have been proposed,310-312 especially with respect to compensating for tumor cell repopulation and differing repair rates between different tissues. However, the lack of robust values at present for the parameters introduced in such calculations (e.g., potential doubling times, half-times for repair, repopulation kick-off times, etc.), ultimately limit their usefulness beyond rough approximations or proofs-of-concept. The current status of some of the existing and proposed parameters of the linear-quadratic model for human tumors and normal tissues is summarized in Table 1-7.**TABLE 1-7Current Status of Existing and Proposed Parameters of the Linear-Quadratic Isoeffect Model for Human Normal Tissues and Tumors**Modified from Bentzen SM: Estimation of radiobiologic parameters from clinical data. In Hagen U, Jung H, Streffer C, editors: Radiation research 1895-1995: volume 2, congress lectures. Wurzburg, 1995, Universitatsdruckerei H. Sturtz AG, pp 833-838, with permission.Radiation Biology in the 21st CenturySince the mid-1980s, most graduate students pursuing careers in oncology necessarily trained as molecular, cellular, or tumor biologists, and not as radiation biologists per se, although a few may have worked with ionizing radiation as a tool for probing fundamental cellular processes or as part of translational research designed to develop new cancer therapies. Even fewer have ever taken a formal course in radiation biology, let alone in its more clinical aspects. This shift in focus and training is part of the natural evolution of the oncologic sciences over the years and surely not an unexpected or unwarranted one. However, the fact remains that the field of radiation biology as a distinct entity, with its rich, 120-year history that has contributed in major ways to fields as diverse as

carcinogenesis, epidemiology, toxicology, DNA damage and repair, genetics and cytogenetics, cell cycle biology, and radiation oncology to name but a few, is threatened with extinction.This too could be viewed as a necessary price of scientific progress, were it not for the fact that there remains a need for all radiologic science professionals (from radiologic technologists and radiation therapists, medical physicists and dosimetrists, to radiologists and radiation oncologists) to be at least reasonably well-versed in the basic principles of radiation biology. Physicians in particular need to be familiar both with the classical and modern aspects of the field, in keeping with the close relationship between the histories of ionizing radiation itself, radiation biology, and the medical specialties of radiology and radiation oncology. And unfortunately, since the events of September 11, 2001, a new mandate has emerged: the need to provide expertise in the basics of radiation biology and radiation protection to emergency responders, civic leaders, and the general public in the event of a radiological or nuclear terrorist attack.Nevertheless, 21st-century radiation biology continues to flourish and remains cutting edge in many respects. Fundamental studies of genomic instability,313,314 epigenetics,315,316 and cell signaling as it applies to radiation response317,318 continue to be active areas of research. Our growing understanding of the complex roles played by cytokines in the etiology of normal tissue complications following radiation exposure319,320 promises to someday deliver novel, molecularly based radioprotectors that may benefit radiation accident victims, first responders during radiation emergencies, and astronauts on deep-space missions. Radiation cytogeneticists study the role telomeres and telomerase play in cellular aging and neoplastic transformation.321 Radiation scientists have also been important contributors to the fields of genomics and proteomics, functional and molecular imaging and molecularly targeted cancer therapy, and to the search for tumor-specific biomarkers that can aid in cancer diagnosis, staging, and the monitoring of treatment progress.As long as the molecular underpinnings of some of these fundamental processes remain poorly understood, and a more comprehensive picture of the full range of radiation-induced gene expression and signaling remains elusive, there will always be a role for radiation biology research.References

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321. Neumann AA, Reddel RR. Telomere maintenance and cancer–look, no telomerase. *Nat Rev Cancer*. 2002;2:879–884. **Chapter 2**Molecular and Cellular Biology*Mary Ann Stevenson, Stuart K. Calderwood*The past two decades have seen revolutions in two areas of cell and molecular biology that are closely related to radiation therapy. The pathways involved in response to genomic stress, including mechanisms for sensing DNA damage and responding by cell cycle changes and DNA repair, have been elucidated by studies of model organisms, particularly the yeast *Saccharomyces cerevisiae*.[1,2] In the second major area, understanding pathways of programmed cell death (PCD), which may play a major role in treatment response, studies of *Caenorhabditis elegans* were the most informative in demonstrating the significance of individual death genes.[3] The challenge is to combine these two areas of knowledge and provide a rational understanding of how tumor cells can be made to enter the death pathways and how the processing of DNA damage could be minimized. Considerable technological improvements also have enabled significant progress in the translation of experimental advances, including rational, *in silico* drug design and high-throughput screening of chemical libraries, which could permit the application of advances in molecular biological knowledge to the development of drugs for use in combination with radiation therapy.Essential Steps in Tumor ProgressionMuch evidence suggests that tumors are formed by a stepwise progression of cells from a minimally altered state, where they are able to grow and form nodules or polyps (e.g., solid tumors) to a state of maximal transformation, characterized by multiply deviated cells that are capable of unlimited growth, manipulation of their local environment, invasion of surrounding tissues, and escape into the circulation to establish new colonies of secondary tumors, or metastases.[4] Tumor progression

involves an array of molecular and morphologic changes. In their landmark review, Hanahan and Weinberg suggested organizing these traits into six essential alterations in cell physiology: self-sufficiency in growth signals, insensitivity to growth inhibition, evasion of PCD, limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis.[5] Although the initial steps are believed to occur earlier in tumor progression, the exact order of occurrence varies among different malignancies, and some phenotypic transformations appear to require more than one molecular change.[6,7] The occurrence in tumor cells of this series of radical, primarily genetic alterations in physiology is in part the result of an evolving instability of the tumor genome resulting from a breakdown in the DNA repair pathways that accompanies tumor progression.In addition to progressing and acquiring enhanced malignant capabilities, most human tumors undergo further selections through exposure to various forms of cytotoxic therapy such as irradiation and chemotherapy, leading to the development of resistant phenotypes in the surviving cells.[8] However a new paradigm is emerging that may challenge our views of tumorigenesis. This is the concept that only a small proportion of the tumor cell population can give rise to tumor initiation and these cells have many of the properties of stem cells.[9] According to this theory, tumors contain a small population of cancer stem cells that renew by dividing slowly and that also give rise to more “differentiated” progeny forming the bulk of tumors. Tumor eradication would thus involve killing this small, slowly dividing, and often treatment-resistant population. Studies in this area could revolutionize the cell and molecular underpinnings of radiation biology.All cancer treatments are aimed at reversing the hallmarks of neoplastic transformation and tipping the balance toward tumor cell death in both the bulk population and the stem cell fraction. Ionizing radiation kills cells almost exclusively through the generation of DNA double-stranded breaks (DSBs).[10] The evolving modification of the cellular phenotype during tumorigenesis leads to the development of resistance to therapy. Our major task is to explore the links among DSB response pathways, engagement of cell death mechanisms, and the outcome of radiation therapy.Radiation Therapy and Radiation BiologyIonizing radiation has long been known to be a potent modality in cancer therapy, killing cells and leading to tumor regression.[11] Killing by ionizing radiation involves the oxygen-dependent generation of free radicals and subsequent damage to multiple molecular structures within the cell.[10] The generation of DSBs is the dominant mechanism of direct lethality. Indeed, it has been estimated that the existence of even one unrepaired DSB can lead to cell death.[12] Within whole organisms, direct and indirect forms of lethality occur. Radiation can cause the death of hematopoietic stem cells in the bone marrow and stem cells in crypts of the small intestine, which leads to secondary damage caused by immunosuppression and infection, as the gastrointestinal lining is eroded.[13,14] The challenge facing radiation therapy is to enhance cell killing within tumors while avoiding dose-related complications in organs containing rapidly renewing stem cell populations.Early studies using the clonogenic cell survival assay established the notion of optimizing radiobiological parameters such as dose, fractionation, and cell cycle inhibition as a means of inhibiting reproductive cell death in critical normal tissues.[15] The clonogenic cell survival assay is a useful measure of cell inactivation because the effects of ionizing radiation seen in vitro often mirror the responses of tumors in vivo.[10] However, a weakness in the informative power of the method is that it does not allow for discrimination or elucidation of the various mechanisms leading to cell death. That kind of detailed information is essential for the successful combination of radiation therapy with other modalities, which may result in sensitization, protection, or both. Irradiation activates a number of pathways that mediate reproductive death, including various forms of PCD (e.g., apoptosis, autophagy), replicative senescence, and necrosis, the default pathway that often dominates when other types of cell death are inhibited.[16,17] The susceptibility to each of these forms of cell death changes over the course of tumor progression, and the tumor cell response to ionizing radiation is likely to reflect the aggregate of genetic changes that accompany tumor progression. The task of radiation biology is to examine mechanisms by which ionizing radiation gives rise to unrepaired DSBs and how such damage is coupled to the pathways of cell killing within the moving target of the evolving cancer cell.DNA Double-Stranded Break ResponseAll cells have become adapted to live in an environment that is more or less mutagenic and have thus evolved responses to permit survival

when the genome is damaged.18 DNA may be damaged during the normal processes of DNA replication and segregation. Within tumor cells, these initial changes become progressively altered because cells compete for survival in the tumor milieu and are subjected to selection by various forms of cytotoxic therapy.19 The response to DSBs involves two principal components: (1) arrest of cell proliferation to prevent replication and segregation of the damaged DNA and, when possible, (2) repair of the lesion.19 A third component in the DSB response in multicellular organisms may be altruistic entry of irreversibly damaged cells into the PCD pathways.20,21 The latter effect is the desired response to cytotoxic therapy. Understanding how the DSB response is regulated at the molecular level may be one of the keys to understanding the differential response to irradiation in individual tumors and the rational design of radiosensitizing drugs. There are several basic questions regarding regulation of this response, including: (1) By which mechanism do tumor cells arrest in the cell cycle? (2) How is the presence of DNA DSBs detected in such cells? (3) What are the major pathways for repair of DSBs? (4) How does the deployment of these pathways affect tumor response to therapy?Mechanisms of Cell Cycle Arrest: The Cell Cycle and CheckpointsThe cell cycle is a series of molecular programs that read out in a preferred order, leading to duplication of the genome and other cellular components, permitting division into two cells, each with a full set of chromosomes and the organelles required for life.22 Orderly, high-fidelity, and complete duplication of the genome is required for cell cycle progression. This is achieved through a surveillance system that detects DNA damage or unreplicated DNA and imposes cell cycle arrest at designated checkpoints, blocking the forward progress of the cell cycle engine (Figure 2-1). Because ionizing radiation produces severe damage to DNA, checkpoints are essential for facilitating repair and subsequent cell survival after radiation exposure, and they provide an ultimate target for strategies aimed at sensitizing cells to radiation therapy.
Figure 2-1 Cell cycle checkpoints. **A,** To progress through G1 to the replication (S) phase, cells require the activity of G1 cyclins (D, E) and cyclin-dependent kinases (CDK2, CDK4, and CDK6) to phosphorylate substrates such as the retinoblastoma protein (RB1). DNA damage is sensed by the protein kinase ATM, which activates TP53 (p53), leading to transcription of the CDKN1A (p21) protein, a powerful inhibitor of cyclin-CDK complex kinase activity, and to arrest in G1. DNA damage may be repaired, leading to deactivation of the checkpoint block and progression into S phase. If it is unrepaired, TP53-dependent programmed cell death may occur. **B,** To progress through G2 into M, the activity of the protein phosphatase CDC25 is required. DNA damage *(red arrows)* also leads to ATM activation, which causes a cascade response as ATM kinase activates the CHK kinases and CHK1 phosphorylates CDC25, leading to recruitment of the activation protein YWHA (formerly designated 14-3-3) and an adapter protein that couples CDC25 to the nuclear export machinery of the cell. Exported from the nucleus, CDC25 is no longer able to mediate dephosphorylation of CDC2 at the G2/M transition and progression through G2.The cell cycle is driven by sequential activation of a series of protein kinases that determine the order and rate of the metabolic events required in each stage. These cell division kinases, or cyclin-dependent kinases (CDKs), are activated by binding proteins originally designated as cell division cycle (CDC) molecules, now known as cyclins.22 These complexes formed between cyclins and CDK molecules are the master regulators that determine the rates of the individual component reactions regulating each stage of the cell cycle.23 Phosphorylation of individual cell cycle effector proteins by the cyclin-CDK complexes acts as a series of switches, turning on cell cycle stages in orderly fashion. In mammalian cells, the cyclins regulate individual stages of the cell cycle, and these include the G1 cyclins (cyclins D and E), S phase-specific cyclins, and G2-specific cyclins (cyclins A and B), which associate, respectively, with G1-specific (CDK2, CDK4, and CDK6), S-specific, and G2-specific (CDC2) cyclin-dependent kinases. The events of G1 (i.e., synthesis of enzymes and other molecules involved in DNA replication), S phase (i.e., DNA replication), and G2/M (i.e., chromosome condensation, disappearance of the nuclear envelope, and mitosis) are regulated by the transient accumulation and subsequent degradation of the cyclins.22-24 Switching off the reactions that characterize individual stages involves targeted degradation of the cyclins by a protein destruction machine called the *proteasome.*24 Targeted destruction of the cyclins after they have carried out their function ensures unidirectional, irreversible progression around the cell

cycle.23 The timing of the various cell cycle phases under normal conditions is related to the time required for cyclin-CDK complexes to reach appropriate, critical concentrations and for the phosphorylation of key substrates.23Irradiation leads to prolongation of the cell cycle and arrests in G1, G2, and S phases because of checkpoint activation (see Figure 2-1).25 Although this phenomenon was observed many years ago, it was only later understood at the biochemical and genetic levels. Arrest of cells at the G1 checkpoint has been particularly well studied (see Figure 2-1, *A*). G1 arrest is caused by the accumulation of a CDK inhibitory protein, CDKN1A (formerly designated p21 or Cip1), and this molecule prevents the key events that are required for transit through G1, including phosphorylation of the retinoblastoma (RB1) protein and activation of the E2F transcription factors (e.g., E2F1, E2F2, E2F4, E2F6) necessary for accumulation of enzymes required to traverse S phase.26,27 CDKN1A can be induced at the transcriptional level by TP53 (also known as p53), which accumulates in irradiated cells and binds to the *CDKN1A* promoter, thereby activating transcription. TP53 accumulation depends on the activation by radiation exposure of a DSB sensor molecule, the protein kinase ATM (ataxia-telangiectasia, mutated).26,27 The exact mechanism involved in detecting accumulation of DSBs and activation of ATM is unclear, although it involves ATM autophosphorylation.28The common mechanism for regulating the G1 and G2 checkpoints is inhibition of the CDK components of the interphase cell cycle engine and a block to phosphorylation and activation of effector molecules (see Figure 2-1).23,27 A variation on this theme is seen at the mitotic spindle checkpoint. Progression through mitosis requires switching off CDK activity through targeted degradation of cyclin B.29 Arrest in M phase, when the mitotic spindle is compromised, involves the TP53-dependent stabilization of cyclin B. The mitotic spindle checkpoint guarantees that replication is followed by cell division, ensuring prevention of aneuploidy. Not surprisingly, the polyploidy nature of many tumor cells is related to TP53 inactivation.29 Most evidence indicates that ATM (and its homologues in other organisms) functions as a sensor for DNA damage through a complex series of interactions with the damaged DNA. This ultimately leads to ATM activation and downstream signaling cascades involving the kinases CHK1 and CHK2 (also called CHEK1 and CHEK2), which couple to the cell cycle engine at the level of individual cyclin-CDK complex molecules (see Figure 2-1, *B*).1,2,19,27,30 The molecular details of these processes are evolving rapidly, and referenced reviews can provide more detailed insight into the mechanisms of DNA damage, sensing, and checkpoint engagement.TP53: Cellular Triage after Ionizing Radiation ExposureTP53 is a nuclear phosphoprotein with sequence-specific DNA binding activity. It can function as both transcriptional activator and repressor and plays a triage role in deciding whether to undergo cell cycle arrest and repair or to enter the pathways of PCD or replicative senescence (Figure 2-2).31,32 When cells are exposed to ionizing radiation or chemotherapeutic agents, the levels of wild-type TP53 protein are increased. TP53 then transcriptionally activates a number of genes, most notably *CDKN1A*, the CDK inhibitor that mediates many of the properties of TP53.33 In addition to G1 arrest, genotoxic stress and ionizing radiation can induce TP53-dependent PCD pathways, including caspase-dependent apoptosis.33 TP53 can transcriptionally activate the proapoptotic *BAX* gene, induce synthesis of JUN kinase, and repress transcription of the anti-apoptotic gene *BCL2*, suggesting that TP53 is a central mediator of PCD processes.31,34 DNA damage in the presence of wild-type TP53 causes G1 arrest, followed by a period of DNA repair; if the damage is too great to be easily repaired, the cell is eliminated through TP53-dependent PCD pathways. However, approximately 50% of human tumors possess inactivating mutations in the *TP*53 gene.35 TP53 becomes inactivated in many tumors as a result of selection against its pro-apoptotic properties. The resultant loss of its central function as “sentinel of the genome” may be a type of collateral damage incurred in cells because of the selection advantage for survival accruing from the loss of TP53 apoptotic function.32,36 The loss of TP53 function is linked to poorer prognosis in malignancies such as lung, breast, colorectal, and hematopoietic tumors.37 Many tumor cell lines containing mutant TP53, including breast, glioma, and lymphoma cell lines, are more resistant to therapy than their wild-type TP53 counterparts.38,39

Figure 2-2 TP53 plays a triage role in deciding a cell's fate after exposure to ionizing radiation. TP53 is activated after irradiation, largely through protein stabilization that results from

phosphorylation by the protein kinase ATM. Most effects of TP53 are mediated through its transcriptional activity, with some gene products (e.g., CDKN1A [p21]) leading to cell cycle arrest and others (e.g., BID, BBC3 [formerly designated PUMA], PMAIP1 [formerly NOXA]) to programmed cell death. A third fate appears to be senescence, which is mediated largely through CDKN1A. The triage decision appears to be related to radiation dose (i.e., degree of DNA damage), with greater damage favoring the death and senescence pathways.The loss of wild-type TP53 function in human malignancies may be a key step in the progression of human cancer, and the TP53 status of cells may control the outcome of many tumor types in response to chemotherapy or radiation therapy.36 Although most of the other DSB response genes have molecular equivalents in yeast, TP53 does not. The TP53 gene appears to be required to determine the fate of damaged cells, which in mammalian cells may be PCD, a sacrifice that contributes to the well-being of the whole organism. TP53 monitors the degree of DNA damage and acts as a master switch, moving the cells from a state of cycle arrest and DNA repair to death or senescence pathways. Loss of TP53 in tumors compromises this critical surveillance and triage function. With the loss of this critical decision-point molecule, damaged and mutated tumor cells may survive to generate new and more malignant phenotypes (see Figure 2-2).*ATM* Gene: Master Regulator of the DNA Double-Stranded Break ResponseOne of the many defects in ataxia-telangiectasia (AT) cells is increased chromosomal instability. Exposure to ionizing radiation also produces an increased number of chromosomal aberrations in AT cells compared with normal cells.40,41 This effect and the sequence similarity between *ATM* and the DNA repair protein DNA-PK suggested that AT cells might have deficient DNA repair. However, most studies have shown that AT cells do not have gross abnormalities in their ability to repair DNA damage. In general, it does not appear that the radiosensitivity of AT cells is caused by faulty DNA repair; it more likely results from an inability to detect the presence of DNA damage. Exposure to ionizing radiation causes normal cells to delay at the G1/S and G2/M transition phases of the cell cycle, and these checkpoints are thought to allow the cells to repair DNA damage before DNA synthesis or mitosis occurs27 Both checkpoints are absent in AT cells, suggesting clues to the function of ATM.27Although ATM and TP53 cooperate in radiation-induced apoptosis, there are ATM-independent pathways for the induction of TP53-dependent apoptosis, and many of the downstream effects of ATM are independent of TP53.27,28 Wild type and knockout mice have been evaluated for acute and late toxicity after whole-body exposure to ionizing radiation. The *ATM*- and *ATM/TP53*-knockout mice had similar severe toxicity profiles, suggesting that TP53 does not play a role in acute radiation toxicity. Both TP53- and *ATM*-knockout mice preferentially developed lymphoid tumors, whereas *ATM/TP53* double knockouts had an accelerated time to tumor formation and a broader spectrum of tumor types. Analysis of the acquired tumors in *ATM*-null/*TP*53-heterozygous mice revealed that three of seven had loss of the remaining *TP*53 allele. These studies showed that ATM and TP53 interact in a complex manner that is specific to cell type and outcome. This interaction most likely relies on a variety of other pathways and will require much additional work before a complete understanding of the ATM/TP53 relationship is obtained.With isolation of the *ATM* gene it became possible to attempt correction of the cellular defects of the AT phenotype using gene transduction techniques. Several groups have reported that the introduction of ATM into AT cells resulted in reversal of AT defects.27,41 The transfection of full-length ATM into AT cells reversed DSBs, restored normal sensitivity to ionizing radiation, and decreased the number of chromosomal abnormalities. Transfection of full-length ATM also reversed the defective activation of CDKN1A (p21) and JUN kinase in response to ionizing radiation.42,43Histones and Chromatin StructureNative DNA exists in the cell in the form of chromatin complexed with a family of proteins called *histones*. Histones are involved in packaging DNA in the nucleus into a compact form. For gene transcription or DNA repair to occur, the histones are altered by posttranslational modifications, most notably acetylation and methylation, that permit decondensation and access of factors to the DNA.44 The histone acetylase TIP60 is important both in this process and in the modification of ATM itself.26,45 For effective DNA repair, acetylation of histones by histone acetylases must occur to permit access of repair proteins to the sites of DSBs.46 DNA is then remodeled by adenosine triphosphate (ATP)-dependent remodeling enzymes to permit repair molecules such as Mre11, RAD50, and

NBS1 to access sites of DSBs.47,48 A novel histone, H2AX, found in low concentrations on chromatin, has been shown to play a crucial signaling role in the response to genomic stresses such as ionizing radiation. H2AX phosphorylation is one of the earliest events occurring after exposure to ionizing radiation, and ATM-dependent phosphorylation of H2AX may be involved in signaling to cell cycle checkpoints and DNA repair enzymes involved in recombination repair.49,50 Mechanisms of DNA Repair after Ionizing RadiationMaintaining undamaged DNA is essential to cell survival. Cells have therefore evolved a wide range of mechanisms to halt cell cycle progression, survey DNA, and repair damage. Radiation causes a number of types of damage either by direct interaction with DNA or indirectly through effects on nearby water molecules and free radical generation. Types of damage include DNA base damage, damage to the deoxyribose sugar backbone, and physical breaks in one or both strands of the DNA. DNA damage induced by ionizing radiation tends to be clustered so that multiple damaged sites are found in proximity along the double helix, known as *locally multiply damaged sites*.51Among the less catastrophic forms of DNA damage induced by ionizing radiation are the singly damaged bases, which can be repaired by base excision repair. This is a process by which the damaged base is recognized and removed by an N-glycosylase, the apurinic or apyrimidinic (AP) site is cleaved by an AP endonuclease, a patch of DNA is excised, DNA is then resynthesized using the other strand as the template, and the repaired strand is ligated.18,52 In settings in which the base damage is not recognized by the N-glycosylase, another mechanism for repair exists, called *nucleotide excision repair*. In a manner somewhat similar to base excision repair, a damaged section of DNA is removed by incision and excision, a patch is resynthesized using the remaining strand, and the repaired strand is then ligated. Although no naturally occurring mammalian mutants have been identified that are defective in base excision repair, there are a number of different excision repair mutants, which are exemplified by *xeroderma pigmentosum* and Cockayne's syndrome.18 They are characterized by abnormalities in the repair of damage caused by ultraviolet light, although the clinical spectrum of these repair-deficiency syndromes varies. The abnormal genes are identified by finding the human gene that corrects rodent cell defects, called *excision repair cross-complementing* (ERCC) groups. For a comprehensive review on repair of single-stranded breaks we recommend Friedberg et al.18DNA Strand BreaksCellular lethality after radiation involves generation of DSBs.53-55 Misjoined or unrepaired DSBs lead to chromosome deletions, translocations, and acentric or dicentrics, with lethal consequences for the cell. As with the excision repair defects (e.g., ERCCs), there are several x-ray repair defects in the x-ray cross-complementation (XRCC) groups involved in the repair of DSBs. DNA single-stranded repair is carried out in a manner similar to base damage repair, with the undamaged DNA strand serving as a template. DSB repair is more complicated because there is no adjacent, undamaged template available to form a template for repair of the broken strands. The ends of the broken DNA must be protected, and the damaged site must be reconstituted by the processes of *homologous recombination* and *nonhomologous end joining* (NHEJ).DNA DSB repair has much in common with the recombinatorial processes involved in immunoglobulin and T-cell receptor gene rearrangement in the immune response. The XRCC groups that have been identified and are involved in DNA DSB repair include genes that produce DNA end-binding proteins XRCC6 (formerly designated KU70 or Ku70) and XRCC5 (formerly designated KU80 or Ku80) and a DNA-dependent protein kinase (DNA-PK). Defects in DNA repair genes are seen in severe combined immunodeficiency (SCID) mice, indicating the importance of recombination in the restoration of DNA integrity after DSBs and in the immune response.18,53Double-Stranded Break Damage and RepairExperiments in yeast and human cells indicate that a single unrepaired DSB can lead to cell death.53,55 However, cells have the capacity to detect such DSBs and repair them. DSB repair would be predicted to require three functional components: (1) a mechanism for detecting and gauging DNA damage, (2) a signal transduction system, and (3) an effector system for DNA repair. These components are discussed in the following paragraphs, commencing with the repair component. DSBs can be repaired by a number of mechanisms, but the most prevalent are homologous recombination and NHEJ. Human cells appear to differ from yeast in that homologous recombination predominates in yeast, whereas the opposite is true in mammalian cells.53,55Genetic analysis has revealed the

existence of a large number of genes that regulate DSB repair. One essential gene, involved in resistance to cell killing by ionizing radiation and the repair of DSBs, is *XRCC5*, which encodes XRCC5, a protein that binds with high affinity to the ends of the double strands (Figure 2-3, *A*).[1,18] XRCC5 functions in normal cellular processes that require DSBs rejoining, most notably V(D)J rejoining in the immunoglobulin and T-cell receptor genes of immature B and T lymphocytes. XRCC5 exists in cells in a heterodimeric complex with XRCC6, the product of the *XRCC6* gene. The XRCC5/XRCC6 heterodimer is required for the end-binding and repair functions.[56] The XRCC (formerly designated KU) proteins recognize the ends of DSBs and protect them from further degradation before the onset of end-joining reactions required for DSB repair. An associated protein in the complex with important functions in DNA repair is the protein kinase DNA-PK, the product of the *XRCC7* gene.[56]

Figure 2-3 **A,** Formation of DNA repair complexes on damaged DNA. Radiation-induced DNA damage causes immediate changes in the vicinity of double-stranded breaks (DSBs). These include rapid phosphorylation of the atypical histone H2AFX and activation of ATM. Protein complexes then begin to assemble on DNA, including the DNA-dependent protein kinase (DNA-PK) complex shown here. This complex is a molecular machine for DNA repair that includes DNA end-binding proteins XRCC6 (formerly KU70) and XRCC5 (formerly KU80); DNA-PK itself, which appears to be a signaling and scaffold protein; and proteins involved in the effector stages of repair. **B,** Another important protein complex mediating DNA DSB repair by the nonhomologous end joining (NHEJ) pathway is the RAD50 complex. The diagram shows the signal transduction pathway leading from ATM, the sensor of DNA DSB, to the phosphorylation of NBN (nibrin, formerly designated NBS1), which induces recruitment of RAD50 and MRE11 into a DNA repair complex at the site of the DNA DSB.DNA-PK is a serine/threonine kinase involved in regulating the cellular response to DNA damage.[56,57] DNA-PK is a member of the phosphatidylinositol-3-kinase gene family (*PIK*) that produce a group of high-molecular-weight proteins that contain a conserved kinase domain at the carboxyl-terminal end. PIK family proteins have been identified in yeast, *Drosophila*, and in mammalian cells, and include the human *ataxia-telangiectasia* gene (*XRCC7*) for DNA-PK and the yeast genes *TEL1* and *MEC1*.[56,57] Homology with genes of other organisms can provide a clue to the function of the family of genes in mammalian systems. For example, the *S. cerevisiae TEL1* gene is a homolog of the human *ATM* gene. *TEL1* mutants have shortened telomeres and exhibit chromosome instability. In mice, the SCID mutation results in the loss of expression of functional DNA-PK.[58] SCID mice are deficient in the repair of DNA DSBs, have faulty V(D)J recombination, and are sensitive to radiation.[58] The binding of the XRCC complex (XRCC5 and XRCC6) to DNA-PK activates its kinase activity. The DNA-PK/XRCC complex is involved in recognizing DNA DSBs. Thus in SCID cells, the catalytic subunit for DNA-PK is absent, and its loss may account for increased sensitivity to ionizing radiation, reduced ability to repair DNA DSBs, and the known immune defects.Genetic evidence indicates the existence of numerous other factors required for efficient DSB repair in human cells.[18,53,55] Among the genes are homologues of the yeast *RAD52* epistasis group, RAD50 and *MRE11*.[26,59-61] RAD50 and MRE11, together with the product of the *XRS2* gene, form a complex in *S. cerevisiae* that is involved in nonhomologous recombination (see Figure 2-3, *B*). RAD50 has been suggested to bind to DNA at the site of DSBs and mark them for repair or recombination. Such a signaling role for RAD50/MRE11 complexes is suggested by studies indicating the formation of nuclear foci containing these proteins in cells after irradiation.[61] The RAD50/MRE11 complexes also contain another important protein, NBN (nibrin; formerly NBS1 or p95), which is inactivated in the Nijmegen breakage syndrome.[26,61] The RAD50/MRE11 foci fail to form in ATM-deficient cells, suggesting a role for this complex downstream of the ATM protein. DSB repair can also be carried out in human cells by homologous recombination, an alternative pathway involving a different group of genes, including *RAD51* through *RAD57*. RAD51 is the eukaryotic homolog of the bacterial protein RecA and forms structures in meiotic chromosomes containing ATM, suggesting functional coupling between these proteins. The complex also contains TP53 and may be involved in coupling of DNA DSB formation to cell cycle arrest, as well as in carrying out recombination and repair.[62]The tumor suppressor genes *BRCA1* and *BRCA2* also have function in DNA repair. Although the these proteins have multiple functions, it

has been shown that *BRCA1* interacts with RAD51 and assists in cell cycle arrest through activation of *CDKN1A*, a gene that is normally activated by TP53.63 RAD51 is thus targeted by three major gene products: ATM, TP53, and BRCA1. Loss of RAD51 leads to cancer development, suggesting that this pathway of DNA repair and replication is crucial in protection of cells from genotoxic stress. That this RAD51 pathway of DSB repair is separate from the RAD50 end-joining pathway is indicated by findings that ionizing radiation causes the formation of nuclear foci containing RAD50 or RAD51 but never both complexes.64 However, further studies are required to understand the relative contributions of the two pathways to cell survival after exposure to ionizing radiation. As with the RAD50 pathway, understanding of mammalian recombination repair is still preliminary and is difficult to assemble into a fully coherent molecular pathway. *BRCA2* mutant cancer cells are also deficient in DSB repair and have increased radiosensitivity.63 It is apparent that DNA damage recognition and repair is complex, involving interaction among molecules that can cause cell cycle arrest, apoptosis, and signal transduction. Defects in any of these pathways could lead to radiation sensitivity in normal tissues (and perhaps the corresponding tumor) or to the development of secondary mutations and the mutator phenotype, as with mismatch repair defects. For radiation therapy, the aim of course is to maximize accumulation of DSBs in tumor cells by targeting these pathways.Pathways of Ionizing Radiation-Induced Programmed Cell DeathThe ability to inhibit the pathways of cell death appears to be a key step in the origin of cancer cells, with the small percentage of cells that are able to evade PCD being the ones most capable of forming tumors.16 Multiple genetic alterations are involved in escape from PCD, most notably in the *TP53* and *BCL2* families.16 Radiation oncologists are thus faced with the undesirable situation in which tumor cells are selected for resistance to PCD, whereas normal cells retain these altruistic pathways. Apoptosis and senescence are key PCD pathways with potential roles in cancer induction and resistance to therapy. Excellent reviews have been provided by Edinger and Thompson32 and Danial and Korsmeyer.65*Apoptosis* is a normal physiologic process that permits altruistic death of cells during tissue remodeling or the elimination of T-cell populations after the reversal of viral infection.65 Apoptosis is characterized morphologically by cell shrinkage, membrane blebbing, chromatin condensation, and ultimate fragmentation of the cell into apoptotic bodies. Molecular characteristics include activation of class of proteases (protein degrading enzymes) the caspases. These are involved in intracellular death signaling. Additionally, activation of enzymes that degrade DNA (endonucleases) is seen. In the final stage of apoptosis, the cell corpses exhibit “eat me” signals a process that involves alteration of the cell surface (including expression of annexin V binding sites on the plasma membrane) permitting neighboring cells to remove the apoptotic bodies without the induction of an inflammatory reaction. Apoptosis is mediated by changes in the outer mitochondrial membrane that lead to the release of death signals such as cytochrome c. BCL2 and related family members function as anti-apoptotic factors by blocking pro-death changes in mitochondrial membrane potential or as anti-apoptotic factors by antagonizing the pro-apoptotic factor BAX. The terminal stages involve DNA degradation, which cleaves DNA into 50- to 300-kb fragments and later into smaller fragments by cleavage of the exposed regions of DNA between nucleosomes.65Replicative SenescenceIn addition to vulnerability to overt killing, all somatic cells possess replicative checkpoints that place limits on the number of permitted cell divisions over the lifetime of the cell. For unlimited growth, cells must bypass crisis, the point at which the telomeres on chromosomes have shortened enough to prevent successful future cell divisions. TP53-sensitive (and CDKN1A-sensitive) expression of the enzyme telomerase in tumor cells is sufficient to bypass crisis and permit unlimited growth in some cells (see Figure 2-2). In addition to crisis, cells undergo telomerase-independent forms of senescence, regulated in many cases by the retinoblastoma protein RB1 and the CDK inhibitory protein CDKN2A (formerly designated p16).66,67 However, TP53 appears to be the primary regulator of senescence and activated this process downstream of DNA damage detected by ATM activation or other pathways.27 Few experiments have been carried out to examine the role of senescence in responses to ionizing radiation, although in one study, ionizing radiation induced premature senescence that occurred independently of ATM expression.68Activation of Anabolic Signaling Pathways by Ionizing RadiationOne of the primary features of cancer is its autonomy in

terms of growth signals. There are three operationally defined steps in growth signaling: (1) transmembrane receptor occupation by growth factors and transmembrane signaling, (2) stimulation of cytoplasmic cascades that amplify primary signals, and (3) activation of downstream effector proteins. Step 1 classically involves dimerization and autophosphorylation of receptor tyrosine kinases (RTKs) or recruitment of nonreceptor tyrosine kinases (NRTKs). Such events occur commonly after ionizing radiation, and a generalized activation of the HER1 through HER4 RTKs occurs in irradiated cells (Figure 2-4).69 Ionizing radiation can induce the release of growth factors such as epidermal growth factor (EGF, TGF-α) and paracrine activation of growth in adjacent cells.69 Increases in cellular phosphotyrosine levels were observed after exposure to x-rays in a number of cells, and activation of NRTKs has been observed.69 Of particular importance in step 2 processes are members of the mitogen-activated protein kinase (MAPK) family, which play crucial roles in cell growth and survival. Ionizing radiation stimulates the activity of MAPK members that carry the mitogenic signal (e.g., MAPK1 [formerly ERK2], MAPK3 [formerly ERK1]).70 The paradigm for step 3 is the activation of factors that bind the promoters of mammalian immediate early genes such as *FOS* and *EGR1* through receptor activation, tyrosine phosphorylation, and MAPK activation. Such transcription factors include serum response factor, ETS domain factors such as TCF62, ELK-1, activating protein-1 (AP1), and cyclic adenosine monophosphate-binding protein (i.e., cAMP response element binding [CREB]).71 Each of these factors is activated by ionizing radiation, and the promoter of at least one immediate early gene (*EGR1*) is induced by radiation treatment. Therefore, exposure to ionizing radiation could amplify the growth signals already active in cancer cells, suggesting that the RTK-MAPK pathway may be a fruitful target in the selection of radiation sensitizers.69,72

Figure 2-4 Induction of transmembrane signals by ionizing radiation (IR), which directly activates several transmembrane receptors. Free radicals induced in cells by ionizing radiation can activate transmembrane tyrosine kinase receptors such as the epidermal growth factor (EGFR) and HER2, HER3, and HER4 (not shown). Receptor activation gives rise to a network of signals that lead to activation of the powerful prosurvival PI3K/AKT pathway and the growth-mediating RAF/MEK/ERK cascade. Further amplification may take place as a result of transcriptional induction and the release from cells of cytokines with growth factors such as EGF and transforming growth factor-α, which give rise to secondary activation of receptors.Radiation-induced signaling may be secondary to the free radical generation. Radiation produces two main species that combine to kill cells in a clinical setting and that may also double as primary stress signals.10 These signals are reactive oxygen species (ROS) and DSBs discussed previously. ROS play a role in signal transduction after stimulation by platelet-derived growth factor (PDGF) and phorbol esters.70,73 ROS produced by cellular stress, such as peroxide, superoxide, hydroxyl radicals, and nitrous oxide, may feed into this ROS-transduced pathway at a number of places.74 Cell kill by radiation is closely correlated with the accumulation of DSBs. The dictates of logic and much preliminary evidence strongly suggest a role for DSBs in the sensing and response to ionizing radiation.27 Cells evidently possess at least one system to sense and respond to radiation, the ATM family of protein kinases, which appear to be situated close to the primary event in DSB-induced signaling as described previously.27,53 ATM activation leads to phosphorylation of TP53, which couples DSB accumulation to cell cycle arrest in G1.30 Vectoral signaling is then coupled to the metronomic events of the cell division cycle. ATM belongs to the lipid kinase family that includes PIK and DNA-PK.27,40 PIK is activated by ionizing radiation and is required for cell survival during irradiation. DNA-PK is intimately involved in cell responses to radiation, and its ability to sense DSBs in combination with DNA end-binding proteins XRCC6 and XRCC5 suggests that it may be able to function independently of ATM in cell signaling after exposure to ionizing radiation.56 Regulatory changes in cells after irradiation may therefore be caused by the aggregate and combinatorial interactions of three distinct types of signaling pathways: classic signal transduction, signaling pathways mediated by ROS, and those initiated in the nucleus by DSBs.Radiation and the RNA WorldOver recent years there has been a revolution in understanding the role of RNA in biology and cancer.75 In addition to its housekeeping function as mRNA and rRNA, novel RNAs have been shown recently to play key regulatory roles. Small interfering RNA and microRNA play

negative regulatory roles in diminishing specific RNA levels and targeting structures in the 3′ and 5′ regions of mRNA.76,77 Large noncoding RNA plays a role in regulation of transcription, although its regulation of radiation responses is not known.78 Study of the roles of these molecules in the radiation response has begun.77,79Tumor Microenvironment and Responses to Ionizing Radiation in VivoIn addition to malignant cells, tumors contain normal cells and structural elements, including endothelial cells, fibroblasts, extracellular matrix molecules, inflammatory cells, and blood vessels. The tumor microenvironment is abnormal in being largely deficient in nutrients such as oxygen and glucose, and abundant in the waste products of metabolism such as lactic acid and carbon dioxide.80,81 Thus, the concentrations of nutrients and cell-cell and cell-matrix contacts are abnormal and may be in a constant state of flux because of intermittent perfusion by the microvasculature. The tumor microenvironment is a key determinant of the response to ionizing radiation. Hypoxic cells are markedly radioresistant because of the requirement for oxygen in the formation of the free radicals that mediate radiation-induced DSBs.10 Defining the tumor milieu and how it is regulated is thus important in understanding the radiosensitivity of tumors.Hypoxia, Microenvironment, and Radiation ResponseThe role of hypoxia in radioresistance has been studied for many years.10,82 Cell killing by radiation is decreased at intermediate- and low-oxygen conditions, and a number of studies have correlated low pretreatment tumor oxygenation with a worse disease outcome.83,84 Whether there is a direct cause-and-effect relationship between low-oxygen concentration and outcome remains to be resolved, primarily through clinical trials that deliberately attempt to correct the poor oxygenation status of the tumors. In addition to directly inhibiting cell killing by ionizing radiation, hypoxia may amplify the mutator phenotype in the severely hypoxic cells in the central cores of tumors, increasing the rate of evolution of resistant clones and amplifying the malignant phenotype.85 Many genes are induced or repressed within the tumor microenvironment.86,87 The largely unregulated environment found in poorly vascularized tumors could be thought of as resembling the conditions under which many unicellular organisms grow. Survival for these organisms depends on their ability to withstand environmental stresses such as nutritional deprivation, temperature and pH changes, radiation exposure (e.g., ultraviolet light, x-rays), and xenobiotics. Among the molecules induced by hypoxia are members of the unfolded protein response family stress proteins (e.g., glucose-regulated proteins [GRPs], redox enzymes such as heme oxygenase, metallothionein IIA, DT-diaphorase; transcription factors such as JUN, FOS, AP1, TP53, nuclear factor-KB [NF-κB], HIF1) and growth factors or cytokines (e.g., erythropoietin, vascular endothelial cell growth factor [VEGF], EGF receptor, interleukin-1α [IL-1α]).86,87 HIF1 appears to be of key significance in the response of cells to the microenvironment at the molecular level because this transcription factor regulates expression of many of the factors that mediate angiogenesis.88Tumor AngiogenesisAnother of the hallmarks of cancer is the ability of cancer cells to induce de novo angiogenesis to sustain growth.36,89,90 Tumors contain multiple angiogenic factors including angiopoietin, acidic fibroblast growth factor (FGF), basic FGF, angiogenin, PDGF, prostaglandins E1 and E2, transforming growth factor-β, tumor necrosis factor-α, and VEGF (also known as vascular permeability factor). The VEGF family has received much attention as potential therapeutic targets. In addition antiangiogenic factors are also expressed and include angiostatin, endostatin, interferon-α and interferon-β, thrombospondin, and tissue inhibitor of metalloproteinase.90,91 The outcome for vascularization and tumor growth depends on a balance between the factors.The use of antiangiogenic therapy with local radiation has been investigated in several laboratories.92,93 One important aspect for combining vascular targeting and radiation therapy and probably also for systemic agents is whether the antiangiogenic therapy increases or decreases tumor oxygenation. The relationship between angiogenesis, antiangiogenic therapy, and the more conventional cancer therapies remains to be elucidated but offers some exciting areas of investigation. The ability to suppress metastases and hold local tumor growth92, 93 offered by vascular targeting may greatly increase the potential role for radiation therapy. However, if antiangiogenic therapy leads to decreased perfusion or hypoxia, the effects are likely to be confounding. Given the role of blood vessels in late radiation injury, the use of antiangiogenic therapy with radiation in clinical treatment will require careful study and

assessment of long-term toxicities and late effects.Radiation and the Cell Biology of CancerStudy of the cell biology of cancer has lagged behind molecular biology studies. However, it is apparent that, rather like normal tissues, tumors contain only a small proportion of cells that can proliferate in the long term. These are known as cancer stem cells by analogy with their normal counterparts. As such cells are resistant to most treatments, targeting them may be important in improving cancer therapy; this is an area of intensive study. In addition to heterogeneity conferred by cell differentiation hierarchies, tumors contain populations of normal cells that confer a growth-promoting niche in the tissue and protect tumor cells by suppressing immunity. Such cells include tumor-associated macrophages and fibroblasts, myeloid suppressor cells, and regulatory T cells. Heterogeneity in terms of cell hierarchy and normal cell infiltration may determine many key features of tumor progression including invasion and metastasis of cancer cells and susceptibility to immune killing. The role of radiation in these processes is under investigation and may be a key area for future development.Targeting Molecular Pathways in the Radiation Response: Discovery of New DrugsThe accumulated data regarding the signaling circuitry underlying the response of cells to DSBs suggests an opportunity for the development of novel adjuvant therapies. Approaches based on small-molecule inhibitors of specific reactions, RNA interference (small interfering RNAs), and antisense and gene therapy are attractive and follow directly from the basic research available. There is much excitement regarding the potential use of kinase inhibitors in cancer treatment, based on the idea that they work catalytically, are present in small amounts, and often regulate key processes in the cell. The most popular paradigm is a kinase inhibitor, known as imatinib (Gleevec), which has greatly influenced the treatment of hematologic malignancies. Given that the cell cycle checkpoint component of the DNA DSB response is regulated largely at the posttranscriptional level by batteries of protein kinases and phosphatases, many inviting targets are available. Proof of principle that such an approach may work is suggested by studies using agents that inhibit the RAS signal transduction pathway, protein kinase C, or the PIK pathway, which were shown to enhance radiation-induced cell killing.[94,95,96,97,98,99,100] Members of the PIK family may be feasible targets for pharmacologic intervention in the clinic. Loss of function of PIK proteins is usually associated with increased radiosensitivity. Compounds that block the function of these proteins would greatly enhance the efficacy of agents that cause DNA damage, such as radiation therapy, and allow for the treatment of tumors that are particularly radioresistant.The fungal metabolite wortmannin is a specific inhibitor of the p110 PIK, now designated PIK3CD. Wortmannin forms a covalent adduct with Lys802 of PIK3CD, inactivating the kinase activity.[101,102] This Lys802 is conserved in DNA-PK and AT proteins, suggesting that both would be sensitive to wortmannin. In vitro studies with a wide range of tumor-derived cells, including those of the breast, colon, and prostate, indicate that wortmannin is an effective radiosensitizer of human cells. Wortmannin can inhibit the kinase activity of DNA-PK in vitro and in vivo. DNA-PK also is involved in the activation of TP53 after irradiation of cells, and wortmannin suppresses the activation of TP53 after irradiation. Other studies have shown that wortmannin may exert its primary effect by inhibiting the repair of DNA strand breaks in cells.In addition to drugs, the development of small interfering RNA approaches offers the promising possibility of “knocking down” selective genes that may mediate resistance.[76] There has been much excitement regarding the use of targeted gene therapy to introduce genes of interest or their antisense partners within viral vectors. Some of the enthusiasm about these approaches has waned because of the potential toxicity of the viral vectors (particularly adenovirus) and a number of other operational problems, such as accurately reaching the target site, achieving sufficient gene production in situ (i.e., drug, enzyme, or toxic molecule) to alter the radiation response, altering the radiation response sufficiently to show an impact on local tumor control, having a positive therapeutic ratio, and ideally, having an impact on survival in addition to enhanced local control. Despite these problems, the approach remains popular, particularly for local expression of products that diffuse from cells, such as cytokines. With the advent of newer, safer vectors for delivery of agents, the approach holds promise.[103]Previous radiation modifiers in clinical use have been developed based on conventional radiation biology models, such as hypoxia (e.g., radiation sensitizers and enhancers, altered oxygen delivery), the competition model (e.g., thiol depletion

and radioprotectors), and increasing susceptibility of DNA to radiation damage (e.g., halopyrimidines).101,102,104 Although such therapies were not based on the new molecular targets that have more recently been elucidated, much can be learned by studying the relationships between these therapies and the molecular processes. For example, it may be possible to understand how better to use the halopyrimidines in relation to cell cycle checkpoints, hypoxic sensitizers in relation to hypoxia-induced processes, and protectors in terms of specific DNA lesions or activity of repair enzymes.As mentioned, tumor cells exist in a unique microenvironment that is depleted of oxygen and glucose and rich in carbon dioxide and lactate. Drugs may be designed to prosper in this altered microenvironment and enhance treatment efficacy. Among these more conventional approaches, the bioreductive agents, such as mitomycin C and tirapazamine, require enzymatic activation that occurs preferentially in hypoxic environments.105Normal TissuesRadiation therapy depends for its selective treatment of cancer on tight control of delivery to tumors and avoidance of normal tissues. Most normal tissues that express wild-type TP53 are sensitive to TP53-induced PCD.106 Renewable tissues such as hematopoietic stem cells and crypt cells in the gastrointestinal tract may be particularly vulnerable. 33 Indirect forms of lethality may occur in whole-body irradiation, leading to the death of hematopoietic stem cells in the bone marrow, or depletion of mature tissues can lead to secondary damage caused by red blood cell depletion, immunosuppression, and gross infection as the gastrointestinal lining is lost.10,13,14Additional changes occur that are thought to be the result of a persistent oxidative state in tissues such as lung, which may lead to chronic inflammatory changes and induction of cytokines and adhesion molecules.107,108 Changes may result from induction of the transcription factor NF-κB by ionizing radiation in an ATM-dependent manner (Figure 2-5).109,110 NF-κB, in addition to its role in cytoprotection, plays a broader part in the acute inflammatory response through transcription of a wide range of proinflammatory cytokines and adhesion molecules.111 Induction of an inflammatory environment in normal tissues may be a side effect of activation of NF-κB in a cytoprotective reaction (see Figure 2-5).

Figure 2-5 Ionizing radiation activates the NF-κB pathway. NF-κB is induced by inflammatory ligands such as bacterial products that stimulate the IKK kinase complex and activate NF-κB through destruction of the inhibitor IK-Bα. This pathway also can be activated by ionizing radiation through a number of mechanisms, particularly IKK activation by the DNA DSB sensor molecule ATM. NF-κB activation has at least two known consequences: increased cell survival through synthesis of death-antagonizing NF-κB products and proinflammatory effects caused by increased expression of cytokines and adhesion factors.Basic Concepts and Techniques of Radiation SciencesTechniques and approaches to molecular and cellular biology in humans and other model organisms, largely developed in the 1960s and 1970s, continue to revolutionize knowledge of basic cancer biology and radiation sciences. The ability to inactivate genes by homologous recombination, which came into frequent use in the 1980s, has permitted the movement of this approach into animal models, allowing study of molecular genetics in vivo. This has permitted us to test the significance of the key genes in the cell cycle, DNA repair, and PCD, which mediate the sensitivity of cells to ionizing radiation and define their role in radiation response. Novel “big biology” approaches, including such genomic techniques as microarray screening, deep sequencing and mass spectrometry permit discovery-based research with the potential for fundamental advances.112-114 Because of the progress in deciphering human and other genomes in the 1990s, a total readout of expression of all human genes can be undertaken. This has opened the way for identification of signature genes in human cancers that correspond to poor prognosis, treatment resistance, and metastasis. The impact of gene expression profiling on radiation biology is perhaps less than in general cancer biology because most responses to genomic stress occur at the posttranscriptional level. The development of proteomics is being developed, based on advances in mass spectrometry, and this approach allied with deep sequencing may permit the rapid study of critical changes in posttranslational modification, such as phosphorylation, acetylation, and ubiquitination, which mediate many regulatory responses to genomic stress. The increased speed and accuracy of drug development,

based on robotic approaches to assaying chemical libraries and in silico design, should also increase the rate of drug discovery and hasten the translation of molecular findings into the clinic.
SummaryThe past 20 years have seen an impressive transformation in understanding of the genes that determine the development of the cancer cell, its subsequent response to radiation and chemotherapy, and the signaling circuitry that links the products of such genes to DNA damage response pathways. The rate of accumulation of new knowledge, particularly in the field of DNA sensing and repair, has been so rapid that its future impact on the practice of radiation oncology is difficult to predict. One area of incomplete knowledge is the exact molecular mechanisms of radiation cell death, their link to the DNA damage response pathways, and how they might be altered by the evolving patterns of gene expression in tumor progression. This dilemma is well illustrated by TP53, the factor that in normal cells links the accumulation of genomic damage to cell death by apoptosis, autophagy, and senescence but that is inactivated in most cancers. In contrast, identification of ATM as the master regulator of the response to genomic stress suggests this pathway as one that can be targeted for radiation sensitization. Another interesting possibility for targeting new treatment approaches is that of necrosis, particularly in tumor cells, in which the pathways of PCD have been closed off through alterations in the TP53 and BCL2 pathways. The success of future approaches to cancer treatment and cure will be determined by their ability to inhibit or facilitate normal and adaptive changes in cell physiology to maximize the deleterious effects on the tumor while preserving viability of the surrounding normal tissues.References

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114. Scherf U, Ross DT, Waltham M, et al. A gene expression database for the molecular pharmacology of cancer. *Nat Genet*. 2000;24:236–244. **Chapter 3**Dose-Response Modifiers in Radiation Therapy*Michael R. Horsman, Jacob C. Lindegaard, Cai Grau, Marianne Nordsmark, Jan Alsner, Jens Overgaard*When cancer patients undergo radiation therapy there is a clear dose-response relationship between the dose delivered and the response of the tumor to the radiation. This is illustrated in Figure 3-1. Unfortunately, there is also an increase in normal tissue damage with increasing radiation dose, and it is this complication that limits the total radiation dose that can be given. Substantial effort has been made to try and modify these dose-response relationships and to thus increase the separation between the tumor and normal tissue dose-response curves. The approach has been either to selectively increase the radiation damage in tumors without affecting the normal tissues or by protecting the normal tissues without having a similar protective effect in tumors.
Figure 3-1 Schematic illustration of the proportion of patients cured and patients with normal tissue complications as a function of the total radiation dose received.Agents capable of enhancing radiation response include certain conventional chemotherapeutic agents, the halogenated pyrimidines, and treatments that specifically overcome radioresistance resulting from the presence of hypoxic cells that occur as a result of the environmental conditions within most

solid tumors. The most widely investigated method applied to the hypoxia problem is radiosensitization of the hypoxic cells with either electron-affinic sensitizing drugs or hyperthermia. Another approach often used to reduce hypoxia—especially in experimental systems—involves increasing oxygen availability (1) by having patients breathe high-oxygen-content gas, (2) introducing perfluorochemical emulsions into the vascular system to increase the oxygen carrying capacity of the blood, (3) modifying oxygen transport or delivery by using agents that affect hemoglobin, (4) using drugs that increase tumor blood perfusion, or (5) a more recent approach of decreasing the oxygen consumption rate of the “nonhypoxic” cell population, thereby increasing the oxygen diffusion distance. Many experimental studies have also demonstrated that hypoxic cells can be preferentially destroyed by bioreductive drugs that are active under reduced oxygen conditions, or again using hyperthermia; each of these hypoxic-cell cytotoxins improve the radiation response of tumors. Another group of agents, which preliminary data suggests have the potential to enhance radiation damage, are the so-called vascular targeting agents. These include drugs that inhibit angiogenesis, the process by which tumors develop their own vascular supply, or agents that preferentially damage the already established tumor vessels.Radiation protectors fall into several categories based on the timing of their administration in relation to radiotherapy. There are the true “radiation protectors,” in particular sulfhydryl compounds, which are used as a prophylactic strategy and administered before radiotherapy and primarily appear to interact with radicals that are formed as a result of radiation exposure. Another group consists of “radiomitigators” that reduce the effects on normal tissues before the emergence of symptoms if given during or shortly after radiotherapy. Finally, there are “therapeutic agents,” which are administered after radiotherapy to treat symptoms that have already developed, especially fibrosis.Radiosensitization by conventional chemotherapeutic agents (e.g., cisplatin, 5-fluorouracil, and mitomycin C), halogenated pyrimidines (e.g., 5-bromodeoxyuridine and 5-iododeoxyuridine), and hyperthermia are discussed in detail elsewhere in this book. In this chapter, the focus will be on hypoxic cell modifiers, vascular targeting drugs, and radioprotectors.
The Hypoxia Problem**Importance of Oxygen**In 1909 Gottwald Schwarz,[1] in a simple but elegant experiment, demonstrated that the radiation response of skin was markedly decreased if the blood flow in the irradiated area was reduced by compression. Although he did not acknowledge that the phenomenon was the result of a lack of oxygen, his study was probably the first radiobiologically oriented clinical study implicating the importance of environmental parameters in the outcome of radiotherapy. This finding was used to introduce the concept of “kompressionsanämie” by which the skin was made anemic, thereby allowing a higher dose to be given to deeply situated tumors. Following the work of Schwarz, in 1910 Müller[2] reported that tissues in which the blood flow was stimulated by diathermia showed a more prominent response to radiation. This early study not only demonstrated the importance of oxygen supply in radiotherapy, but it was also the first clinical approach showing how resistance could be overcome by using hyperthermia. Subsequently, sporadic clinical and experimental observations indicated the importance of sufficient blood supply to secure an adequate radiation response. These observations led Gray et al[3] in the early 1950s to postulate that oxygen deficiency or hypoxia was a major source of radiation resistance.The first clinical indication that hypoxia existed in tumors was made around the same time by Thomlinson and Gray[4] when, from histological observations in carcinoma of the bronchus, they reported seeing viable tumor regions surrounded by vascular stroma from which the tumor cells obtained their nutrient and oxygen. As the tumors grew, the viable regions expanded and areas of necrosis appeared at the center. The thickness of the resulting shell of viable tissue was found to be between 100 and 180 µm, which was within the same range as the calculated diffusion distance for oxygen in respiring tissues. It was thus suggestive that as oxygen diffused from the stroma, it was consumed by the cells and, although those beyond the diffusion distance were unable to survive, the cells immediately bordering the necrotic area might be viable yet hypoxic. In 1968, Tannock[5] described an inverted version of the Thomlinson and Gray picture, with functional blood vessels surrounded by cords of viable tumor cells outside of which were areas of necrosis. This

“corded” structure, illustrated in Figure 3-2, is the more typical picture found in most solid tumors.6 It arises because the tumor blood vessels, which are derived from the normal tissue vessels by a process of angiogenesis, are inadequate to meet the needs of the rapidly growing tumor cells. This hypoxia is more commonly called *chronic hypoxia*.

Figure 3-2 Schematic representation of the interrelationship between tumor cells and the vascular supply. On the left, cells are seen growing as a “corded” structure around a functional vessel from which the cells receive their oxygen supply. As oxygen diffuses out from the vessel it is used up, thus the outermost viable cells (shown by the shading) are oxygen deprived or chronically hypoxic. A similar arrangement is seen on the right, but here flow through the vessel is transiently stopped, thus making all the cells oxygen deprived. Reprinted from Horsman MR: Measurement of tumor oxygenation. Int J Radiat Oncol Biol Phys 42:701–714, 1998. Copyright 1998, with permission from Elsevier Science.It was also suggested that hypoxia in tumors could be acute in nature.7 However, it was not until later that Chaplin et al8 were able to confirm the existence of acutely hypoxic cells in tumors and demonstrate that these cells were the result of transient stoppages in tumor blood flow (see Figure 3-2). To date, these temporary cessations in blood flow have been observed in mouse and rat tumors, as well as human tumor xenografts, with anywhere from around 4% to 8% of the total functional vessels involved,9 although the exact causes of these stoppages are not known. The current use of chronic or acute to explain hypoxia in tumors is probably an oversimplification of the real situation. Chronic hypoxia generally refers to prolonged and reduced oxygen concentrations that influence radiation response, but there is evidence that oxygen concentrations that are higher, yet below normal physiological levels, are often found.10 Furthermore, reduced perfusion can be both partial as well as total,11 and while cells under the former condition would be oxygen deprived, with the latter they would be starved of oxygen and nutrients, and as such, their survival and response to therapy would be expected to be different.Evidence for Hypoxia in TumorsIn experimental tumors it is not only relatively easy to identify hypoxia, but one can also quantitatively estimate the percentage of cells that are hypoxic. Three major techniques are routinely used.12 These are the paired survival curve, the clamped tumor growth delay, and the clamped tumor control assays. All involve a comparison of the response of tumors when irradiated under either normal air-breathing conditions or when tumors are artificially made hypoxic by clamping. Using these procedures hypoxia has been directly identified in most animal solid tumors, with the values ranging from less than 1% to well more than 50% of the total viable cell population.12 Unfortunately, none of these procedures can be applied to the clinical situation. One therefore must rely on indirect techniques.Estimating hypoxia in human tumors has generally involved the use of indirect methods.13 Some of the earliest attempts focused on the vascular supply because it was only via the tumor vasculature that oxygen could be delivered. The endpoints included immunohistochemical estimates of intercapillary distance, vascular density, and distance from tumor cells to the nearest blood vessel14-16; oxyhemoglobin saturation determined using cryophotometry, or noninvasively with near-infrared spectroscopy or magnetic resonance imaging (MRI)17-19; or measurements of tumor perfusion using MRI, computed tomography (CT), or positron emission tomography (PET).20-22 With the finding that hypoxia could up-regulate gene/protein expression, it was suggested that endogenous markers could be used to identify hypoxia.23 The principal markers have included hypoxia inducible factor 1 (HIF-1), carbonic anhydrase IX (CAIX), the glucose transporters GLUT-1 and GLUT-3, and osteopontin (OPN).24-27 These have been applied either individually or combined with other endogenous markers in gene signatures.13 More popular techniques involve measurements of the binding of exogenous markers. This can be achieved following immunohistological analysis of biopsied sections, using, for example, pimonidazole or EF5.28,29 It can also be done noninvasively with PET, single-photon emission computed tomography (SPECT), or MRI analysis of radioactively labeled nitroimidazoles (i.e., [18F] labeled misonidazole or FAZA; [123I] labeled azomycin arabinoside), or PET imaging of [60-64Cu]-ATSM.30-33The most direct method involves determining oxygen partial pressure (PO2) distributions with polarographic electrodes.34-39 How this approach can be used to detect hypoxia and relate the measurements to radiotherapy outcome is illustrated in Figure 3-3. In this

international multicenter study in head and neck cancer patients, their tumor's pO2 was measured before radiation therapy and was found to correlate with overall survival, in that those patients with lower tumor oxygenation status, did significantly worse.40
Figure 3-3 Oxygen levels were measured with Eppendorf electrodes before radiation therapy in 397 patients with squamous cell carcinomas of the head and neck. Tumors were stratified by whether the fraction of pO2 values ≤ 2.5 mm Hg (HP2.5) were above or below the median value for the whole group (i.e., 19%). The lines show Kaplan Meier estimates of actuarial overall survival probability for patients with less hypoxic tumors (HP2.5 ≤ 19%; *red line*) compared with more hypoxic tumours (HP2.5 > 19%; *blue line*), *p* = 0.006. From Nordsmark M, Bentzen SM, Rudat V, et al: Prognostic value of tumor oxygenation in 397 head and neck tumors after primary radiation therapy. An international multi-center study. Radiother Oncol 77:18–24, 2005, with permission.Probably the best evidence for the existence of hypoxia in human tumors comes from the large number of clinical trials in which hypoxic modification has shown some benefit.41 The latter situation constitutes a circular argument: if hypoxic modification shows an improvement then hypoxic clonogenic cells must have been present in tumors. It is, however, likely that even tumors with the same histological makeup and of the same type have substantial heterogeneity with respect to the extent of hypoxia. It must be admitted that today, a century after the first clinical description, the importance of hypoxia and its influence on the outcome of radiotherapy is still the subject of substantial debate. However, we will now discuss in detail how the different hypoxic modifiers have been used to modify the radiation dose response of tumors.Overcoming Tumor Hypoxia
High-Oxygen-Content Gas Breathing Because the oxygen supply to tumors is insufficient to meet the needs of all the tumor cells, radiation-resistant hypoxia develops; therefore, an obvious solution to improving the tumor's radiation response would be to increase the oxygen supply. This has been tried, both experimentally and clinically, by simply allowing the tumor bearing host to breathe high-oxygen-content gas mixtures before and during irradiation.Early experimental studies reported that breathing either oxygen and carbogen (95% O2 + 5% CO2) could substantially enhance the response of murine tumors to radiation and that the best effect was generally seen when the gasses were inspired under hyperbaric (typically 3 atmospheres [3 atm]) rather than normobaric conditions.42,43 This is not surprising because hyperbaric conditions would be expected to saturate the blood with oxygen more than normobaric conditions. However, later studies indicated that the radiosensitizations produced by normabaric oxygen or carbogen were quite substantial;44,45,46 because it is quicker and easier to breathe gas under normobaric conditions, the use of cumbersome, expensive, and complex hyperbaric chambers is probably not necessary.Clinically, the use of high-oxygen-content gas breathing, specifically under hyperbaric conditions, was introduced relatively early by Churchill-Davidson et al.47 Most trials were fairly small, and suffered from the applications of unconventional fractionation schemes, but it appeared that the effect of hyperbaric oxygen was superior to radiotherapy given in air, especially when few and large fractions were applied.47-49 In the large, multicenter clinical trials conducted by the British Medical Research Council (Table 3-1), the results from both uterine cervix and advanced head and neck tumors showed a significant benefit in local tumor control and subsequent survival.48,50-53 The same findings were not observed in bladder cancer nor were they seen in a number of smaller studies.53 In retrospect, the use of hyperbaric oxygen was stopped somewhat prematurely. This was partly the result of the introduction of hypoxic radiosensitizers and partly because of problems with patients' compliance; it has been claimed that hyperbaric treatment caused significant suffering, but the discomfort associated with such a treatment must be considered minor compared to the often life-threatening complications associated with chemotherapy, which is used with less restrictive indications.**TABLE 3-1 Multicenter Randomized Trials with Hyperbaric Oxygen (HBO)***Endpoints were control (locoregional control) or survival. See Overgaard53 for additional information.*fx,* Fractions; *NS,* not significant.The use of high-oxygen-content gas breathing under normobaric conditions to radiosensitize human tumors has also been tried clinically, but it failed to show any dramatic improvement.54-56 In the most recent study this may have been the result of size limitation,56 but

in previous studies it may have been caused by the failure to achieve the optimum preirradiation gas breathing time.54,55 Experimental studies have shown that the amount of time is critical for the enhancement of radiation damage and that it can vary from tumor to tumor.43-45,57Hypoxic Cell RadiosensitizersAn alternative approach to the hypoxia problem is the use of chemical agents that mimic oxygen and preferentially sensitize the resistant population to radiation. The advantage of these drugs over oxygen is that they are not rapidly metabolized by the tumor cells through which they diffuse and thus the drugs can penetrate further than oxygen and so reach all the tumor cells. In the early 1960s, researchers found that the efficiency of radiosensitization was directly related to electron-affinity58 and that ultimately led to in vitro studies demonstrating preferential radiosensitization of hypoxic cells by highly electron-affinic nitroaromatic compounds.59,60 Several of these compounds were later shown to be effective at enhancing radiation damage in tumors in vivo,61 and as a result, they underwent clinical testing.The drugs reaching clinical evaluation include metronidazole, misonidazole, benznidazole, desmethylmisonidazole, etanidazole, pimonidazole, nimorazole, ornidazole, sanazole, and doranidazole. Initial clinical studies were with metronidazole in brain tumors and were followed, in the latter part of the 1970s, by a boom in clinical trials exploring the potential of misonidazole as a radiosensitizer.53,61,62 The results from the multicenter randomized trials are summarized in Table 3-2. Most of the trials with misonidazole were unable to generate any significant improvement in radiation response, although a benefit was seen in some trials, especially the second Danish Head and Neck Cancer study (DAHANCA 2), which found a highly significant improvement in the stratification subgroup of pharynx tumors but not in the prognostically better glottic carcinomas.63 The overall impression of the “misonidazole-era” was a prolongation of the inconclusive experience from the hyperbaric oxygen trials, namely, that the problems related to hypoxia had not been ruled out indefinately.61 Therefore, the search for more efficient or less toxic hypoxic sensitizers continues. Furthermore, the experience from the misonidazole trials has been taken into account to select a more homogeneous tumor population in which hypoxia is more likely to be present.**TABLE 3-2 Multicenter Randomized Trials with Nitroimidazoles***Endpoints were control (loco-regional control) and survival (median survival in months). See Overgaard61 for additional information. *DAHANCA,* Danish Head and Neck Cancer study; *EORTC,* European Organization for Research and Treatment of Cancer; *ETA,* etanidazole; *MISO,* misonidazole; *MRC,* Medical Research Council; *NIM,* nimorazole; *NS,* not significant; *PIM,* pimonidazole; *RT,* radiation therapy; *RTOG,* Radiation Therapy Oncology Group.Results from subsequent randomized trials with other nitroaromatic compounds have been conflicting. The European pimonidazole trial in uterine cervix was disappointing,64 whereas the two other multicenter trials in head and neck cancer, using etanidazole, showed no benefit.61,65 On the other hand, studies with the low toxic drug nimorazole given to patients with supraglottic and pharynx carcinomas (DAHANCA 5) showed a highly significant benefit in terms of improved locoregional tumor control and disease-free survival rates (Figure 3-4),66 thereby confirming the result of the DAHANCA 2 study. More recent trials with the 3-nitrotriazole compound, sanazole (AK-2123), in uterine cervical cancer67 and doranidazole in pancreatic cancer 68 demonstrated significant improvements in both local tumor control and overall survival.

Figure 3-4 Actuarial estimated loco-regional tumor control and disease-specific survival rate in patients randomized to receive nimorazole or placebo in conjunction with conventional radiotherapy for carcinoma of the pharynx and supraglottic larynx. Reprinted from Overgaard J, Sand Hansen H, Overgaard M, et al: A randomised double-blind phase III study of nimorazole as a hypoxic radiosensitizer of primary radiotherapy in supraglottic larynx and pharynx carcinoma. Results of the Danish head and neck cancer study [DAHANCA] protocol 5-85. Radiother Oncol 46:135–146, 1998, with permission form Elsevier Science.The potential benefit of using hypoxic radiosensitizers to improve radiotherapy is probably best illustrated from a recent meta-analysis of randomized clinical studies in squamous cell carcinoma of the head and neck.69 These results are summarized in Figure 3-5 and clearly showed that radiosensitizer modification of tumor hypoxia significantly improved locoregional tumor control and overall survival with odds ratios of 0.71 and 0.87, respectively. Although the overall observed gain were small (5% to 10% for local control and 0% to 6% for survival) they are actually relevant. We can

conclude that the nonsignificant outcome of most clinical trials (see Table 3-2) is not the result of a biological lack of importance of hypoxia, but in most cases is considered a consequence of poor clinical trials methodology with an overly optimistic study design and an expected treatment gain that goes far beyond what is reasonable. Overall, the results with nitroimidazoles add to the general consensus that if a nontoxic hypoxic modification can be applied, then such treatments may certainly be relevant as a baseline therapy together with radiotherapy for cancers such as advanced head and neck cancer. Such a strategy has been adopted in Denmark where nimorazole has become part of the standard radiotherapy treatment in cancer of the head and neck.
Figure 3-5 Meta-analysis of hypoxic modification of radiotherapy in squamous cell carcinomas of the head and neck. Results show summary data from 32 randomized trials (including 4805 patients). Patients received radiation alone or radiation with a hypoxic modifier that included high-oxygen-content gas breathing under normobaric or hyperbaric conditions, or a hypoxic radiosensitizer. Adapted from Overgaard J: Hypoxic modification of radiotherapy in squamous cell carcinoma of the head and neck—a systemic review and meta-analysis. Radiother Oncol 100:22–32, 2011.Dose Modification Based on HemoglobinOne of the major factors influencing the delivery of oxygen to tumors is the concentration of hemoglobin. It is, therefore, not surprising that low hemoglobin concentration in general has a negative impact on tumor radiation response. In a review of 51 studies involving 17,272 patients the prognostic relationship between hemoglobin concentration and local tumor control were analyzed, and of these, 39 studies (14,482 patients) showed a correlation, whereas only 12 (2790 patients) did not.[70] However, the relationship between hemoglobin concentration and tumor oxygenation status is not clear because a large (397 patients) international multicenter study in head and neck cancer failed to show a correlation between these parameters.[40]Although a well-documented causal relationship between hemoglobin concentration, tumor oxygenation, and response to radiotherapy has not been shown, it is likely that such a relationship does exist and there is thus a rationale for investigating the possibility of improving the outcome of radiotherapy in relevant tumor sites in patients with low hemoglobin concentration given curative radiotherapy. This was investigated in two randomized trials using transfusion to raise the hemoglobin levels.[66,71] Despite an initial positive report from the Canadian trial in uterine cervix carcinoma, both studies concluded that the use of such transfusions did not significantly improve treatment outcome. In the DAHANCA 5 study, transfusion was given several days before radiotherapy and adaptation may have occurred. Based on preclinical data it was hypothesized that any increase in tumor hypoxic fraction induced by anemia will be only transient, with tumors adapting to the lowered oxygen delivery[72]; transfusing anemic animals decreased tumor hypoxia, but this effect also was only transient and the tumors were able to adapt to the increased oxygen level. This suggests that when correcting for anemia it may not necessarily be the final hemoglobin concentration by itself that is important. Rather, an increasing hemoglobin concentration occurring at the time when the tumors are regressing during radiotherapy may be more likely to result in an increased oxygen supply to tumors and a subsequent improvement in response to radiotherapy.Increasing the hemoglobin concentration by stimulation with erythropoietin (EPO), a hormone normally secreted from the kidney in response to tissue hypoxia and low serum levels, has also been investigated. Several preclinical studies have shown that the induction of anemia in animals could be corrected by serial injection with EPO and that this EPO treatment also overcame the anemia-induced radiation resistance.[73,74] The concept of using EPO to correct for anemia has also been tested in several clinical trials. However, although low hemoglobin can be effectively and safely improved by EPO,[75,76] patients treated with radiation and EPO had a poorer outcome than the control arms not treated with EPO.[77] Although this clearly raises concerns about the use of such agents to improve radiation therapy through a manipulation of hemoglobin levels, it does not make the concept of having a high hemoglobin concentration during radiation therapy an irrelevant issue.Other “hemoglobin-related” methods for improving tumor oxygenation have been investigated.[78] These include the use of artificial blood substances, such as perfluorocarbons,[79] which are small particles capable of carrying more oxygen than hemoglobin, or by manipulating the oxygen unloading capacity of blood by modifying the oxy-hemoglobin

dissociation curve. This can be achieved either by increasing the red blood cell 2,3-DPG content,[80] 2,3-DPG being one of the most important allosteric factors controlling the hemoglobin-oxygen dissociation curve, or using antilipidemic drugs.[81] Although each of these approaches has been shown to improve the oxygenation status of experimental tumors or enhance radiation damage, none of them have yet reached controlled clinical testing; thus, their potential usefulness in the clinic is uncertain.Changing Oxygen ConsumptionA novel approach that is currently receiving attention focuses on reducing tumor hypoxia by decreasing the oxygen consumption of cells close to blood vessels and thereby increasing the oxygen diffusion distance, which makes more oxygen available to the hypoxic cells. This may be achieved using the drug metformin, which has undergone extensive clinical evaluation for the treatment of diabetes and has been linked to decreased rates of certain types of cancer.[82] One preclinical study has clearly shown that high doses of metformin can decrease cellular oxygen consumption in vitro.[83] Additional in vivo data from that study demonstrated that metformin could improve tumor radiation response, but whether or not this was the direct result of reduced oxygen consumption is not entirely known. Further studies in this area are clearly warranted.Dealing with the Problem of Fluctuating (Acute) HypoxiaAlthough several of the procedures used to combat radiation resistance caused by hypoxic cells have met with some success, the results are far from satisfactory. A possible explanation is that most of the procedures used clinically seem to operate primarily against diffusion-limited chronic hypoxia and have little or no influence on fluctuating hypoxia, which is caused by transient variations in tumor blood flow.[9]Experimental studies have clearly demonstrated that the vitamin B3 analog, nicotinamide, can enhance radiation damage in a variety of murine tumor models using both single and fractionated treatments (Figure 3-6).[9] The enhancement of radiation damage appears to depend on the tumor type, drug dose, and time of irradiation after drug administration.[9] The drug can also enhance radiation damage in certain normal tissues, but generally the effects are less pronounced than those seen in tumors.[9] Nicotinamide seems to primarily prevent or reduce the transient fluctuations in tumor blood flow that normally lead to the development of acute hypoxia.[9] This finding led to the suggestion that the optimal approach would be to combine nicotinamide with treatments that specifically overcome chronic hypoxia. This was subsequently demonstrated with hyperthermia,[84] perfluorochemical emulsions,[85] and carbogen breathing.[57,86-88] Combining nicotinamide with carbogen has undergone testing in a number of European clinical studies, and the results in head and neck[89] and bladder cancer[90] demonstrated an improved response to radiation therapy.

Figure 3-6 Effect of nicotinamide (500-1000 mg/kg) on local tumor control measured as a function of the total radiation dose given either as a single treatment to C3H mammary carcinomas or in fractionated schedule to the carcinoma NT. The drug was i.p. injected 30-60 minutes before irradiation. Modified from Horsman MR, Chaplin DJ, Overgaard J: Combination of nicotinamide and hyperthermia to eliminate radioresistant chronically and acutely hypoxic tumor cells. Cancer Res 50:7430–7436, 1990, and Kjellen E, Joiner MC, Collier JM, et al: A therapeutic benefit from combining normobaric carbogen or oxygen with nicotinamide in fractionated x-ray treatments. Radiother Oncol 22:81–91, 1991. Copyright 1991, with permission from Elsevier Science.Bioreductive DrugsThe early preclinical studies with electron-affinic radiosensitizers showed that these agents, which were relatively nontoxic to cells under normal oxygenated conditions, were reduced to a more toxic form under hypoxia.[91] This led to the development of various bioreductive drugs that preferentially killed the radiation-resistant hypoxic tumor cell population. Basically, these drugs can be divided into three major groups, as illustrated in Figure 3-7. They are the quinones, nitroaromatics, and N-oxides.[92]

Figure 3-7 Survival of mammalian cells exposed to mitomycin C, RSU 1069, or tirapazamine under aerobic *(red symbols)* of hypoxic *(blue symbols)* conditions. Adapted from Stratford IJ, Stephens MA: The differential hypoxic cytotoxicity of bioreductive agents determined in vitro by the MTT assay. Int J Radiat Oncol Biol Phys 16:973–976, 1989, and Hall EJ: Radiobiology for the radiobiologist. ed 4. Philadelphia, 1994, JB Lippincott.The quinine derivative, Mitomycin-C (MMC), is probably the prototype bioreductive drug.[93] It has been used clinically for many years as a chemo-radiosensitizer, long before it was realized it had preferential effects against hypoxic

cells. It is activated by bioreduction to form products that crosslink DNA and therefore produce cell killing. Several randomized clinical trials in patients with squamous cell carcinoma of the head and neck were undertaken, specifically using MMC to counteract the effects of hypoxia, but not all showed a benefit.94-98 This may not be surprising when one considers that MMC actually has a small differential killing effect between aerobic and hypoxic cells (see Figure 3-7) and was only administered once or twice during the entire course of radiotherapy. Attempts to find more efficient quinones have been undertaken and to that end porfiromycin, RH1, and EO9 were developed.92 Of these EO9 is currently being evaluated in a phase II trial in bladder cancer.The finding that misonidazole was preferentially toxic toward hypoxic cells led to numerous efforts to find other nitroimidazoles that were better. The first drug developed was RSU-1069 (see Figure 3-7). This compound has the classic 2-nitroimidazole radiosensitizing properties, but also an aziridine ring at the terminal end of the chain, which gave the molecule substantial potency as a hypoxic cell cytotoxin, both in vitro and in vivo.99 In large-animal studies it was found to cause gastrointestinal toxicity and a less toxic prodrug was therefore developed (RB-6145), which is reduced in vivo to RSU-1069. Although this drug was found to have potent antitumor activity in experimental systems, further animal studies revealed that this drug induced blindness; this is perhaps not surprising when one realizes that the retina is hypoxic, thus further development of this drug was halted. However, other nitro-containing compounds have been developed, including NLCQ-1, CB 1954, SN 23862, PR-104, and TH-302,92 of which the latter is currently under Phase II/III clinical evaluation albeit in combination with chemotherapy.Perhaps the most promising group of bioreductives is the organic nitroxides, of which the benzotriazene di-N-oxide, tirapazamine, is the lead compound (see Figure 3-7). The parent moiety shows limited toxicity toward aerobic cells, but after reduction under hypoxic conditions, a product is formed that has been shown to be highly toxic and can substantially enhance radiation damage to tumors in vivo.100 Most clinical studies have involved combining tirapazamine with chemotherapy, although there have been a few trials with radiation with or without chemotherapy.92 The results from the phase II trials generally showed promise, but in the few randomized trials that have been completed the results were somewhat disappointing. However, it has now been suggested that the benefit of tirapazamine might be achieved if one could select patients who had hypoxic tumors before treatment. Other N-oxides currently under development include chlorambucil N-oxide, SN30000, and AQ4N (Banoxantrone), the latter being combined with radiation in a number of clinical trials.92Vascular Targeting Agents**Angiogenesis Inhibitors**The tumor's vascular supply plays a critical role in determining the tumor's microenvironmental factors that influence radiotherapy.101 This vasculature develops from the normal tissue vessels via the process of angiogenesis,102 which is a highly complex process triggered by the release of specific growth factors from the tumor cells.103 These growth factors initiate a series of physical steps, including local degradation of the basement membrane surrounding capillaries, invasion of surrounding stroma by the endothelial cells in the direction of the angiogenic stimulus, proliferation of the endothelial cells, and, finally organization of the endothelial cells into three-dimensional structures that connect with other similar structures to form the new blood vessel network.103 The importance of this process makes it an attractive target for therapy and numerous approaches for inhibiting the various steps in the angiogenic process have been tested in preclinical models.104,105 Many of these therapies have now moved into clinical evaluation106 and, of these, the antivascular endothelial growth factor (anti-VEGF) antibody bevacizumab (Avastin) has been shown to improve outcome in a number of chemotherapy-based trials.107 Preclinical studies using rodent and human tumor xenografts show that certain angiogenesis inhibitors can be effectively combined with radiation to improve tumor response (Table 3-3),105 and as a result, a limited number of clinical studies have been initiated combining certain angiogenesis inhibitors with radiation therapy.106**TABLE 3-3List of Vascular Targeting Agents That Have Been Combined with Radiation**See Horsman and Siemann105 for additional information.The consensus opinion is that the improvement in radiation response found in preclinical studies is the consequence of normalization of the tumor vasculature, resulting in a decrease in tumor hypoxia.108 Although there are certainly preclinical studies showing an improved tumor oxygenation status with such treatment, there are just as many studies showing

no change and even a decrease in tumor oxygenation.105 These findings not only make it unclear as to the role of vessel normalization in influencing the combination of angiogenesis inhibitors with radiation, but they also indicate that timing and sequencing of the two modalities may be critical for an optimal benefit.Vascular Disrupting AgentsAn alternative approach for targeting tumor vasculature involves using agents that can damage the already established tumor vessels.104,105 This is not a new concept; it was first demonstrated with the tubulin binding agent colchicine back in the 1940s.109 Since then a number of vascular disrupting agents (VDAs) have been proved capable of preferentially damaging tumor vessels, leading to a reduction in tumor perfusion which results in an increase in tumor ischemia and necrosis, subsequently producing an inhibitory effect on tumor growth.105 The VDAs include physical treatments (e.g., hyperthermia, photodynamic therapy, and even radiation), chemotherapeutic agents (e.g., tumor necrosis factor, vinca alkaloids, and arsenic trioxides), and small molecule agents (e.g., flavonoid derivatives such as 5,6-dimethylxanthenone-4-acetic acid; tubulin binding drugs such as Combretastatin A-4 phosphate).As with the angiogenesis inhibitors, several of the VDAs have been combined with radiation (see Table 3-3), and significant improvements in response have been seen in preclinical models.105 This is illustrated in Figure 3-8 using a C3H mammary carcinoma grown in CDF1 mice. The radiation dose needed to control 50% of treated animals (±95% confidence intervals) following single dose radiation treatment alone was found to be 53 Gy (51-56). This was significantly reduced (Chi-squared test; $p < 0.05$) to 46 Gy (42-49) when tumors were locally irradiated, but 30 minutes later mice were given a single intraperitoneal injection of a large but nontoxic dose of Combretastatin A-4 phosphate (CA4P), the lead VDA in clinical evaluation.105,107 On its own, in this tumor model, such a drug dose will only slow the growth of the tumors by about 2 days. The enhancement of tumor radiation damage is known to be time and schedule dependent, with the greatest effect seen when the drug is given within a few hours after irradiating.105 It is also tumor specific with no enhancement of radiation response seen in normal tissues. This has been shown for CA4P in acutely responding normal skin (see Figure 3-8) or late-responding bladder and lung.105 These differences between the tumor and normal tissue results are entirely consistent with the drugs' ability to induce damage in tumor vessels but not vessels in normal tissue.105

Figure 3-8 Effect of Combretastatin A-4 disodium phosphate (250 mg/kg) on either local control of a C3H mammary carcinoma **(A)** or the development of moist desquamation in the foot skin of CDF1 mice **(B)** following radiation treatment. Radiation was either given alone *(red symbols)* or 30 minutes before intraperitoneal injection of the drug *(blue symbols)*. Adapted from Murata R, Siemann DW, Overgaard J, et al: Interaction between Combretastatin A-4 disodium phosphate and radiation in murine tumors. Radiother Oncol 60:155–161, 2001.

Radiation Protectors**Sulfhydryl-Containing Compounds**More than 50 years ago it was realized that certain amino acids, glutathione, and ascorbic acid were able to modulate radiation induced inactivation of biological material. Based on those observations Patt et al investigated the effect of treating mice with the thiol-containing amino acid, cysteine.110 They found that administering this compound to mice before whole-body irradiation resulted in a remarkable increase in animal survival (Figure 3-9). In contrast no effect was observed when cysteine was given after irradiation. During the Cold War, this finding led to a large research program at the Walter Reed Army Institute of Research aimed at developing a drug that could protect soldiers from nuclear weapons.111 Numerous sulfhydryl-containing substances with substantial radioprotective properties were detected, but only WR-2771 (amifostine) was found to exhibit acceptable toxicity. The idea of using amifostine in oncology arose when preclinical studies, performed mainly in the 1970s and 1980s, suggested a selective protection of normal tissue from damage induced not only by irradiation but also from chemotherapy.112,113 Despite these findings, the interest in amifostine over the years has waxed and waned, although this interest was boosted by commercialization of the drug, US Federal Drug Administration (FDA) approval, and the establishment of authoritative guidelines.114 The lack of interest was probably related to the fact that there is no fail-safe modification of traditional radiotherapy.115 Many normal tissues are dose limiting; therefore, evaluating the therapeutic benefit of a radioprotector requires either that the

protector have absolute normal tissue selectivity (and thus fewer complications with an unchanged rate of tumor control for a given radiation dose) or, if selectivity is uncertain, that an increase in radiation dose may be needed to maintain the same rate of tumor control. However, this scenario requires that the protective effect on tumor tissues is predictable and exceeded by the protection offered to all relevant normal tissues. In addition, the “perfect” radioprotector must have an acceptable toxicity profile and must be easy to handle if generalized clinical use is to be expected with routine fractionated radiotherapy.116-118

Figure 3-9 Percentage of rodents surviving after whole-body irradiation with 8 Gy. Animals were either control irradiated (blue symbols) or given radiation after injection with 575 mg of cysteine *(red symbols)*. Adapted from Patt HM, Tyree EB, Straub RL, et al: Cysteine protection against X irradiation. Science 110:213–214, 1949. Copyright 1949 American Association for the Advancement of Science.It is not entirely clear how amifostine induces radioprotection. The drug must first undergo dephosphorylation to its active metabolite WR-1065, which is further metabolized to the disulfide WR-33278; the latter metabolite may also afford some protection, although to a lesser extent.119 Several mechanisms are involved in radioprotection, depending on the quality of the radiation. Protection against sparsely ionizing radiation, such as x-rays, is mainly obtained by scavenging of free radicals120,121; such scavenging has also been observed with glutathione, superoxide dismutase and its mimetics, and isoflavones like genistein. Because WR-1065 and WR-33278 react with free radicals in competition with oxygen, the protection obtained by scavenging is highly influenced by oxygen tension (Figure 3-10). Here the protection is maximal at intermediate levels of oxygen (20% to 50% oxygen in the inspired air). At higher oxygen tensions, WR-1065 is counterbalanced by excess oxygen and the protection is gradually lost. The degree of protection is also diminished at low-oxygen tensions where scavenging of free radicals is no longer important because the lack of oxygen by itself provides radioprotection. Additional and complex mechanisms are undoubtedly involved. Some of these may involve hypoxia created locally by direct interaction of thiols with oxygen, chemical repair by thiol-donation of hydrogen, or decreased accessibility of radiolytic attack sites by induction of DNA packaging.116

Figure 3-10 The variation in normal skin protection in mice given 400 mg/kg WR-2721 30 to 45 minutes before irradiation in mice that breathed various oxygen concentrations. Modified from Denekamp J, Michael BD, Rojas A, et al: Radioprotection of mouse skin by WR-2721: the critical influence of oxygen tension. Int J Radiat Oncol Biol Phys 8:531–534, 1982, with permission from Elsevier Science.Preclinical studies have shown that many tissues can be protected from radiation damage by amifostine (Table 3-4). However, the protection observed in different normal tissues is unfortunately heterogeneous. Some normal tissues—such as central nervous system, which often is dose limiting in radiotherapy—are not protected because amifostine probably does not cross the blood-brain barrier.122 In other normal tissues such as salivary glands and hematopoietic system, amifostine affords significant radioprotection. These variations are probably explained by tissue variations in oxygen concentration, dephosphorylation activity, and distribution of amifostine and its metabolites.119,121,123 To make things even more complicated, tumor protection has been impossible to rule out by preclinical experiments.115 In addition, large single doses of irradiation have often been used, and relevant comparison with tumor effects have been absent or difficult to translate into a clinical meaningful context.116

TABLE 3-4Protection Factors Achieved by Amifostine in Different Normal Tissues and Tumors

Tissue	Protection Factor
Salivary gland	2.3-3.3
Bone marrow	1.8-3.0
Jejunum	1.5-2.1
Skin	1.4-2.1
Testis	1.5-1.6
Kidney	1.3-1.5
Bladder	1.3-1.5

Lung1.2-1.4
Heart>1.0
Tumor1.0-2.8Data from references 115 and 121,123-127.On the clinical side there have been far too many publications of phase I-II studies with limited number of patients and a few underpowered randomized studies. In addition, chemotherapy has often been applied together with radiotherapy, making it difficult to evaluate the results (Table 3-5). Despite the long list of preclinical normal tissues studies with a proven effect of amifostine, disappointingly few have been confirmed in the clinical setting. Amelioration of acute radiation toxicity has been observed in studies which often employed various types of treatment intensification like concomitant chemotherapy or accelerated radiotherapy. However, definite confirmation regarding late morbidity such as fibrosis has not been obtained. A pivotal trial by Brizel et al128 showed that amifostine significantly protected against radiation-induced xerostomia with no apparent loss of tumor control in head and neck cancers. That was followed by amifostine being approved by the US Federal Drug Administration for use in patients undergoing postoperative fractionated radiotherapy in the head and neck region to decrease the incidence of acute and chronic xerostomia. In light of the paucity of relevant clinical data this recommendation seems premature. A later large meta-analysis of published data concluded that amifostine could significantly reduce acute and late side effects associated with radiation therapy.129 However, that review has since been criticized for publication and classification bias and the quality of the trials included.130 A more comprehensive meta-analysis found that amifostine did not change radiation-induced overall or progression free survival,130 thus further studies are needed to explore the potential benefits of amifostine.**TABLE 3-5Randomized Clinical Trials Investigating the Effect of Amifostine on Outcome in Radiotherapy (RT) Administered Alone or with Chemotherapy (CT)**Modifiers of the Oxygen SupplyBecause hypoxia reduces radiation sensitivity, decreasing the availability of oxygen to tissues may be way to achieve radiation protection. One way to accomplish this might be to modify hemoglobin-oxygen affinity. The most widely studied agents in this context are the substituted benzaldehyde, BW12C, and derivatives.149,150 These agents preferentially bind to oxy-hemoglobin and so increase the affinity of the hemoglobin for oxygen.151,152 Consequently this decreases the amount of oxygen available to the tissues. Although this radioprotects some normal tissues,149,153 there is evidence that in certain normal tissues BW12C can actually increase perfusion,154 which should increase oxygen delivery. Additionally, experimental studies have also shown that BW12C will significantly radioprotect tumors.149,150 Carbon monoxide reduces oxygen transport to tissues by binding to hemoglobin, thereby decreasing the amount available for oxygen transport, as well as causing a left shift of the hemoglobin-oxygen dissociation curve. Therefore, any oxygen that binds to the hemoglobin does so more strongly.155 This approach to increase hypoxia and reduce radiation response has been well documented in experimental systems.156,157 Unfortunately, this effect was demonstrated in tumors and not normal tissues. The effect of carbon monoxide in patients has also been observed, as shown in Figure 3-11, which illustrates that patients with head and neck carcinomas who were smokers (and therefore had higher carboxyhemoglobin levels) had a poorer response to radiation therapy than nonsmokers.
Figure 3-11 Influence of smoking (smk) during treatment on the outcome of radiotherapy in 232 patients with advanced head and neck carcinoma. Results show 10-year survival when comparing nonsmokers and quitters to moderate or heavy smokers (>20 cigarettes or one pack per day). Redrawn from Hoff CM: Importance of hemoglobin concentration and its modification for the outcome of head and neck cancer patients treated with radiotherapy. Acta Oncol 51:419–432, 2012.Because most modifiers of the oxygen supply already discussed also provide radioprotection for tumors as well as normal tissues, their use in this context must be considered limited. However, this may not be true for pentoxifylline. This is a drug that alters red blood cell deformability and inhibits platelet aggregation and fibrinolytic activity.158 As a result, red blood cells are better able to transverse narrowed arterioles and capillaries. When given before irradiation, pentoxifylline enhances radiation damage in tumors,159 presumably because of an improvement in oxygen delivery,160,161 but it has no effect on the response of normal

tissues.159 However, when administered on a daily basis after irradiation, pentoxifylline had no effect on early skin reactions in mice but did significantly reduce the incidence of late reactions.162 Other RadioprotectorsVarious other agents have been reported to be capable of radioprotecting certain normal tissues.163-165 These include cytokines, such as granulocyte-macrophage colony-stimulating factor, interleukin-1, tumor necrosis factor-α (TNF-α), transforming growth factor-β (TGF-β), and basic fibroblast growth factor.166-170 Another interesting group of agents inhibit the process of apoptosis. Many tumors have acquired mutations that prevent them from undergoing radiation-induced apoptosis. Proliferating normal cells, however, are often killed by radiation-induced apoptosis, and several apoptosis inhibitors have shown radioprotective potentials in animal models. These include p53 inhibitors (pifithrin-α and pifithrin-µ), growth factors [KGF-1 (palifermin), KGF-2 (repifermin), FGF-20 (velafermin)], activators of NRF2 (triterpenoids), and inhibitors of NF-κB (CBLB502).163-165 Another recent approach, designed to take advantage of a typically acquired defect in tumor cells, is to induce a G1 cell cycle arrest in normal cells, thereby temporarily arresting them in the relatively radioresistant G1 phase allowing for improved DNA repair (e.g., PD0332991 and 2BrIC).163-165 Agents are also being developed that target molecular pathways involved in radiation-induced effects, including the TGF-β1/Smad, CTGF/RHO/ROCK, TNF-α, and PDGF/PDGFR pathways.163-165 Finally, there is a group of miscellaneous inhibitors that include the angiotensin-converting enzyme inhibitor captopril, corticosteroids, prostaglandins, and essential fatty acids.171-175 However, the mechanisms of action are not entirely clear, nor is there evidence that these agents do not also radioprotect tumors.SummaryThe use of radiation to treat certain types of cancer with curative intent is a well-established and effective therapy, but there is still room for improvement. The additional use of treatments that can either increase radiation damage in tumors without affecting normal tissues, or protect normal tissues without having a similar protective effect in tumors, is clearly warranted. Because the vasculature and microenvironment of tumors differs from those of normal tissues, targeting these parameters should lead to tumor specificity. Many preclinical studies have demonstrated this to be a viable approach. But despite numerous clinical studies confirming the potential of such methods to significantly improve outcome to radiation therapy only one agent has become established in standard radiation therapy protocols. That agent is the radiosensitizer nimorazole, which is only used in Denmark and only in head and neck cancers. The use of radioprotectors is more controversial. Experimentally, several agents have been shown to radioprotect certain normal tissues, but data also demonstrate that some of these agents induce radiation protection in tumors. Results from clinical studies investigating the potential of radioprotectors have also been inconclusive, and until good human data demonstrating radioprotection of normal tissues, but not tumors, become available the use of radioprotectors must be considered experimental.References

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175. Hopewell JW, Robbins MEC, van den Aardweg GJMJ. The modulation of radiation-induced damage to pig skin by essential fatty acids. *Br J Cancer*. 1993;68:1–7. **Chapter 4**Interaction of Chemotherapy and Radiation*Christopher Douglas Willey, Eddy Shih-Hsin Yang, James A. Bonner*
Oncology has increasingly become a multidisciplinary field of medicine: (1) Surgery remains the definitive local treatment modality; (2) Chemotherapy remains the definitive systemic treatment modality; and (3) Radiation therapy is the definitive loco-regional treatment modality. Although, historically, these approaches have predominantly been used exclusively of one another, the past 25 years have seen an explosion of both preclinical and clinical efforts that have sought to combine these therapies for improved outcomes, including improved local and regional control, overall survival, cosmesis, and organ preservation. We have learned a great deal about the interactions between chemotherapy and radiation from clinical trials that have combined these treatment modalities in sequential and concomitant regimens. In addition, laboratory investigations have demonstrated key molecular targets and pathways that can potentially be exploited for improved outcome. Indeed, the combination of chemotherapy and radiation has changed the management approach in several disease sites, which are broadly reviewed here.Historical PerspectiveRadiosensitization and chemosensitization are complex concepts that have many different interpretations and have been used to describe many different interactions.[1,2] The use of radiation and chemotherapy for mutual or even simultaneous sensitization adds to the intricacies of these interactions. Indeed, more than 100 years ago, radiation treatment and benzene systemic

therapy were combined for leukemia treatment.3 However, probably the best historical model of chemotherapy and radiation therapy interaction is that of 5-fluorouracil.5-FluorouracilIn the 1950s, the halogenated pyrimidine, 5-fluorouracil (5-FU), was combined with external beam irradiation (EBRT) after this class of drug was determined to have anticancer properties.4 In the last 50 years, 5-FU has been successfully combined with radiation to treat a variety of gastrointestinal cancers, as well as cervical cancer and head and neck cancers.5 The route of administration and scheduling of 5-FU has been manipulated many times in an attempt to reduce toxicity and maximize tumor control. What began as bolus delivery at the beginning and end of a fractionated radiation treatment course (“Moertel” regimen) has progressed to protracted venous infusion (PVI) and now to twice daily oral 5-FU analog formulations. These approaches have allowed for an increase in cumulative dose of the drug, decreased chemotherapy toxicity, and improved radiosensitization. Indeed, 5-FU has proven to be a staple drug in the armamentarium of medical oncologists and a key radiosensitizer for the radiation oncologist.
Rationale**Limitations in Current Therapeutic Approach**Over the past several decades, we have seen the great technological advances in surgery and radiation while novel systemic agents are being developed at a never-before-seen pace. Nevertheless, cancer morbidity and mortality remain major problems. The advent of combined modality therapy has sought to improve on the limitations that surgery, chemotherapy, and radiation carry independently. For several decades, radiation has complemented surgery by improving loco-regional control. Unfortunately, tumor-specific and patient-specific factors limit both surgical and radiation success. In this chapter, we will focus on the multiple ways that systemic therapies are used in an attempt to overcome the shortcomings of radiation treatment. The presence of micrometastatic disease, disease outside of our treatment fields, and the inability to deliver adequate dose to the target region as a result of normal tissue toxicity risk are some of the most frequently cited reasons. In addition, tumors may contain regions of relative hypoxia or subpopulations of cells with intrinsic or acquired resistance to radiation damage. We will briefly review the current understanding of these topics.**Tumor Detection**If ionizing radiation were without normal tissue toxicity, tumor detection would be immaterial, and radiotherapy could be delivered to the entire body much like chemotherapy. Obviously this is not the case, and much like surgeons, we must be able to identify the tumor so that we can precisely and accurately target it with our radiation akin to “carving out” a tumor with a scalpel. Fortunately, advances in radiology have dramatically improved our ability to detect tumor location and extent. Whereas computed tomography (CT) and magnetic resonance imaging (MRI) provide excellent anatomic information, when combined with biological or functional imaging such as positron emission tomography (PET) and single photon emission computed tomography (SPECT), the radiation oncologist can more confidently define tumor vs. nontumor. Emerging MRI sequences including dynamic contrast enhanced and fast imaging employing steady-state acquisition (FIESTA) ultrafast pulse sequence approaches, and MR spectroscopy are providing improved anatomic (and biologic in the case of MR spectroscopy) imaging for surgeons and radiation oncologists. Nevertheless, the resolution of our current techniques (on the order of 5 mm for PET resolution6) and high false-negative rates with small tumors still limit our ability to identify microscopic tumor extent and micrometastatic disease. Future technologies such as PET/MRI as well as novel radiopharmaceuticals may provide improvements.**Inherent and Acquired Resistance**We know, empirically, that certain tumors have inherent radiation resistance pathways that manifest with high rates of local failure following radiation. In some cases, such as in pancreatic cancer, it is difficult to deliver adequate doses of radiation to the target because of the limitations in dose tolerance of surrounding bowel, kidney, and liver. However, there are other tumors with extremely high local failures despite dose escalation. A prime example is glioblastoma multiforme, which has local recurrence rates approaching 100%. Biological factors within the tumor or tumor microenvironment also generate resistance. Figure 4-1 summarizes some of the major resistance pathways within tumors. Later in this chapter we will describe how chemotherapeutics can potentially mitigate these resistance pathways.
Figure 4-1 Schematic of radiation-induced signal transduction cascades indicating

pathways leading to antiapoptosis, proliferation, DNA repair, and angiogenesis.**Increased Toxicity**In the original treatise by Steel and Peckham on combining chemotherapy and radiation,[7] it was assumed that each modality functioned independently in both beneficence and toxicity. However, it is abundantly clear that concurrent chemoradiation has increased toxicity suggesting some level of overlapping toxicity, chemosensitization by radiation, or radiosensitization by the chemotherapy. Because chemoradiation is often used when tumors have wide anatomic extension (thus, precluding surgery), the volume of normal tissue irradiated, and therefore, at risk of toxicity, is larger. In some cases, a patient has comorbid conditions that prevent aggressive therapy as well.Therapeutic IndexThe features described previously generate the need for a metric to determine efficacy relative to toxicity so that newer approaches can be compared. This metric, known as the *therapeutic index* (or therapeutic ratio) refers to the ratio of the probability of tumor control to the probability of normal tissue toxicity. Typically, the ratio is calculated based on the 50% control rate of tumor versus the 50% normal tissue toxicity. These sigmoidal-shaped curves determine the estimated efficacy versus toxicity of treatment. Figure 4-2 depicts an idealized form of these curves. Therapeutic index has been, and will continue to be, the “holy grail” of cancer therapy. For this reason, it is no surprise that it takes careful treatment planning to try to achieve maximal tumor cell kill while also sparing normal tissue in hopes of preserving function. There are a host of technological factors that impact this therapeutic ratio. Certainly our ability to correctly identify tumor versus normal tissue will affect therapeutic ratio. The expanding use of PET, MRI, and SPECT imaging, as described previously, are allowing radiation oncologists to better differentiate target from nontarget. Obviously, the ability to precisely deliver radiation through techniques such as intensity modulated radiation therapy (IMRT), stereotactic procedures, and proton therapy allow us to avoid normal tissue while targeting tumor. Moreover, our ability to accurately deliver radiation via image guidance (IGRT) has grown by leaps and bounds, which enables smaller margin expansions and will also limit dose to normal tissue. Nevertheless, based on the anatomical location of the tumor, there are technological limits to what can be accomplished with radiation in and of itself. Therefore, additional improvement will likely rely on the interaction of systemic agents with our technologically advanced radiation delivery methods.
Figure 4-2 Graph of an idealized therapeutic index curve. The therapeutic index is indicated as the difference in probability of tumor response and probability of normal tissue toxicity. The greater the separation of these two curves, the greater the therapeutic index.**Strategies to Improve Therapeutic Index**The fundamental approach to improving outcomes through combined modality therapy has its basis on the theoretical strategies set forth by Steel and Peckham in 1979.[7] Their seminal paper defined four potential means by which combined therapy could improve the therapeutic index and were described as (1) independent toxicity, (2) normal tissue protection, (3) spatial cooperation, and (4) enhanced tumor response. As will be discussed, the first theoretical concept may not actually function as in the original intent. However, the latter three concepts are relevant for modern strategies of combining drugs with radiation. Additional mechanistic considerations have been identified in recent years that expand on Steel and Peckham's “exploitable mechanisms in combined radiotherapy-chemotherapy” that was described more than three decades ago.[7] These newer concepts of biological cooperation and temporal modulation are impacting current investigative strategies for improving the therapeutic index.**Independent Toxicity**One of the main concepts suggested by Steel and Peckham as a means to improve the therapeutic index is to select a chemotherapeutic regimen with a distinct toxicity profile from that of radiation. This ideal selection of nonoverlapping toxicities could allow for increased tumor cell kill with minimal impact in terms of tissue toxicity. Although this has been pursued in therapy selection, the actual success in finding independent toxicity has been elusive. However, the inverse relationship has been implemented to a great extent. Indeed, the avoidance of drugs with overlapping toxicities is standard of care practice, for instance, avoiding methotrexate with cranial radiation, Adriamycin with breast irradiation, or bleomycin with lung irradiation.**Normal Tissue Protection**The identification of clinically relevant agents that promote normal tissue protection without protecting tumors has provided little in terms of therapeutics. Limited success has been achieved with the free radical scavenging agent, amifostine (WR-2721),

which appears to be selectively taken up by normal tissue relative to tumor where it is converted into the active thiol metabolite, WR-1065.[8] Although amifostine has been shown to protect against xerostomia in head and neck cancers treatment (Table 4-1) and to limit renal toxicity from cisplatin, several clinical trials have failed to show an advantage to amifostine use. Undoubtedly, investigations into novel radioprotectors will continue with the potential to impact the therapeutic ratio.**TABLE 4-1Possible Drug–Radiation Interactions**

Mechanism	Example	Notes
Normal Tissue Protection	Amifostine in head and neck cancers	Reduces xerostomia rates from RT alone
Spatial Cooperation	Early stage breast cancer with adjuvant chemotherapy	PCI in SCLC
	RT provides locoregional control for breast cancer but no impact on DM	SCLC chemotherapy does not effectively cross BBB → RT can effectively treat the brain
Biological Cooperation	Targeted therapies inhibit prosurvival/proliferation pathways within tumors	Kinase targeted agents including tyrosine kinase inhibitors such as dasatinib and sunitinib as well as monoclonal antibodies such as cetuximab and bevacizumab; mTOR inhibitors
Temporal Modulation	Drugs that impact tumor response in between fractions, namely targeting repair, repopulation, reoxygenation, and redistribution.	This is essentially a composite of several of the other mechanisms but requires concomitant delivery of the drug rather than sequential.
Increased DNA Damage	Drugs that incorporate into DNA	5-FU and platinum are classic examples
Inhibition of DNA Repair	DNA intercalators and nucleoside analogs can disrupt repair and enhance radiation cytotoxicity	Alkylators, antimetabolites, platinum, and topoisomerase inhibitors are a few examples
Cell Cycle Effects	Most chemotherapeutics are cell cycle specific (except alkylators)	Cell cycle arrest in radiosensitive phases (microtubule targeting agents at M-phase)
	Elimination of radioresistant cells (S-phase)	Taxanes, epothilones, 5-FU, gemcitabine, and topoisomerase inhibitors are good examples
Targeting Repopulation	Conceivably any systemic agent that has at least cytostatic properties can prevent repopulation	Molecularly targeted agents as well as chemotherapeutics (particularly antimetabolites) can function this way
Hypoxia Targeting	Mitomycin C and tirapazamine selectively targeting hypoxic cells	Tumor shrinkage by chemotherapy decreases interstitial pressure and improves oxygenation
	Taxanes and other chemotherapies that can produce tumor shrinkage are indirect means (given as induction therapy) whereas mitomycin C and tirapazamine are directly affecting hypoxic cells.	
Tumor Microenvironment Targeting	Antiangiogenesis promotes vascular renormalization	Bevacizumab in glioma

5-FU, 5-Fluorouracil; *BBB,* blood-brain barrier; *DM,* distant mets; *PCI,* prophylactic cranial irradiation; *RT,* radiation therapy; *SCLC,* small-cell lung carcinoma.

Spatial Cooperation The concept of spatial cooperation implies that chemotherapy and radiation therapy are independent players with systemic therapy acting systemically, that is, targeting micrometastatic disease, and radiation therapy acting loco-regionally. Because these therapies function independently, it could be assumed that a full dose of each will be required to achieve the desired outcome. If the drug and radiation did function completely independently, then concurrent administration should be possible with nonoverlapping toxicities. It is unclear whether a completely independent action can actually be achieved with the chemotherapies that are currently used with radiation treatment, however, because in-field toxicities do occur suggesting some level of localized radiation sensitization. Therefore, sequential therapy is probably the best means to exploit spatial cooperation. Many clinical examples exist for this approach, such as breast cancer with adjuvant chemotherapy followed by radiation, consolidative radiation to bulky disease in lymphoma, or prophylactic cranial irradiation in small cell lung cancer (see Table 4-1).**Enhanced Tumor Response (Cytotoxic Enhancement)**Currently, a tremendous amount of investigative effort is focused on achieving cytotoxic enhancement with combined modality treatment. In other words, the combination of therapies leads to an interaction on some level that generates improved antitumor effect relative to each treatment alone. Interestingly, we have some clinical examples that subtherapeutic, or radiosensitizing, doses of chemotherapy can impact distant disease control

suggesting either that increased loco-regional control can diminish distant metastatic disease potential or that lower dose chemotherapy can treat micrometastatic disease (i.e., spatial cooperation).**Biological Cooperation**The term, *biological cooperation*, is a newer concept[9] that involves independent targeting of subpopulations of cells within the tumor itself (see Table 4-1). Although similar to the spatial cooperation concept, biological cooperation implies that some portion of the actual radiation target (i.e., in-field) is resistant to radiation, which is the target of the drug given concomitantly. The most prominent example for biological cooperation is hypoxic cell cytotoxins such as tirapazamine. Because hypoxia is a known radiation resistance condition, tirapazamine will target these subpopulations of cells since it is most potent in anoxic conditions. Tirapazamine is discussed in more detail later in this chapter.**Temporal Modulation**The four R's of classical radiobiology, which include reoxygenation, repair, redistribution, and repopulation,[10] refer to factors that are particularly important for fractionated radiation therapy. For example, antiproliferative therapies could prevent accelerated repopulation between fractions, which might not be detectable using single fraction assays, in vitro. Conversely, although DNA damage repair blockade may enhance radiation sensitivity in the tumor, if DNA repair is also inhibited in normal tissue, outcomes may be worse in fractionated therapy.[9] Depending on which factors are most prominent in normal and tumor cells, the therapeutic index can be shifted in either beneficial or detrimental directions. Therefore, temporal modulation implies therapeutics that optimize these four radiobiology factors in between fractionated radiation treatments (see Table 4-1).[9]Potential Biological Mechanisms of Drug Radiation InteractionThere are a host of potential mechanisms by which a drug may impact radiation efficacy that are summarized in Table 4-1. Classical definitions of radiosensitizers indicated an enhancement of DNA damage as the critical factor. However, with increased understanding of cancer cell biology, it is apparent that targets other than DNA damage can enhance radiation efficacy. Therefore, a broader defined “radiation enhancer” can impact several potential mechanisms to increase radiation effect.Increasing Radiation DamageThe classical radiobiology definition of a radiosensitizer implied that the drug would enhance DNA damage. This is accomplished when the drug incorporates itself into the DNA or causes damage to the DNA itself by forming adducts, thereby increasing susceptibility of the DNA to radiation damage. Examples of this type of interaction include 5-FU and cisplatin.Inhibition of DNA RepairCancer cells that can effectively repair DNA damage will have resistance to radiation effect. Therefore, compounds that can interfere with the DNA damage repair signal transduction pathway can potentially enhance radiation damage. Several chemotherapeutics target this process, particularly those that disrupt nucleotide biosynthesis and utilization. Modified nucleotides such as 5-FU, bromodeoxyuridine, gemcitabine, fludarabine, methotrexate, etoposide, hydroxyurea as well as cisplatin fall into this category. Additionally, as will be described, compounds that alter the cell cycle may indirectly inhibit DNA repair.Cell Cycle EffectsA multitude of preclinical work has identified the G2/M as the most radiation-sensitive and S as the most radiation-resistant phases of the cell cycle.[11,12] In addition, many cytotoxic chemotherapeutics are cell cycle specific. Therefore, agents that can maintain cells in radiation-sensitive phases or eliminate those cells in radiation-resistant phases will cooperate with radiation for enhanced efficacy. Although this is clearly seen in preclinical settings, there is much less direct evidence for this phenomenon in clinical data. Nevertheless, taxanes and nucleoside analogs and modified pyrimidines appear to work in this manner.[13-16]RepopulationIn normal adult tissue, the rate of cell loss is balanced by that of cell proliferation. When increased cell loss occurs from injury, including radiation treatment, signaling for proliferation occurs resulting in a repopulation. Cancers, however, have an excess of cell proliferation relative to cell loss by their very nature. Therefore, when a subtotal cell loss occurs during fractionated radiation, cancers can also promote increased proliferation. This is known as *accelerated repopulation*. Chemotherapeutics with cytotoxic or even cytostatic effects when given concurrently with radiation, can counteract this repopulation and enhance efficacy.Hypoxia/Tumor MicroenvironmentSolid tumors, particularly those that have grown to any significant size, will contain regions of lower oxygen tension because of the limitations in vascular flow as well as oxygen diffusion within the tumor. Although many tumors trigger angiogenic factors within the tumor, these stimulants manifest as aberrant

vasculature often with disorganized architecture. Moreover, larger tumors may have increased interstitial pressure that leads to further collapse of blood vessels creating hypoxic regions and overt necrosis at times.Hypoxia is one of the most potent factors of radiation protection known because radiation relies on the production of oxygen free radicals (hypoxia generates two- to threefold less radiation sensitivity).17 Therefore, drug therapies that mitigate this hypoxia can enhance radiation efficacy. There are four general chemotherapeutic approaches for accomplishing this: (1) chemotherapy can shrink the tumor through cytotoxic action thereby decreasing interstitial pressure. Moreover, because chemotherapy typically targets the fastest proliferating cells, those cells located next to blood vessels are removed bringing the hypoxic regions into closer proximity with the oxygenated region. A good example of this process is demonstrated by taxanes such as paclitaxel.18 (2) Antiangiogenic therapies such as bevacizumab, an antibody targeting the vascular endothelial growth factor (VEGF), can potentially normalize vascular flow by eliminating the aberrant neovasculature of the tumor. Work by Rakesh Jain19,20 and others21,22 provide evidence of this phenomenon. (3) Hypoxic cell targeting agents, such as tirapazamine, can provide biological cooperation by eliminating the most radiation-resistant cells. (4) Hypoxic cell radiation sensitizers can reverse the inherent radiation resistance of the hypoxic cells. Drugs such as misonidazole, a nitroimidazole compound, can mimic the effects of oxygen within the hypoxic regions.23,24Cell Death Pathway EffectsAll of the preceding potential mechanisms of drug-radiation interaction display their efficacy through the consequence of cytotoxicity. However, in recent years, it has become clear that there are several ways in which cytotoxicity manifests itself. In 2005, the Nomenclature Committee on Cell Death (NCCD) was created and a classification system based purely on morphological criteria defined four modes of cell death: apoptosis (Type 1), autophagy (Type 2), necrosis (oncosis) (Type 3), and mitotic catastrophe.25 Although these are distinct forms of cell death, the stimuli and processes involved interrelated. Moreover, there is evidence that ionizing radiation can manifest its cytotoxicity by each type of cell death. Therefore, as our understanding of these cell death pathways improve, novel therapeutics targeting these forms of cell death could enhance radiation efficacy. These cell death mechanisms are briefly described here.**Apoptosis**Apoptosis is the most clearly defined and studied mechanism of cell death. This programmed cell death involves characteristic morphologic changes including chromatin condensation (nuclear pyknosis) and nuclear fragmentation (karyorrhexis). Apoptotic bodies ultimately form and the cell is removed through phagocytosis but without generating inflammatory response. Apoptosis can occur with or without caspase activation 26,27 and does not require DNA fragmentation,25 although this is a classical hallmark. Apoptosis is considered the major mechanism for chemotherapy-induced cell death. As a mechanism for radiation-induced cytotoxicity, apoptosis occurs readily in “liquid tumors” as opposed to most solid tumors in which apoptosis is a minor component of cell death. As such, drugs targeting the apoptosis pathway may enhance radiation cytotoxicity in solid tumors. **Autophagy**Whereas apoptosis is a clear self-destruct mechanism for the cell, the role of autophagy in cell death is more controversial. Autophagy, literally meaning “self-eating,” can provide a protective mechanism for a cell during times of stress (such as nutrient deprivation) because it allows recycling of cellular building blocks through a controlled break down of cytoplasmic components. However, autophagic cell death does occur, which principally differs from apoptosis because of the lack of chromatin condensation.25 **Necrosis**Type 3 death, or necrosis, is a cell death mechanism in which the cell swells (oncosis), ruptures the plasma membrane, and releases its contents resulting in a local inflammatory response.25 The best example of this type of cell death is ischemic injury. Large, single fraction radiation, or radio-ablative doses, can produce this type of cell death as seen in stereotactic radiosurgery of brain lesions.**Mitotic Catastrophe**Mitotic catastrophe is a unique form of cell death that involves failed mitotic events.25 Typically, this is manifest as micronucleation and multinucleation suggesting a series of mitotic divisions occur without cytokinesis that ultimately leads to cell death.Analyzing Drug–Radiation InteractionSeveral methodologies for determining the interaction between a drug and radiation have been detailed in the literature. Moreover, several definitions for the possible interactions have also been described. The concept

of radiosensitization originated many years ago, and classic radiosensitization has been defined as an increased amount of radiation-induced cell death that results from exposure to a second agent, after correction for the cytotoxicity of this agent. Clonogenic survival assays, that measure all forms of cell death as well as prolonged or irreversible cell cycle arrest, is the most encompassing method of measuring radiation cytotoxicity in vitro (Figure 4-3). Survival curves are generated by plating known quantities of cells on plates and treating them with various doses of radiation or drug and plotting the surviving fraction of colonies formed in a semilogarithmic fashion. Normalization is performed by dividing the surviving fraction for treated groups by the plating efficiency, which is defined as the surviving fraction of the untreated cells. Modification in radiosensitization, therefore, is demonstrated in clonogenic survival curve data in which a downward or leftward shift of the normalized surviving fraction implies a radiosensitizing interaction, whereas an upward or rightward shift implies a radioprotective shift. Although survival curves can show interaction between chemotherapy and radiation, a better description of radiation modulation is necessary because both chemotherapy and radiation cytotoxicity do not typically follow a linear relationship.

Figure 4-3 Graph of the concept of radiation modulation. The solid line indicates the control clonogenic survival assay plotted as surviving fraction relative to dose in Gy. A combined treatment that causes a rightward shift of the curve indicates a radioprotection effect, whereas a leftward shift of the curve indicates a radiosensitization effect.One of the early attempts at providing a more descriptive system was provided by Tyrell,28 which may be a better starting point for describing various interactions among therapies:

Antagonism: used in all cases in which the action of two treatments is less than would be expected from the addition of the two treatments given independently.

Zero interaction: used when two treatments lead to the effect expected from the addition of the two treatments given independently.

Positive interaction: used in all cases in which the action of two treatments is greater than would be expected from the addition of the two treatments given independently.

Synergism: a special case of a positive interaction; strictly, used when kinetic data are available.

These terms seem to have been supplanted by the “additivity” descriptors including supraadditive, additive, and infraadditive. Once again, the classic paper by Steel and Peckham7 describes the construction of an “envelope of additivity” for evaluating the interaction of two treatments using isobologram analysis. This envelope of additivity is constructed from cytotoxicity data by calculating a mode 1 curve that assumes that both agents have completely independent mechanisms of action as well as a mode 2 curve that assumes that the two agents have exactly the same mechanism of action. When plotting combination therapy data points on the isobologram, they can either fall between mode 1 and 2 (additive interaction—within the envelope), above mode 1 (infraadditive), or below mode 2 (supraadditive, or synergistic). An idealized isobologram is shown in Figure 4-4 and a step-by-step method for constructing an isobologram is included in the Expert Consult Website.

Figure 4-4 Graph of an isobologram for examining the interaction of radiation (RT) and a drug. Isoeffective doses of A (RT) and B (drug) are indicated on the axes. This diagram shows regions of infraadditivity, supraadditivity, as well as an envelope of additivity.The following is a step-by-step procedure for calculating isobolograms using Steel and Peckham's method7,29 (see eFigure 4-1).

eFigure 4-1 Step-by-step construction of an isobologram to define an envelope of additivity for two cancer treatments. Isobologram analysis is used to evaluate the interaction of two treatments, and it requires the construction of an envelope of additivity that is bordered by mode 1 and mode 2 lines. The mode 1 line assumes that the two agents have completely independent mechanisms of action, whereas the mode 2 line assumes that the two agents have the same mechanism of action.

Step 1. The investigator must choose to make the assessments at one level of cytotoxicity (e.g., construct an isobologram that represents the interaction of the agents for a cumulative cytotoxicity of 50%, 10%, or 1%). The example in Figure 4-3 depicts the chosen level of cytotoxicity as

horizontal line Z: 1% cytotoxicity (0.01 surviving fraction) in this case.
Step 2. Plots are made of dose-response data for both agents. In eFigure 4-1, the dose-response data for the two agents are represented by curves A and B.
Step 3. The extreme points of the envelope of additivity are determined. Initially, a separate cartesian graph is created. The *y*-axis represents the dose of agent B, and the *x*-axis the dose of agent A. The first extreme point of the envelope is placed on the *y*-axis at the dose of agent B alone that causes a specific level of cytotoxicity, as determined by the dose-response curve of agent B, at the intersection of line Z (see eFigure 4-1). The second extreme point of the envelope is placed on the *x*-axis, at the dose of agent A alone that results in that level of cytotoxicity at the intersection of the dose-response curve with line Z (see eFigure 4-1).
Step 4. The mode 1 line is constructed assuming that the agents function independently. The individual dose-response curves are used for this construction. After exposure to dose X of agent A (XA), a level of cytotoxicity is obtained at a point on the dose-response curve that is above line Z. This level of cytotoxicity is identified as Y. Next, the dose of agent B (XB) that is required to produce cytotoxicity equal to the difference in cytotoxicity at line Z and point Y (identified as C) is determined. The cartesian coordinate (XA, XB) is plotted and becomes a point on the mode 1 line. The entire mode 1 line is constructed in this manner by varying the dose of agent A (for a resulting level of cytotoxicity that falls above line Z) and subsequently calculating the appropriate complementary dose of agent B as described.
Step 5. The mode 2 line is constructed assuming that the two agents have the same mechanism of action. As for mode 1 line construction, exposure to dose X of agent A (XA) results in a level of cytotoxicity identified as Y. The dose-response curve for agent B is then examined. The dose of agent B required for cytotoxicity equivalent to Y is determined and identified as YB. The change in dose of agent B that is required to increase cytotoxicity from YB to line Z is determined and labeled ΔB. The cartesian coordinate (XA, ΔB) is plotted. Similar points are calculated for various initial doses of agent A, and the mode 2 line is formed. The mode 2 line varies in shape depending on whether agent A or agent B is selected first for step 5. Generally, the mode 2 line that results in the greatest separation from the mode 1 line is chosen for the envelope of additivity.
Step 6. The two agents are given concomitantly, and a dose-response curve for concomitant treatment is obtained (typically by holding the dose of one agent constant while varying the dose of the other). The doses of the individual agents that result in combined cytotoxicity equivalent to the level represented by line Z are plotted (J, P).This procedure allows for characterization of experimental data. The experimental point (J, P) represents an antagonistic interaction if the point falls above the envelope of additivity. The effect of the combination treatment is less than would be expected if the agents had completely independent mechanisms of action. An experimental point that falls directly on the mode 1 line suggests that the agents have independent mechanisms of action and the interaction is additive. An experimental point that falls below the mode 2 line suggests a synergistic interaction between the two agents for the particular concomitant treatment used.The most difficult result to interpret is an experimental point that falls within the envelope of additivity. In some respects, the envelope of additivity is a misnomer because experimental points that fall in this range display an interaction that is greater than the additive effect that is achieved if the agents function by completely independent mechanisms. The interaction between the agents may be positive if the agents have independent mechanisms of action, or it may be negative if they have identical mechanisms of action.Although the isobologram analysis is useful, it is somewhat limited because interactions in each analysis are investigated at a single level of cytotoxicity. The investigation of interactions at several levels of cytotoxicity requires the construction of several envelopes of additivity. The ambiguity associated with experimental points that fall within the envelope can be disconcerting and may lead to erroneous conclusions, especially if several levels of cytotoxicity are not investigated. Other mathematical modeling systems have been developed to assess the interaction of agents that cause cytotoxicity. These assessments aim to account for the kinetics of cytotoxicity of the involved agents and to assess multiple levels of cytotoxicity.Median Dose Effect PrincipleA mathematical modeling system that has gained fairly widespread use for interactions of cytotoxic agents is the median effect principle of Chou and Talalay.[29-31] This

system was derived from Michaelis-Menten equations and basic mass-action law considerations. This system has been useful for describing competitive enzyme interactions and interactions of cytotoxic agents. The primary relationship of the median effect principle is described by the following equation: fa/fu = (D/Dm)m, in which D is dose, fa is the fraction affected, fu is the fraction unaffected, Dm is the dose required to produce the median effect (50%), and m is a Hill-type coefficient used to describe the sigmoid nature of the curve. For first-order Michaelis-Menten kinetics, m = 1.The following manipulation of this equation can be performed, with surviving fraction (SF) substituted for fraction unaffected in the last step:The general equation is *y* = *mx* + *b*.A plot of log[(1/SF) − 1] on the *y*-axis and log(D) on the *x*-axis results in a line with a slope of *m* and a *y*-intercept of mlog(Dm). The survival curves for the individual agents and for the combination treatment (the individual agents given together in some fashion) can be fitted to the equation for a line by linear regression. If the interaction of two agents is assessed, three lines (i.e., median effect plots) are produced: one for each agent and one for the combination treatment. A graph of the median effect plots for mock individual agents A and B and for the combination of A and B is shown in Figure 4-5. For the combination treatment, D is the sum of the doses of the two agents given concomitantly; it is helpful to perform the experiments with the two agents given together in a fixed ratio of doses (e.g., 1 : 2). By using various total doses (i.e., the sum), with the agents given in the same ratio, it is possible to determine the contribution of the individual agents to the combination treatment in a later calculation.
Figure 4-5 The hypothetical graph *(left)* demonstrates the median effect principle analysis for agents A and B given alone or in combination. The combination treatments are given in a fixed ratio so that the individual contribution of each agent to the combined effect can be calculated. The combination index *(right)* is then calculated at various levels of cytotoxicity as measured by surviving fraction (SF). The areas of antagonism, additivity, and synergism are indicated.This concept can be visualized in Figure 4-5. For instance, in the case of log[(1/SF) − 1] = 0, where SF is surviving fraction, the corresponding log(D), D indicating the sum of the doses of the two agents, can be calculated from the median effect plot for the combination treatment. An example of an actual combination treatment that has been assessed in this manner is radiation followed by a 24-hour exposure to etoposide.29 In this example, a set of experiments was performed with the dose ratio fixed as 32 Gy to 1 µg/mL of etoposide. In this example, the dose D that resulted in log[(1/SF) − 1] equaling a given value was a combination of radiation and etoposide given in the ratio of 32 : 1. The radiation and etoposide components could be discerned, from the median effect plot of the combination treatment, by dividing the resulting dose into the appropriate components based on the ratio of delivery of the two agents.Definitions used in the median effect principle include the following:
Mutually exclusive: the agents of interest have similar modes of action and do not act independently.
Nonmutually exclusive: the agents of interest have different modes of action or act independently.
Combination index (CI): the derivation of this index is beyond the scope of this chapter. Calculation of CI allows characterization of an interaction as synergistic (CI <1), antagonistic (CI >1), or a summation (CI = 1). Chou and Talalay31 provide a full description.CI can be calculated for any surviving fraction and for mutually exclusive or mutually nonexclusive interactions. For a mutually exclusive interaction,For a mutually nonexclusive interaction,in which
(Dx)1 = Dm[(1/SF) − 1]1/m, solving the general equation for agent 1 given alone in a dose x.
(Dx)2 = Dm[(1/SF) − 1]1/m, solving the general equation for agent 2 given alone in a dose x.
(Dx)1,2 = Dm[(1/SF) − 1]1/m1,2, solving the general equation for the agents given in combination for dose x, which represents the sum of the doses of the agents given in a fixed combination.
D1 = (Dx)1,2 × (fraction of the mixture that is agent 1).
D2 = (Dx)1,2 × (fraction of the mixture that is agent 2).CI represents the doses of the agents required for a given effect when they are given together, divided by the doses required when the agents are given alone; in this way, CI less than 1 represents a synergistic interaction. A diagram of a CI plot for various levels of surviving fraction is shown in Figure 4-5.Despite the advent of these

robust statistical tools for determining the additivity relationship between treatments, the applicability outside of in vitro models is limited based on time and expense necessary to complete dose response experiments for both drug and radiation. Therefore, preclinical in vivo experimentation typically involves the use of a single drug dose at a concentration that can be achieved clinically.Chemotherapy and Radiation and Combinations of Cytotoxic Agents**General ConceptsFrom the Bench to the Clinic**Occasionally, the process of quantifying interactions of chemotherapy and radiation has frustrated clinicians attempting to interpret in vitro and in vivo laboratory information, as exemplified by Charles Moertel (quoted by Tannock32) in his keynote speech at the first International Conference on Combined-Modality Therapy in 1978:*Based on the results of various individual studies, I could conclude that it is most ideal to administer the nitrosourea 15 hours before irradiation, 2 hours before irradiation, simultaneously with irradiation, or 6 hours after irradiation. While we will continue to cheer our radiation biology colleagues on from the sidelines, I am afraid that we are not yet at the stage where we can comfortably incorporate their results into our clinical practice.*This was not meant as disrespect for the radiobiology community but to point out that, at that time, the laboratory models were potentially quite different from the clinical setting. Because it has been difficult to extrapolate from laboratory results to clinical results, many clinicians have used combination treatments on a trial-and-error basis. However, the reverse order of study has occasionally been fruitful, and efficacious combinations of treatment demonstrated in clinical studies have inspired laboratory investigations that revealed interesting molecular bases of interaction.29,30 Translational research ideally occurs with a concept that arises from laboratory findings and subsequently is shown to have clinical efficacy. However, preclinical model systems have not always allowed investigators to take findings from the laboratory to the clinic, as indicated by the quotation of Moertel and by many of the early hypoxic cell sensitizer studies.
Therapeutic Benefits Tannock32 mentioned another problem with translating findings from the laboratory to the clinical setting, emphasizing that investigators must not merely explore combinations of therapeutic agents to find synergistic interactions but must also find interactions that will produce a therapeutic benefit (e.g., provide greater cytotoxicity in tumor cells than in normal cells). To categorize potentially exploitable differences, Tannock32 described three main categories of biologic diversity between tumor cells and normal cells: tumor cells may display genetic instability compared with normal tissues; tumor cells and normal cells may be different with respect to cellular proliferation or proliferation that occurs after treatment; and environmental factors such as oxygenation and pH may affect tumor cells and normal cells differently. As findings are translated from the laboratory to the clinical setting, it is important to consider the effects of the host mechanisms on these three areas.Chemotherapeutic ClassesIn the next section, several classes of systemic agents will be presented followed by a brief review of clinical data describing combination treatment of these agents with radiation. There are a host of chemotherapeutic classes that are used in patients that will undergo radiation treatment. Although not all of these agents are used concurrently with radiation, it is helpful to understand their predominant mechanism of action. A brief description of several of the major classes of chemotherapeutics with some information regarding possible means of interaction with radiation follow. In addition, Figure 4-6 summarizes the cell cycle phase specificity of these agents.
Figure 4-6 Diagram of the cell cycle with the cell cycle phase dependence of various chemotherapeutics.**Antimetabolites**The origin of antimetabolite chemotherapy dates back to the 1940s when aminopterin was used to treat pediatric leukemia.33 Since then, a large number of antimetabolite chemotherapeutics have been developed with tremendous success. The targets for these drugs include folate metabolism and nucleoside analogs. The major antimetabolites are presented here.**Fluoropyrimidines: Fluorouracil, Fluorodeoxyuridine, and Capecitabine**The fluoropyrimidines, as the name implies, are halogenated pyrimidines that function as antifolates by inhibiting thymidylate synthesis. As mentioned previously in historical perspectives, 5-FU is one of the most established drugs used in combination with radiation. It has been used in both a bolus infusion as well as a continuous venous infusion when combined with radiation and appears to target the radioresistant cells in S-phase.16 The two delivery methods have some differences in terms of side effect profile, but both seem to have good efficacy. In a phase III rectal cancer

postoperative adjuvant chemoradiation trial, concurrent continuous infusion 5-FU during EBRT was more effective than the bolus delivery.[34] Moreover, data shows that 5-FU plasma levels and intracellular metabolite levels are rather short-lived,[35] also suggesting a need for continuous administration of the drug to be effective with radiation. Because of this, oral formulations have been developed, most notably capecitabine, a fluoropyrimidine carbamate prodrug of 5-FU, which must be converted through the action of thymidine phosphorylase. In addition to the improved patient comfort of taking an oral medication rather than having an infusion pump, another potential advantage of capecitabine in combination with radiation is that it appears that radiation increases thymidine phosphorylase levels in tumors, which allows potential bioaccumulation of active metabolite within irradiated tumor.[36,37]**Gemcitabine**Gemcitabine is an analog of deoxycytidine that specifically functions during the S-phase by preventing the dNTP production. There is both preclinical and clinical evidence demonstrating dramatic radiation sensitizing properties to combined gemcitabine and radiation.[38-41] In fact, a significant amount of toxicity has been demonstrated in clinical trials for pancreatic cancer[42] necessitating either decreased dose of gemcitabine or limited field size for radiation treatment ports.**Antifolates: Methotrexate, Trimetrexate, and Pemetrexed**The antifolate, methotrexate, tightly binds dihydrofolate reductase (DHFR) thereby inhibiting folate metabolism. Through this inhibition, thymidylate synthesis is blocked, and thus, purine biosynthesis. In addition, some amino acid synthesis is impaired through blockade of this enzyme resulting in cytotoxicity.[43] Pemetrexed is a pyrrolopyrimidine that functions as an antifolate that inhibits multiple enzymes including thymidylate synthase, dihydrofolate reductase, glycinamide ribonucleotide formyl-transferase, and aminoimidazole carboxamide formyl-transferase in a cell cycle independent manner. Pemetrexed is effective against many solid tumors and has shown radiosensitizing propertied in preclinical systems.[44,45,46]**Alkylating Agents**Alkylating agents are composed of several classes of electrophilic compounds that share the antitumor characteristic that they form covalent bonds with (“alkylate”) DNA bases. Some alkylators interact with a single strand of DNA, whereas others can cross-link two strands. The induced DNA damage leads to the cytotoxicity of these agents. The various classes of alkylators that are potentially used with irradiation are briefly presented here.**Nitrogen Mustard: Chlorambucil and Melphalan**The first of a long series of alkylating agents developed for clinical use, mustard gas was discovered based on clinical observations of people and animals exposed to mustard gas during World War I, particularly the effect on bone marrow.[33,47] Later, nitrogen mustards were developed for lymphoma therapy as mechlorethamine or Mustargen, eventually used in the MOPP (Mustargen, vincristine [Oncovin], procarbazine, prednisone) regimen for Hodgkin's lymphoma. The most commonly used nitrogen mustards are chlorambucil and melphalan (L-phenylalanine mustard) and are principally used for the treatment of chronic lymphocytic leukemia and multiple myeloma, respectively. Because of the bone marrow effect of these drugs, caution should be used when irradiating large volumes of bone marrow.**Oxazaphosporines: Cyclophosphamide and Ifosfamide**The oxazaphosphorines are nitrogen mustard-like compounds that include cyclophosphamide and its structural isomer, ifosfamide. These agents are used in combination with radiation in both pediatric and adult cancer treatment.**Mitomycin C**Mitomycin C is an antibiotic with alkylating characteristics derived from *Streptomyces*. This agent, is an aziridine ring–containing compound that resembles nitrogen mustard as well. This drug blocks DNA synthesis but also causes cell cycle arrest during G2/M phase transition.[48,49] Mitomycin C has also been shown to function well as a hypoxic cell radiosensitizer,[50] which may help explain why mitomycin C-based chemoradiation is so effective in anal cancer treatment,[51,52] as discussed later in the chapter.**Triazenes: Procarbazine, Dacarbazine, and Temozolomide**Temozolomide has revolutionized the treatment of high-grade gliomas in combination with irradiation. It effectively crosses the blood-brain barrier. (The cerebrospinal fluid [CSF] can achieve 30% of plasma levels).[53] Temozolomide generates DNA damage through methylation of DNA at the O-6 position of guanine. Interestingly, the O-6 methylguanine DNA-methyltransferase (MGMT) is a p53 DNA repair enzyme that can be regulated epigenetically silenced by promoter methylation, which appears to predict for response to temozolomide-based chemoradiation because of the inability of the cancer cell to remove the O-6

methylguanine causing cytotoxicity.54 Indeed, this DNA alkylation/methylation from temozolomide triggers the mismatch repair pathway with a G2/M phase arrest yielding apoptosis and radiosensitive cells.55 Ongoing clinical trials are examining the importance of MGMT status and combining temozolomide with other agents.54,56**Nitrosoureas: BCNU, Methyl-CCNU, CCNU, and Streptozotocin**Several members of the nitrosourea group of alkylating agents are capable of crossing the blood-brain-barrier and cross-linking DNA. BCNU, or carmustine, has been used to treat brain tumors, predominantly gliomas, but it has also been used for multiple myeloma and high-dose transplant regimens. Gliadel (BCNU impregnated wafer) can be placed in a glioma resection cavity, which biodegrades slowly to release the chemotherapeutic. CCNU, or lomustine, is a related compound with increased lipid solubility also used for brain tumors.57**Platinums: Cisplatin, Carboplatin, Oxiliplatin, and Satraplatin**Cisplatin, (cis-Diammine dichloroplatinum[II]), the prototypical and most widely studied member of the platinum family, has been used for decades as an anticancer treatment. Preclinical work in the late 1970s by Soloway et al58 demonstrated radiosensitization in a murine model of transitional cell carcinoma. Since then, a host of both clinical and preclinical data suggests several mechanisms of interaction between cisplatin and radiation. Potential cooperation between the two modalities can occur at the level of DNA because radiation often causes repairable single-stranded breaks in DNA, which can be converted to lethal double-stranded breaks when they occur close to cisplatin-DNA adducts (intra- and interstrand cross-links). This may also be as a result of the ability of cisplatin to function as a free-electron scavenger that impairs the DNA repair mechanism, thus, “fixing” the radiation-induced DNA damage.59 In addition, radiation may enhance the uptake of cisplatin into the cell as well as help generate active platinum metabolites.1 The other family members, carboplatin, oxiliplatin, and the orally active, satraplatin, appear to have similar mechanisms of action though differences among the members may be because of the three-dimensional structure of the DNA adducts that each platinum generates, which influences binding to various polymerases and DNA repair enzymes.60 For these reasons, the platinums function independent of the cell cycle phase.**Microtubule Targeting**Because microtubule polymerization and depolymerization are critical for spindle formation and chromosome segregation during mitosis, microtubule targeting agents have the ability to enhance radiation effect by creating a cell cycle blockade during M-phase, which is a radiation-sensitive phase of the cell cycle. Moreover, these agents promote apoptosis as well. The three predominant classes of microtubule targeting agents are discussed here.**Estramustine**Estramustine is an interesting hybrid molecule that is derived from both nitrogen mustard and 17β-estradiol. Estramustine effectively blocks microtubules by binding β-tubulin and microtubule associated proteins resulting in destabilization of microtubules. This targets the mitotic spindle and leads to cell cycle arrest during M-phase causing radiosensitization.61 This drug has been approved for hormone-refractory prostate cancer for almost three decades.**Vinca Alkaloids: Vincristine, Vinblastine, and Vinorelbine**The vinca alkaloids have been used as anticancer agents for more than 40 years and function by targeting microtubules. They are able to force depolymerization of microtubules and, thus, disrupt the mitotic spindle resulting in an M-phase blockade.62 These drugs have been used for a wide variety of malignancies, both pediatric and adult. In terms of radiosensitization, vincristine, vinblastine, and vinorelbine impact cell cycle effects and DNA damage repair.9,62**Taxanes: Paclitaxel, Docetaxel, and Albumin-Bound Paclitaxel**As opposed to the vinca alkaloids, the taxanes stabilize microtubules and promote further tubulin polymerization, which inhibits centrosome mechanics during mitosis. In terms of enhancing radiation effect, the taxanes appear to manipulate several of the factors listed in Table 4-1. First, the taxanes will block the metaphase-anaphase cell cycle checkpoint, which could allow for accumulation of cells in the radiosensitive G2/M phase.62,63 Furthermore, taxanes can cause tumor shrinkage,64,65 thereby decreasing interstitial pressure and allowing for improved oxygenation.18 In addition, taxanes can manipulate signal transduction cascades involved in radiation response.66**Epothilones (Epothilone B, Aza-Epothione B [Ixabepilone])**The epothilones are considered next-generation microtubule targeting agents that function similar to taxanes but are derived from mycobacterium. They are able to stabilize microtubules with high

potency and halt mitosis similar to taxanes.62 These drugs were developed to be independent of the p-glycoprotein efflux resistance mechanisms that target taxanes and vinca alkaloids.62,67 These drugs will likely become a popular choice for concurrent chemoradiation regimens in the future.**Topoisomerase Inhibitors**Topoisomerases are critical enzymes in DNA replication of all cells because of their ability to unwind DNA. There are two major classes of topoisomerases in mammalian cells that are clinically relevant for oncology therapeutics, topoisomerase I and II. Topoisomerase I (TopI) is involved in DNA replication fork movement and unwinding supercoils during DNA transcription, whereas topoisomerase II (TopII) is important for untangling DNA during transcription and remodeling chromatin.35,43 These classes are named based on how many DNA strand breaks are created during enzymatic action, a single-stranded break for TopI and double-stranded break for TopII.43 These breaks are required for TopI and II to unwind and disentangle DNA, but they are temporary because the enzyme will reconnect the broken strands (religation). TopI/II inhibitors have cytotoxicity by disrupting the process and generating DNA double-stranded breaks.**Topoisomerase I Inhibitors (Camptothecins—Irinotecan, Topotecan)**Camptothecin is a naturally occurring alkaloid derived from the plant *Camptotheca acuminata* that was identified in an anticancer drug discovery screen in the 1960s.68 Camptothecin is believed to form a stable ternary complex that prevents normal DNA religation and a collision of the complex with the replication fork occurs leading to a DNA double-stranded break and cytotoxicity.69 The S-phase specificity of this drug class provides some of the rationale for radiosensitization. The drug was unsuccessful in the clinic because of severe urinary complications, though camptothecin is still used as a research tool and positive control for cytotoxicity and apoptosis. However, derivatives of camptothecin, notably irinotecan and topotecan, are used as chemotherapeutics. Irinotecan is FDA approved for colorectal cancer, but data related to concomitant administration with radiation has been generated in patients with both small cell70-72 and non–small cell lung carcinoma.73,74 Topotecan is approved for ovarian, small cell, and cervical cancer, but it has been combined with radiation in glioblastoma clinical trials.75,76 **Topoisomerase II InhibitorsPodophyllotoxins: Etoposide, Etoposide Phosphate, and Teniposide.**The plant extract, podophyllotoxin, has microtubule binding activity, yet, the clinically used derivatives do not function through microtubule action but actually are TopII poisons.77 These epipodophyllotoxins, most notably etoposide and teniposide, are glycoside derivatives that are used in both childhood and adult tumors.35 In addition, these drugs have been used with radiation in both sequential and concomitant regimens.78-81**Anthracyclines: Idarubicin, Doxorubicin, Epirubicin, and Daunorubicin.**Anthracyclines are naturally occurring substances that intercalate into the DNA when they target TopII leading to DNA double-stranded breaks.35,82 These drugs have a wide range of clinical indications, including both liquid and solid tumors. In terms of radiation sensitization, the interaction of doxorubicin and radiation are well known such that concurrent administration is generally avoided. In fact, when doxorubicin is given after radiation, an inflammatory reaction known as “radiation recall” can occur.83,84 **Others: Mitoxantrone and Dactinomycin.**Mitoxantrone is an anthracenedione that was designed to function like an anthracycline but have less cardiotoxicity85 because it is less likely to form free radicals35 and may affect calcium release differently.86 Like anthracyclines, mitoxantrone can intercalate in DNA and poison TopII to form DNA double-stranded breaks. This drug is approved for hormone-refractory prostate cancer. Dactinomycin is a *Streptomyces* derived antibiotic that can intercalate in DNA, block TopII and cause DNA double-stranded breaks.35 Dactinomycin is used in pediatric sarcoma therapeutic regimens, including rhabdomyosarcoma. Chemoradiation Clinical ExamplesAn all-encompassing review of the clinical examples of the use of chemoradiation is beyond the scope of this chapter. More comprehensive information regarding disease site-specific trials are included in the individual chapters devoted to each site. What follows is merely a brief account of some of the landmark trials that have demonstrated improved organ preservation, local control, and overall survival in the modern era. Specifically, aerodigestive, genitourinary, gynecological, and central nervous system cancer examples are presented.Gastrointestinal Cancers**Anal Cancer**A classic example of the evolution of an efficacious interaction of chemotherapy and radiotherapy is combined modality treatment for anal cancer. In

the early 1970s, it was discovered that anal cancer could be treated successfully with a combination of 5-FU, mitomycin C (MMC), and irradiation.52 In 1974, Nigro et al52 reported on three patients who received variations of these three treatments, with excellent responses to the preoperative therapy (eTable 4-1). This article became a classic in the oncology literature, and the regimen became prominent in the treatment of anal cancer. Because of this regimen, many patients were spared abdominal perineal resection.**eTABLE 4-1Combined-Modality Treatment for Anal Cancer: A Study of Three Patients***Dose of 1500 mg of 5-fluorouracil in the form of a continuous 24-hour infusion for 5 days.*5-FU,* 5-Fluorouracil; *APR,* abdominoperineal resection; *Fx,* fractions; *NED,* no evidence of disease; *RT,* radiation therapy.Data from Nigro ND et al.52 After the initial report of Nigro et al,52 several other groups confirmed the efficacy of chemotherapy and irradiation (without surgery) as the standard treatment for primary anal cancer (Table 4-2).52,87-89 Subsequently, an intergroup effort was undertaken by the Radiation Therapy Oncology Group (RTOG) and Eastern Cooperative Oncology Group (ECOG) to determine whether MMC could be removed from the regimen because its inclusion resulted in increased toxicity compared with that of 5-FU and radiation without MMC. With 5-FU, however, fewer patients were able to avoid colostomy (see Table 4-2).**TABLE 4-2Concomitant Radiation and Chemotherapy for Anal Cancer**

Study	Regimen	Outcome
Nigro et al52 (1987), Wayne State University	RT (30 Gy/15 Fx) with CI 5-FU (1000 mg/m2) for 4 days × 2 cycles and mitomycin C (15 mg/m2) on day 1	Of 104 patients, 31 required APR
Sischy et al89 (1989), RTOG/ECOG	RT (40 Gy/20 Fx) with CI 5-FU (1,000 mg/m2) for 4 days × 2 cycles and mitomycin C (10 mg/m2) on day 2	Of 79 patients, 8 required APR
Flam et al88 (1996), RTOG/ECOG	RT (45 Gy/25 Fx) with CI 5-FU during weeks 1 and 4, with randomization to mitomycin C (10 mg/m2) on days 1 and 29 vs. no mitomycin C	Colostomy-free survival improved with MMC, 71% vs. 59% (p = 0.014)
Bartelink et al87 (1997), EORTC	RT (60-65 Gy*) alone vs RT plus 5-FU (750 mg/m2 on days 1-5, 29-33) and mitomycin C (15 mg/m2) on day 1	Improved event-free survival with RT and chemo compared with RT alone (p = 0.03)
Ajani et al51 (2008), Gunderson et al90 (2012); RTOG 98-11 (GI INT)	RT (55-59 Gy) with CI 5-FU CI 5-FU (1000 mg/m2, for 4 days, wks 1 & 5) and MMC (10 mg/m2 on days 1 and 29) vs CI 5-FU (1000 mg/m2) + cisplatin (75 mg/m2 on days 1 and 29) with induction chemotherapy	(N = 682); Improved DFS and OS at 5 y (67.8% vs. 57.8%; p = 0.006 and 78.3% vs. 70.7%; p = 0.026, respectively) for MMC arm

*Surgery 6 weeks after initial 45 Gy if no response.*5-FU,* 5-Fluorouracil; *APR,* abdominoperineal resection; *chemo,* chemotherapy; *CI,* continuous infusion; *ECOG,* Eastern Cooperative Oncology Group; *EORTC,* European Organization for Research and Treatment of Cancer; *Fx,* fractions; *GI INT,* gastrointestinal intergroup; *MMC,* mitomycin-C; *N,* total number of patients; *RT,* radiation therapy; *RTOG,* Radiation Therapy Oncology Group.Additional attempts at replacing MMC with less toxic concurrent chemotherapy have been undertaken, most notably in the U.S. GI Intergroup study (see Table 4-2) coordinated by RTOG (RTOG 98-11). This trial compared an induction chemotherapy (5-FU and cisplatin) regimen followed by the same chemotherapy concurrently with radiation versus standard concurrent chemotherapy (5-FU and MMC) with radiation. The hypothesis was that the induction chemotherapy would decrease tumor bulk making radiotherapy more effective and thus improving local control and that the additional cycles of induction chemotherapy may improve overall survival (OS) by decreasing distant metastases. However, these hypotheses were disproven because the cisplatin arm not only failed to show a benefit in terms of local control, disease-free survival (DFS), and OS, but was clearly inferior to MMC in terms of colostomy-free survival (CFS).51 Long-term followup of trial results not only confirmed initial results regarding CFS but, more importantly, demonstrated a statistically significant superior DFS and OS with 5-FU and MMC compared to induction and concurrent 5-FU and cisplatin.90 Therefore, the combination of 5-FU, MMC, and irradiation remains the standard regimen for anal cancer. IMRT strategies combined

with 5FU-MMC based chemoradiation have been investigated by multiple groups and demonstrate a reduction in normal tissue toxicities.91-93The clinical finding of the efficacious combination of 5-FU, mitomycin C, and radiation led to laboratory studies. Dobrowsky et al94 performed a complex isobologram analysis using the same agents reported by Nigro et al.52 The assessment by Dobrowsky et al,94 using an in vitro system of a squamous tumor cell line, illustrated some of the difficulties with the ideal progression of taking laboratory discoveries to the clinic. Two different endpoints were used: colony formation (cells plated after treatment and allowed to form colonies) and viable cells per flask (obtained by multiplying the cell number per flask at 96 hours by the surviving fraction, as stipulated by a standard colony formation assay). In an attempt to duplicate the clinical treatment of Nigro et al,52 mitomycin C was given as a 1-hour exposure and 5-FU as a 4-day exposure after initial radiation.The first experiments assessed the interaction of 5-FU and mitomycin C without radiation. Initially, a single dose of mitomycin C (0.5 µg/mL for 1 hour) was combined with various doses of 5-FU. Isobolograms were constructed for the colony formation endpoint at a surviving fraction of 0.04. Isobologram construction showed that the combination treatment resulted in an experimental point below the envelope of additivity at this level of cytotoxic assessment. Isobolograms also were constructed for the viable cells per flask endpoint at the 1% viability level; the experimental point for combined 5-FU and mitomycin C was directly on the mode 2 line. This endpoint was included because it was believed to account for the cytotoxic and cytostatic effects of the treatment.Because the results of this synergy analysis varied with the endpoint used, the optimal endpoint, whether colony formation or viable cells per flask, is not known. That the use of these slightly different endpoints produced slightly different isobologram results illustrates some of the problems in interpreting in vitro data and in attempting to extrapolate this information to the clinical setting. In the future, it may be possible to assess which endpoints may be most useful for various cytotoxic agents and various tumors on the basis of the relative contribution of cytotoxic and cytostatic effects for a given situation.On the basis of the experiments without irradiation, specific concentrations of mitomycin C (0.5 µg/mL) and 5-FU (0.15 µg/mL) were selected for subsequent experiments involving radiation94; these concentrations resulted in 60% and 80% surviving fractions, respectively. With colony formation as the endpoint, it was discovered that the interaction of irradiation and 5-FU or irradiation and mitomycin C (at the levels of cytotoxicity assessed) produced experimental points below the envelope of additivity. These results corroborated those reported previously by Byfield et al,95 in which some level of 5-FU cytotoxicity was required for a positive interaction with irradiation. However, the results of radiation in conjunction with mitomycin C were not entirely consistent with those of previous reports, which had suggested that a positive interaction of these agents did not exist.96The previous example illustrates several important points. If the protocol of Nigro et al52 had been designed on the basis of laboratory studies (if all of the aforementioned studies had existed in 1974), it would have been difficult to assess where to begin. First, the investigator would need to decide which in vitro endpoint would be most relevant to anal cancer (viable cells per flask or colony formation), and this decision would affect whether one believed that 5-FU and mitomycin C interacted synergistically. Second, the investigator would need to decide which assessment of mitomycin C and radiation was most relevant to the treatment of anal cancer because authors disagreed about whether this interaction was synergistic. This example also illustrates the challenges that are faced when interpretations of in vitro or in vivo experimental data are used to guide the design of clinical trials. These challenges can be exciting as we learn more about the significance of various endpoints at the molecular level and how these molecular events may be manipulated in a particular tumor.**Esophagus/Esophago-Gastric Junction**

Esophageal/esophago-gastric junction cancer remains a challenging cancer to treat, primarily because of the locally advanced stage that is typically found at diagnosis. For nonsurgical approaches to treatment, radiation alone has been shown to be quite limited in terms of controlling the disease. Indeed, chemoradiation has been clearly shown to be the treatment of choice following several landmark trials comparing chemoradiation to radiation alone. The Intergroup trial coordinated by RTOG (85-01) originally published by Herskovic et al97 and

later updated by Cooper et al98 randomized patients to either 64 Gy radiation alone at 2 Gy/fraction or 50 Gy in 2 Gy/fraction concurrent with 5-FU (1000 mg/m2/day for days 1-4) and cisplatin (75 mg/m2 on day 1). In the concurrent arm, the chemotherapy was given every 28 days during radiation and then every 21 days thereafter for two additional cycles. This trial established that radiation alone was inferior to combined modality chemoradiation (5 year OS 0% vs. 26%, $p = 0.0001$).98 A meta-analysis by Wong et al99 confirmed that concurrent chemoradiation was beneficial in terms of survival with a hazard ratio of 0.73, 95% confidence interval of 0.64-0.84, $p < 0.0001$. Sequential chemotherapy and radiation did not show a statistically significant benefit, however.99The role of trimodality therapy with neoadjuvant chemoradiation followed by surgery has also been investigated. Tepper et al100 published the results of the U.S. GI Intergroup study (CALGB 9781) that randomized patients to neoadjuvant cisplatin/5-FU/EBRT (50.4 Gy) before esophagectomy versus esophagectomy alone. Although the trial was closed early because of poor accrual, the 56 patients enrolled were analyzed on an intent-to-treat analysis. This revealed a significant difference in median and 5-yr OS with trimodality treatment and surgery alone (median: 54 vs. 21.6 mo.; 5-yr OS: 39% vs. 16%, $p = 0.002$).100 More recently, the CROSS group completed a large randomized phase III study of 366 analyzable patients comparing neoadjuvant carboplatin/paclitaxel/EBRT (41.4 Gy) before esophagectomy and esophagectomy alone.101 Results of this study revealed that preoperative chemoradiation improved OS (median, 49.4 vs. 24 mo.; 5-yr OS 47% vs. 34%; $p = 0.003$; hazard ratio, 0.657) without significant increases in acute side effects or postoperative complications. Pathologic complete response was noted in 29% of patients with neoadjuvant therapy. A complete resection of the tumor (R0 resection) was accomplished in 92% of patients who underwent neoadjuvant therapy compared to 69% in patients who had surgery alone.Combining molecularly targeted agents with standard chemotherapies with radiation have also been tested. Most heavily investigated has been the addition of EGFR targeting agents to standard chemoradiation regimens. Results from the SCOPE1 trial, a European multicenter phase II/III trial evaluating the addition of cetuximab to concurrent cisplatin, capecitabine, and radiation (50 Gy), has revealed a lack of benefit and possible detriment with the addition of cetuximab.102 Initial reports of RTOG 0436, a randomized phase III trial evaluating the addition of cetuximab to concurrent cisplatin, paclitaxel, and daily radiation (50.4 Gy), also confirm these findings with little or no benefit of cetuximab as evidenced by no improvement in OS or clinical complete response.103 **Gastric**The U.S. GI Intergroup 0116 phase III trial, reported by McDonald et al, compared adjuvant postoperative chemoradiation to surgery alone for patients with resected high-risk gastric or gastroesophageal cancers. Bolus 5-FU/leucovorin was given before EBRT (one 5-d cycle), concurrently with EBRT (2 cycles: 4-d wk 1, 3-d wk 5), and after EBRT (2 additional 5-d cycles). A survival advantage of concurrent chemoradiation was shown (3-yr OS—50% vs. 41%, $p = 0.005$; 3-yr relapse free survival—48% vs. 31%, $p = 0.001$),104 and this treatment has been the standard of care for gastric cancer in the United States. In the United Kingdom, the MAGIC trial established a nonradiation regimen that involves perioperative (neoadjuvant and adjuvant) epirubicin, cisplatin, and 5-FU (ECF) chemotherapy as an appropriate standard of care for resectable gastric cancer.105 The logical followup study was a postoperative adjuvant U.S. GI Intergroup phase III trial (CALGB 80101) that essentially married the INT-0116 and the MAGIC trial by investigating the role of chemoradiation in the setting of more modern ECF chemotherapy as the experimental arm versus the control arm of GI INT 0116. Although the experimental arm did not improve survival, the ECF regimen had a more favorable toxicity profile.106**Rectal**Although rectal cancer is a surgically managed disease, the addition of adjuvant therapy is well recognized as a vital component of therapy. Four randomized trials107-110 investigating the addition of chemotherapy to neoadjuvant EBRT in Stage II and III rectal cancer is summarized in Table 4-3 (the Bujko trial did not use the same dose/duration of EBRT so it is not a true comparison of EBRT ± concurrent chemotherapy). Furthermore, a meta-analysis reviewed these four trials.111 Although this analysis showed improved complete pathological response rate and local control with the addition of chemotherapy to preoperative radiation, no benefit was found in terms of

sphincter preservation, DFS, or OS. Of note, preoperative chemoradiation was found to produce increased grade 3 and 4 toxicity compared to preoperative EBRT alone.**TABLE 4-3Randomized Trials of Neoadjuvant Concomitant Radiation and Chemotherapy for Rectal Cancer**

Study	Regimen	Outcome
Boulis-Wassif et al[108] (1984)	Preop RT (34.5 Gy at 2.3 Gy/fx) ± 5 FU 10 mg/kg/d day 1-4 followed by surgery	Trend toward improved 5 y OS (59% vs. 46%, $p = 0.06$)
Bosset et al[107] (2006)	4 arm study: preop RT (45 Gy) + S vs. preop CT-RT with 5 FU (325 mg/m2/d)/LV (20 mg/m2/day) d1-5, 28-32+ S vs. preop RT + S + adjuvant 5 FU/LV vs. preop CT-RT + S + adjuvant 5 FU/LV	5 y LR was worse in the RT only arm (17.1% vs. 8.7%, 9.6%, and 7.6% in the CT containing arms; $p = 0.002$)
Bujko et al[109] (2006)*	Preop RT (25 Gy at 5 Gy/fx) + S vs. Preop CT-RT (50.4 Gy at 1.8 Gy/fx with 5 FU(325 mg/m2/d)/LV (20 mg/m2/day) d1-5, 28-32 + S	No difference in LC, OS, or late toxicity, but CT-RT had more early toxicity (8.2% vs. 3.2%; $p < 0.001$)
Gerard et al[110] (2006)	Preop RT (45 Gy at 1.8 Gy/fx) ± 5 FU (350 mg/m2/d d1-5 days + LV) followed by S + adjuvant 5 FU (350 mg/m2/d d1-5 days + LV)	Improved pCR (11.4% vs/ 3.6%; $p < 0.05$) and less LR (8.1% vs. 16.5%; $p < 0.05$) with CT-RT*

Not a true comparison of adjuvant EBRT ± concurrent chemo as used markedly different adjuvant EBRT regimens*5-FU,* 5-Fluorouracil; *CT,* chemotherapy; *CT-RT,* concurrent chemoradiation; *fx,* fractions; *LR,* local recurrence; *LV,* leucovorin; *pCR,* pathologic complete response; *RT,* radiation therapy.The current standard of care approach, however, was defined in the phase III German Rectal Trial.[112] Preoperative chemoradiation was shown to be superior to postoperative chemoradiation in terms of local control, sphincter preservation rates, and toxicity.As in other disease sites, more recent trials are investigating the addition of molecularly targeted agents to the standard preoperative chemoradiation regimen. The antivascular endothelial growth factor antibody, bevacizumab, has shown promising results in phase I/II trial in which all 32 patients showed tumor regression following neoadjuvant therapy with an actuarial 5-year DFS of 75%.[113] In addition, EGFR targeted agents have shown promise in KRAS wild-type tumors.[114,115] However, these agents remain investigational in this setting.Head and Neck CancersHead and neck cancers management for locally advanced tumors were traditionally managed with surgery and postoperative radiation. However, over the past two decades, an explosion of chemoradiation trials shifted the management toward an organ preservation approach (summarized in Table 4-4). One of the most impressive results for a randomized trial in head and neck cancers was that of Intergroup 0099 (RTOG 8817) originally published by Al-Sarraf et al in 1998.[116] In this trial of patients with nasopharyngeal cancer, radiation alone (70 Gy at 2 Gy/fx) versus radiation with concurrent cisplatin with adjuvant cisplatin/5-FU demonstrated a dramatic 67% to 37%, respectively, 5-year OS advantage in favor of the chemoradiation arm. More than 90 randomized clinical trials have been performed examining chemoradiation in head and neck cancers. Several meta-analyses have been published showing an absolute survival benefit to chemoradiation. Indeed, the most recent update of the MACH-NC in 2009 analyzed more than 17,000 patients in 93 randomized trials and showed an absolute OS benefit of 6.5% at 5 years.[117] Although two large randomized trials of neoadjuvant chemotherapy followed by radiation have shown a benefit in terms of laryngeal organ preservation,[118,119] subsequent studies, including RTOG 9111,[120] and the MACH-NC[117] suggest that concurrent chemoradiation is more effective than sequential administration.**TABLE 4-4Selected Concomitant Radiation and Chemotherapy for Head and Neck Cancers**

Study	Regimen	Outcomes
INT 0099 (1998)[116]	RT (70 Gy) vs. RT (70 Gy) + cisplatin (100 mg/m2 q3 wks × 3) with adjuvant cisplatin (80 mg/m2)/5-FU (1 g/m2/d for 96 h q 4wks × 3)	At 5-yr update, PFS (58% vs. 29%), DFS (74% vs. 46%), and OS (67% vs. 37%) favors the CT-RT arm ($p < 0.001$)
Brizel et al (1998)[121]	RT (75 Gy at 1.25 Gy BID) vs. RT (70 Gy at	

1.25 Gy BID) with concurrent cisplatin (12 mg/m2/d) and 5-FU (600 mg/m2 days 1-5) on weeks 1 and 6.3-yr LRC favored CT-RT (70% vs. 44%, $p = 0.01$). 3-yr OS trends in favor of CT-RT (55% vs. 34%, $p = 0.07$).
RTOG 9111 (2003)[120]3 arm trial of glottic and supraglottic cancer patients: RT(70 Gy) vs. sequential chemo (cisplatin 100 mg/m2 + 5-FU g/m2/d for 120 h q3wks × 3) then RT(70 Gy) vs. concurrent ChemoRT (cisplatin 100 mg/m2 q3wks × 3)No difference in OS but concurrent arm had superior local control (2-yr: 78% vs. 61% sequential vs 56% RT alone; p ≤ 0.003) and highest organ preservation rate (88% vs. 75% vs. 70%; p ≤ 0.005)
Adelstein et al (2003)[122]3 arm trial: RT (70 Gy) vs. concurrent RT (70 Gy) + cisplatin (100 mg/m2 q3wks × 3) vs. split course RT (30 Gy with cycle 1 and 30-40 Gy with cycle 3) + concurrent 5-FU (1 g/m2/d for 96 h) and cisplatin (75 mg/m2) q4wksThe concurrent non-split cisplatin/RT arm had superior 3-ry OS (37% vs. 27% in split course CT-RT vs. 23% in RT alone; $p = 0.014$). Concurrent cisplatin/RT had highest rate of grade 3+ toxicity (89% vs. 77% vs 52%; $p < 0.0001$)
EORTC 22931 (2004)[123]Postoperative RT (up to 66 Gy) vs. postoperative CT-RT (up to 66 Gy with cisplatin 100 mg/m2 q3wks × 3) for potential high-risk head and neck cancer patients (Stage III/IV except T3N0 or T1-2N0-1 with +margins, +PNI, +ECE, +VSI, OC/OP primary with + LNs at levels 4-5)Improvement in 5-yr OS (53% vs. 40%; $p = 0.02$), 5-yr PFS (47% vs 36%; $p = 0.04$), and 5-yr LRC (82% vs. 69%; $p = 0.007$) with CT-RT; Grade 3/4 mucositis was higher in CT-RT arm (41% vs. 21%; $p = 0.001$)
RTOG 9501 (2004)[124]Postoperative RT (up to 66 Gy) vs. postoperative CT-RT (up to 66 Gy with cisplatin 100 mg/m2 q3wks × 3) for potential high risk head and neck cancer patients (2 or more +LNs, +ECE, + margins)Improvement in 2-yr DFS (54% vs. 44%; $p = 0.04$) and LRC (82% vs. 72%; $p = 0.01$) with trend toward better OS (63% vs. 57%, $p = 0.19$); Higher rate of grade 3 or greater acute toxicity in chemoRT arm (77% vs. 34%; $p < 0.001$)
Bonner et al (2006[125] and 2010[126])Once Daily RT (70 Gy at 2 Gy/d), concomitant boost (72 Gy in 42 fxs) or hyperfractionated (72-76.8 Gy at 1.2 Gy BID) ± cetuximab (given 1 week before RT at 400 mg/m2 then given weekly at 250 mg/m2 × 7 wks)Improvement in MS (49 vs. 29.3 months) and 5-yr OS (45.6% vs. 36.4%) in the cetuximab arm (HR of 0.73; $p = 0.018$). No difference in grade 3 or 4 toxicity, including mucositis, (except acneiform rash and infusion reaction).
MACH-NC meta-analysis[117]Meta-analysis of 93 randomized trials of CT in head and neck cancer, with 17,346 patients.Concomitant CT-RT provides absolute 5-yr OS benefit of 6.5% whereas induction chemo showed only 2.4% (HR of 0.81; $p < 0.0001$).*5-FU,* 5-Fluorouracil; *BID,* twice per day; *CT,* chemotherapy; *CT-RT,* chemoradiation; *DFS,* disease-free survival; *ECE,* extracapsular extension; *EORTC,* European Organization for Research and Treatment of Cancer; *INT,* intergroup; *LN,* lymph nodes; *LRC,* loco-regional control; *MACH-NC,* meta-analysis of chemotherapy in head and neck cancer; *MS,* median survival; *OC/OP,* oral cavity/oropharynx; *OS,* overall survival; *PFS,* progression-free survival; *PNI,* perineural invasion; *RT,* radiation therapy; *RTOG,* Radiation Therapy Oncology Group; *VSI,* vascular space invasion.As mentioned previously, the RTOG 9111 was a landmark Intergroup trial for patients with what would currently be staged as T2 and T3 glottic and supraglottic squamous cell carcinomas.[120] This trial randomized patients to one OS was not different between the groups, the DFS and local-regional control favored the concurrent chemoradiation arm.[120] This benefit is not restricted to organ preservation studies. Indeed, two trials published in the same issue of the *New England Journal of Medicine* detailed the RTOG[124] and EORTC[123] trials randomizing patients to postoperative radiation with or without concurrent chemotherapy. Although the two trials had slight differences in their inclusion criteria, they both established the importance of adjuvant chemoradiation in the setting of high-risk postoperative patients. A pooled data analysis from the two trials identified positive margins and extracapsular extension as the two significant risk factors for combining chemotherapy with radiation in the adjuvant setting.[127]Recently, the question regarding whether induction chemotherapy followed by concurrent chemoradiation is superior to concurrent chemoradiation alone in locally advanced

head and neck cancers was addressed in the PARADIGM trial.128 In this study, patients were randomized to receive either induction chemotherapy with docetaxel, cisplatin, and 5-FU followed by concurrent chemoradiation with docetaxel or carboplatin compared to concurrent chemoradiation using cisplatin. Although the trial was terminated early because of poor patient accrual, results suggest no difference in overall survival between the two groups. However, a greater incidence of febrile neutropenia was noted in patients who received induction followed by concurrent chemotherapy compared to concurrent chemoradiation alone.In 2006, Bonner et al published the results of a randomized phase III trial incorporating a molecularly targeted therapy with radiation in locally advanced head and neck cancers.125 This trial used cetuximab, which is a monoclonal chimeric (mouse and human) antibody to EGFR. This trial showed not only a local control benefit but also an overall survival benefit when cetuximab was combined with radiation, including altered fractionation schedules. In addition, the incidence of serious toxicity (other than rash and transfusion reactions) was similar between the groups, which is in stark contrast to chemoradiation regimens that invariably have increased toxicity. These results have held up at the most recently published update126 showing a 49-month median survival in the cetuximab arm and 29.3 months in the radiation alone arm. Five-year OS was 45.6% in the cetuximab group and 36.4% in the radiation alone group (hazard ratio, 0.73; p = 0.018).Several trials are accruing or recently closed combining cetuximab with chemoradiation including the Phase III RTOG 0522 randomizing patients with stage III and IV squamous cell carcinoma of the oropharynx, hypopharynx, and larynx to cisplatin (100 mg/m2 every 3 weeks) with or without a 1-week pretreatment (400 mg/m2) and concurrent (250 mg/m2/week) cetuximab with accelerated fractionation. Initial reports suggest the addition of cetuximab to concurrent chemoradiation does not improve clinical outcomes.129 Final results should be forthcoming.The human papilloma virus (HPV) has been implicated as a major cause of head and neck cancers.130 Interestingly, patients with HPV-associated head and neck cancers have improved outcomes over patients with cancers not associated with HPV. It has been suggested that this may be as a result of an inherent DNA repair defect in HPV-associated head and neck tumors. Thus, efforts by ECOG and RTOG are under way to deescalate therapy in attempts to reduce the morbidities of therapy. The ECOG E1308 is a phase II trial, which tests the efficacy of reducing radiation doses and using concurrent cetuximab rather than chemotherapy in patients who have achieved a complete response to induction chemotherapy. Initial results are promising, with an 86% overall response rate observed in enrolled patients, and more importantly, decreased toxicities compared to historical controls.131 The RTOG 1016 is a phase III trial randomizing patients to concurrent chemoradiation or cetuximab/radiation. This study is currently nearing completion of accruing the necessary number of patients.Non–Small Cell Lung CarcinomaNon–small cell lung carcinoma (NSCLC) is the number-one cause of cancer death in the United States, which is at least partly the result of the late presentation that typically occurs with this disease. As such, most patients will present with stage III disease or stage IV.The treatment of stage III NSCLC has evolved over the past two decades. For unresectable stage III patients, EBRT alone produced poor 2-year OS, on the order of 20%. A modest improvement (up to 29%) was achieved with more intense radiation schedules such as the continuous hyperfractionated accelerated radiotherapy (CHART) regimen.132To improve on these outcomes, the approach that was most heavily tested was the addition of systemic agents to EBRT, which is summarized in Table 4-5. Three major trials of sequential chemotherapy followed by radiation were published in the 1990s that showed improvement with the addition of platinum-based chemotherapy.133-135 Around the same time, a meta-analysis demonstrated a small, but significant improvement with the addition of chemotherapy.136 However, because the various trials included many different sequences of chemotherapy and radiation, it was unclear what would be the best regimen. Therefore, several randomized phase III trials compared sequential to concurrent chemoradiation.137-140 In all but one trial,140 the concurrent arm showed a significant improvement relative to sequential chemotherapy and irradiation. This established concurrent chemoradiation as the treatment of choice. What was not clear, however, was whether adjuvant chemotherapy could be added to concurrent chemoradiation regimens, and if so, if it was

better to administer the chemotherapy neoadjuvantly or adjuvantly.**TABLE 4-5Concomitant Radiation and Chemotherapy for Non–Small Cell Lung Cancer**

Study	Regimen	Outcome
Dillman et al[133] (CALGB 8433 updated[141])	Sequential cisplatin (100 mg/m2 d1 and 29) and vinblastine (5 mg/m2 weekly × 5) followed by RT (60 Gy) vs. RT alone (60 Gy)	Improved MS (13.7 vs. 9.6 mo.; $p = 0.012$) with CT-RT
Sause et al[134] (RTOG 8808 updated[142])	Three-arm trial: sequential cisplatin (100 mg/m2 d1 and 29) and vinblastine (5 mg/m2 weekly × 5) followed by RT (60 Gy) vs. HyperFx RT (69.6 Gy at 1.2 Gy BID) vs. RT alone (60 Gy)	Improved MS (13.2 vs. 12 vs. 11.4 mo.; $p = 0.04$) with CT-RT
Furuse et al[138]	Chemo of cisplatin (80 mg/m2 on d1 and 29), vindesine (3 mg/m2 on d1, 8, 29, and 36), and mitomycin C (8 mg/m2 on d1 and 29) given either concurrently with split course RT (28 Gy × 2, 10 days apart) or sequentially with RT (56 Gy)	Improved MS (16.5 vs. 13.3 mo.; $p = 0.03998$) with concurrent CT-RT
Curran et al[137] (RTOG 9410)	Three-arm trial: cisplatin (100 mg/m2) and vinblastine (5 mg/m2) given before (Arm 1) or concurrently with once daily RT (Arm 2, 60 Gy), or concurrent cisplatin (50 mg/m2) and oral etoposide (50 mg BID) with HyperFx RT (Arm 3, 69.6 Gy at 1.2 Gy BID)	Improved MS (17 mo.) in concurrent once daily CT-RT arm (arm 2) va. 15.6 mo (arm 3) va. 14.6 mo. (arm 1) ($p = 0.038$)
Albain et al[143] (INT 0139)	Stage IIIA patients received two cycles of chemo: cisplatin (50 mg/m2 on d1, 8, 29, and 36) and etoposide (50 mg/m2 on d1-5 and 29-33) plus 45 Gy. If no progression, they were randomized to surgery or 16 Gy boost. All patients received 2 adjuvant cycles of cisplatin/etoposide	No difference in MS, or 5-yr OS; improved median PFS in trimodality arm (12.8 vs. 10.5 mo.; $p = 0.017$); in subset analysis, improved OS in patients who underwent lobectomy vs. CT-RT, but CT-RT was better if patient underwent pneumonectomy.
Thomas et al[144] (GLCCG)	Patients received three cycles of cisplatin and etoposide and were randomized to CT-RT with concurrent carboplatin and vindesine followed by S or S followed by RT alone.	Preoperative CT-RT resulted in increased pathological response but no improvement in PFS. For patients requiring pneumonectomy, preop CT-RT had trend to increased Tx-related mortality (14% vs. 6%; $p = 0.14$)

BID, Twice daily; *CALGB,* Cancer and Leukemia Group B; *CT-RT,* chemoradiation; *INT,* intergroup; *GLCCG,* German Lung Cancer Cooperative Group; *HyperFx,* hyperfractionation; *mo.,* months; *MS,* median survival; *OS,* overall survival; *PFS,* progression-free survival *RT,* radiation therapy; *RTOG,* Radiation Therapy Oncology Group; *S,* surgery; *Tx,* treatment.A phase II trial called the Locally Advanced Multimodality Protocol (LAMP) trial attempted to determine what would be the best approach.[145] The three arms in this trial included (1) neoadjuvant chemotherapy followed by radiation, (2) neoadjuvant chemotherapy followed by concurrent chemoradiation, and (3) concurrent chemoradiation followed by adjuvant chemotherapy. Although the concurrent followed by adjuvant arm had the best outcome, the trial had some limitations including insufficient power to determine the best regimen.[145]Although concurrent chemoradiation is the standard approach for stage III NSCLC, there are certain instances in which surgery can be combined with chemoradiation in a trimodality approach. Two major randomized trials have been published regarding this trimodality approach, which are also included in Table 4-5 (U.S. Lung Intergroup 0139[143] and the German Lung Cancer Cooperative Group[144] trials). Both trials appeared to show that trimodality therapy was feasible, but that preoperative chemoradiation should be avoided if a pneumonectomy would be performed due to excessive treatment related mortality.Cervical CancerOne of the clearest clinical examples demonstrating improved outcomes of combined chemoradiation is in locally advanced cervical cancer. Cisplatin-based regimens have had clear success in a series of randomized phase III trials showing not only improvement in local-regional control but in also OS. This series of clinical trials (summarized in Table 4-6) had differing inclusion criteria and various treatment approaches, yet, these trials presented convincing evidence that cisplatin, when combined with radiation, improves outcome in locally advanced cervix cancer. An excellent review of the historical context and

remaining controversies regarding these trials is available,146 so they will only briefly be discussed here.**TABLE 4-6Concomitant Radiation and Chemotherapy for Cervix Cancer**

Study	**Regimen**	**Outcome**
GOG 85 (1999)147	IIB-IVA patients; RT + HU (3 g/m2 twice per week) vs. RT + Cisplatin (50 mg/m2)/5-FU(4 g/m2/96 h)	Improved PFS (RR of 0.79, $p = 0.033$) and improved OS (5-yr: 62% vs. 50%, RR of 0.74, $p = 0.018$) in cisplatin containing arm
GOG 120 (1999)148	IIB-IVA patients; three arms: Cisplatin (40 mg/m2/week) vs. Cisplatin (50 mg/m2)/5-FU (4 g/m2/96 h) with HU (2 g/m2 twice per week) vs. HU (3 g/m2 twice per week)	Improved PFS (RR of 0.57 and 0.55, $p < 0.001$) and improved OS (3-yr: 65% vs. 47%, RR of 0.61 and 0.58, $p < 0.005$) in both cisplatin containing arms
GOG 123149	IB (tumors ≥4 cm) patients; RT ± cisplatin (40 mg/m2/week)	Improved PFS (RR of 0.51, $p < 0.001$) and improved OS (3-yr: 83% vs. 74%, RR of 0.54, $p < 0.008$) in cisplatin containing arm
SWOG 8797 (2000)150	I-IIA patients after hysterectomy with high-risk features (positive nodes, positive margins, or parametrial involvement); RT ± cisplatin(70 mg/m2)/5-FU(4 g/m2/96 h)	Improved PFS (HR of 2.01, $p = 0.003$) and improved OS (4-yr: 81% vs. 71%, HR of 1.96, $p = 0.007$) in cisplatin containing arm
RTOG 9001151	IB-IIA (≥5 cm), IIB-IVA (or positive pelvic nodes) patients; RT ± cisplatin (75 mg/m2)/5-FU(4 g/m2/96 h)	Improved 5-yr DFS (67% vs. 40%, $p < 0.001$) and improved 5-yr OS (73% vs. 58%, $p = 0.004$) in cisplatin containing arm
NCIC152	IB-IIA (≥5 cm), IIB-IVA (or positive pelvic nodes) patients; RT ± cisplatin (40 mg/m2/week)	No difference in PFS or OS

5-FU, 5-Fluorouracil; *DFS,* disease-free survival; *GOG,* Gynecological Oncology Group; *HR,* hazard ratio; *HU,* hydroxyurea; *NCIC,* National Cancer Institute Canada; *OS,* overall survival; *PFS,* progression-free survival; *RR,* relative risk; *RT,* radiation therapy; *RTOG,* Radiation Therapy Oncology Group.The Gynecological Oncology Group (GOG) had three positive trials for chemoradiation as a component of treatment. The GOG 85 trial investigated two different concurrent chemotherapy regimens (hydroxyurea and cisplatin/5-FU) with radiation for patients with locally advanced cervix cancer.147 This trial demonstrated that concurrent cisplatin/5-FU was superior to hydroxyurea (5-yr OS 62% vs. 50%, $p = 0.018$). GOG 120 was a three-arm trial that compared radiation alone to two different cisplatin containing regimens (one with cisplatin alone and the other with cisplatin plus 5-FU and hydroxyurea).148 The two cisplatin-containing arms were superior to the control arm (3-yr OS for both cisplatin based arms of 65% vs. 47%, $p < 0.005$). Since the cisplatin alone arm was less toxic than the 5-FU/hydroxyurea combination, the concurrent cisplatin/radiation approach was preferred. The GOG also investigated the importance of chemotherapy with radiation for patients in bulky stage IB treated with hysterectomy in GOG 123. In this trial, the patients were randomized to radiation alone versus radiation plus weekly cisplatin before undergoing hysterectomy.149 Once again, the cisplatin-containing arm was superior (3-yr OS 83% vs. 74%, $p = 0.008$).In the 1990s, the RTOG also examined the importance of cisplatin with radiation for patients with locally advanced cervix cancer. RTOG 9001 compared extended field radiation alone to pelvic radiation plus cisplatin and 5-FU.150 The chemoradiation arm was superior to the extended field irradiation (5-yr OS 73% vs. 58%, $p = 0.004$).The fifth trial to support concurrent cisplatin/radiation was the Intergroup 0107/SWOG 8797, which tested chemoradiation in the adjuvant setting for patients that underwent hysterectomy with high-risk features at time of surgery including positive pelvic nodes, positive margins, and parametrial involvement.151 Patients were randomly assigned to receive radiation alone or radiation with cisplatin/5-FU. The chemoradiation group was again superior (4-yr OS 81% vs. 71%, $p = 0.007$).Only one major trial, from National Cancer Institute of Canada (NCIC), failed to demonstrate an OS benefit for this approach.152 However, that trial has been criticized because of the small size and wide confidence intervals that may have prevented a difference from being detected.The next generation of chemoradiation trials is adding molecularly targeted agents, including cetuximab and tirapazamine, to standard cisplatin-based regimens.Genitourinary CancerChemoradiation is established as the standard bladder preserving management strategy for muscle invasive bladder cancer. Shipley et al153 published a

single institution experience using a neoadjuvant chemoradiation approach similar to the larynx preservation trials discussed previously in which a cisplatin-containing chemoradiation regimen is given (following a maximal transurethral resection of bladder tumor [TURBT]), an evaluation for tumor response is then performed followed by either surgery (for anything less than a complete response) or consolidative chemoradiation. With this approach, the 5-year OS is comparable to surgical series at 54%. Bladder preservation rates without invasive local recurrence are ~50%.Another genitourinary example involving the use of hormone ablation therapy plus EBRT for high-risk prostate cancer demonstrates a local control, DFS, biochemical DFS, and possibly an OS benefit based on both RTOG154-156 and EORTC157,158 experience. It has been postulated that androgen-deprivation therapy used in concomitant radiation regimens not only provides a spatial cooperation interaction but also a biological cooperation function. Currently, the RTOG is investigating the addition of TAK-700 (Orteronel), which suppresses adrenal androgen production, to concurrent EBRT and standard androgen deprivation therapy for high-risk prostate cancer patients (RTOG 1115) in attempts to maximally suppress androgen production.GlioblastomaThe treatment of glioblastoma has dramatically changed in recent years based on the encouraging findings from the phase III trial from EORTC and NCIC. This trial, first published by Stupp et al in 2005159 and updated with 5-year data,160 demonstrated a remarkable improvement in median survival (14.6 vs. 12.1 months) and OS (9.8% vs. 1.9% at 5 years; hazard ratio, 0.63; $p < 0.0001$) with the use of concurrent temozolomide and radiation with adjuvant temozolomide. Prognostic161-163 and possibly predictive164 information can be garnered from evaluating the MGMT promoter methylation status because it has been shown that patients whose tumors have MGMT epigenetic silencing as a result of promoter hypermethylation do better.Future Directions**Molecular Prediction**One of the current ideals for the medical field is to develop personalized or “precision” medicine for patients. For the oncology field, the identification of biomarkers for prognosis and response to therapy is of critical importance for achieving this ideal. Perhaps the most promising strategy toward personalized medicine in oncology has been the investigation of synthetic lethal interactions between two distinct pathways, which can be exploited therapeutically.165 This concept of synthetic lethality is one in which mutation or loss of either of the two pathways individually has no effect on survival; however, mutation or loss of both leads to cell death. To this end, inhibitors of poly(ADP)-ribose polymerase (PARP) have come to the forefront for patients with BRCA-associated cancers.166,167 PARP is involved in the base excision repair of single-stranded DNA damage, which if left unrepaired, is converted into a double-stranded DNA break that can be repaired by the homologous recombination repair pathway mediated by BRCA. In patients with BRCA-associated tumors, which are homologous recombination repair defective, inhibition of PARP leads to significant cytotoxicity of tumors while sparing normal tissues, which are proficient in homologous recombination repair. This exciting therapeutic strategy has shown promise in multiple clinical trials and exemplifies the ultimate goal of personalized therapy—that is, to maximize therapeutic ratio.The predominant investigative tools for biomarker discovery are related to genomics and proteomics, the study of global gene expression and protein expression, respectively. An emerging science is that of kinomics in which global kinase activity is determined. These expression or activation patterns of individual tumors can be correlated to outcomes including treatment response and survival endpoints. The hope is that diagnostic tests can be developed that will guide clinicians in selection of therapy. A brief description of each discovery tool is found here.

Genomics/Transcriptomics The most actively studied component of molecular prediction uses genomic technology. Genomics refers to the genome-wide evaluation of individual gene expression. Pharmacogenomics refers to the study of genetic information to predict treatment response. There are a host of platforms for analyzing genetic information within a biologic specimen. One means is through evaluation of polymorphisms, which refers to a variation within a gene such that at least two alleles occur in 1% or more of the general population. When the variation occurs at a single nucleotide, this is termed a *single nucleotide polymorphism*, or SNP. Several groups have identified SNPs within DNA synthesis/DNA repair genes that could potentially serve as markers of response to radiation,168 chemotherapy,169 or chemoradiation.170

Genome-wide SNP arrays are commonly used these days and have been applied to translational studies. Historically, though, the most widely used approach has been microarray-based, such as the GeneChip from Affymetrix (Santa Clara, CA). These arrays enable the analysis of relative expression of more than 38,000 genes on a single chip. However, with the advent of next-generation sequencing (NGS or “deep sequencing”) ushering in lower costs and higher throughput, NGS approaches are largely replacing microarray-based methods. For example, the RNASeq approach can provide gene expression information (transcriptome) but also provide information about genomic alterations (at least for the expressed genome). Focused exome NGS is being used to identify drug-targetable mutations in patient tumors. NGS approaches are now the methodology of choice for The Cancer Genome Atlas (TCGA) project that is cataloging molecular data on 20 tumor types that has already identified many molecular subtypes among several cancer types.171No matter what platform is used, the identification of genomic/transcoptomic differences between good and poor responders may eventually guide therapy decisions. A significant amount of work has been done in predicting breast cancer response to chemotherapy in vivo.172 Similar studies for radiation and chemoradiation response are in various stages of development.**Proteomics**Proteomics is becoming an established platform for biomarker discovery. The predominant approach involves mass spectrometry to identify levels of proteins based on peptide fragments generated from enzymatic digestion of all of the proteins within a biologic specimen such as a biopsy, tissue sample, blood, urine, etc. This approach has been used for prognostic purposes in NSCLC173 as well as prediction of sensitivity to chemoradiation in cervical cancer patients.174 Miniaturization with nanofluidic assays175 and novel dynamic-proteomics176 approaches are providing more information and understanding regarding cellular response to drugs.**Kinomics**Although genomic and proteomic strategies are being used in translational components of several clinical trials with RTOG and other cooperative groups, these technologies have significant difficulty in detecting transient signaling events such as kinase activation. This is as a result of the fact that kinases are predominantly regulated posttranslationally, that is, they are subject to phosphorylation events, conformational changes, subcellular translocation, binding partnerships, etc. Kinomics, thus, refers to the global detection of kinase signaling events within a cell or tissue. There is a tremendous amount of preclinical data that demonstrates robust but transient activation of kinase-based resistance pathways downstream of ionizing radiation and chemotherapy.177-181 Arguably, the most prominent class of next-generation therapeutics for oncology is kinase-targeted agents. In the near future, there may be a large repertoire of available kinase inhibitors that are available for therapy if we could only identify the patients most likely to benefit from them. Kinomic analysis may help guide therapy by identifying the critical kinase activations that predict for sensitivity or resistance to particular drugs. Although this technology is still in its infancy, kinomics may one day provide a complementary clinical tool to the genomic and proteomic strategies that are making their way into oncology practice. Indeed, the TCGA selected reverse phase protein arrays (RPPA) as their platform technology for functional proteomic evaluation of tumors.182 Other translational research examples include kinomic profiling of pediatric brain tumors,183 chondrosarcomas,184 preclinical xenograft treatment response prediction,185 chemoradiation response prediction in locally advanced rectal cancer patients,186 and even for identification of novel radiation modulators.187**Patient-Derived Xenografts**One potential strategy to improve molecular prediction is to use better preclinical model systems for combination testing. Patient-derived xenografts (PDX, xenolines, or tumor avatars) have been increasingly used as preclinical model systems. PDX appear to more faithfully represent clinical reality because they are passaged within immunocompromised mice rather than cultured in plastic with high levels of serum. “-Omic” characterization and therapy response data have shown considerable promise leading many pharmaceutical companies and some academic institutions to incorporate PDX into their preclinical testing program. Indeed, several companies specializing in PDX production and therapy testing have emerged in recent years. One potential strategy for integrating PDX into therapeutic development is the concept of a “parallel mouse” clinical trial that can be performed in conjunction with an actual human clinical trial. The

incorporation of the parallel mouse trial allows for a larger cohort of tumor specimens to be available for molecular characterization and biomarker development. The potential for PDX-informed clinical decision making could be possible with this approach.
SummaryMultimodality therapy has become the mainstay of the vast majority of solid malignancies. Understanding the interaction between chemotherapy and radiation is, therefore, vital to improving patient care. With the addition of biologically targeted agents to the armamentarium of anticancer therapy, it may be better to refer to chemotherapeutics and biologics simply as systemic agents. It is expected that clinical investigation will help validate as well as generate novel strategies in the laboratory. In addition, the establishment of useful biomarkers will hopefully usher in a new era of personalized oncologic medicine. In this way, exploitable differences between tumor tissue and normal tissue will be optimized for each patient.
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187. Jarboe JS, Jaboin JJ, Anderson JC, et al. Kinomic profiling approach identifies Trk as a novel radiation modulator. *Radiother Oncol*. 2012;103:380–387. **Chapter 5**Biologics and Their Interactions with Radiation*Timothy V. Waxweiler, David Raben*Over the past decade, radiation oncology researchers have rapidly developed the capability of delivering “targeted” therapy using photon- or proton-based energies with the evolution of intensity-modulated radiation therapy (IMRT) and the incorporation of image-guided motion tracking. During this same decade, advances in molecular pathology have resulted in powerful predictive biomarkers such as KRAS, ALK, and BRAF that have ushered in a new era in cancer therapy, that of personalized cancer therapeutics. Improved understanding of the molecular mechanisms underlying malignant processes has allowed the development of novel therapeutic agents that target specific cellular processes. Molecularly targeted therapeutic agents, or biologics, are a class of agents that have been developed to specifically interfere with functions central to the pathophysiology of the malignant phenotype.Although initially targeted in the single-agent setting and then with chemotherapy, many of these biologic pathways are also central to the radiation response; this finding raised the likelihood that some biologic agents may be effective radiosensitizers. A radiosensitizing effect has indeed been demonstrated clinically and in model systems. As biologically targeted drugs continue to gain prominence in the cancer therapy landscape, radiation oncologists should be familiar with therapies that their patients are likely to be receiving in conjunction with radiation. Furthermore, the potential for radiosensitization mandates that radiation oncologists become educated regarding the potential for these agents to exacerbate toxicity or improve efficacy in the standard radiotherapy setting. Finally, the potential to take advantage of the new biologics as radiosensitizers offers an exciting opportunity to improve local control for many tumor types treated with radiotherapy.Before we can move forward we must ask why many of our preclinical studies, that at first glance appear promising, have failed to translate successfully in phase III clinical trials when combined with chemoradiation.[1,2] Over the past decade or so, we have, in fact, only demonstrated success in one phase III trial using an anti-EGFR antibody with radiation alone.[3] Perhaps the optimal preclinical studies were never performed to adequately evaluate optimal sequencing and whether adding a targeted agent to a chemoradiation backbone in fact could be antagonistic. Perhaps some of these targeted drugs

might be best used as maintenance therapies after completion of chemoradiation. Perhaps dual targeting of specific pathways might provide improved local-regional control over traditional chemoradiation combinations and should be tested against standard of care. Do we need to dose biologically based approaches in a maximum tolerated drug (MTD) format similar to what we traditionally do with chemoradiation phase I trials? We have even gone as far as adding dual biologics to a chemoradiation backbone with little or no preclinical data to support its clinical translation, resulting in added toxicity.4-7 These are critical questions that must be answered if we are to realistically move our field forward in a molecular age.In a previous version of this chapter, the authors emphasized developments in the laboratory and in the clinic related to epidermal growth factor inhibition, antiangiogenesis, proteasome interference, DNA repair inhibitors, mTOR inhibitors, and insulin growth factor receptor inhibitors; and how these factors cooperate with radiation. In this edition, the clinical experience with familiar molecularly targeted drugs and radiation has been updated and streamlined; however, we intend to place an emphasis on selected newer agents that appear promising in a variety of disease sites. These include PI3K/Akt/mTOR pathway inhibitors, PARP inhibitors, and immunomodulating agents. Many additional agents are under investigation including “dirty” biologics, agents such as the multityrosine kinase inhibitors that target multiple pathways, although a detailed discussion of these agents is beyond the scope of this chapter. To provide some perspective, since the previous edition of this chapter was published in 2011, the number of biologics approved by the Food and Drug Administration (FDA) has more than tripled, from ~14 to more than 50 agents (Table 5-1). This update particularly focuses on emerging biologics believed likely to play an increasing role in clinical radiation oncology in the near future. What does the practicing radiation oncologist need to know as we witness a remarkable evolution in our understanding of molecular oncology and develop new insights in to radio-genomics to predict response and toxicity to radiation therapy? Next-generation sequencing is assisting in defining new targets within a cancer to personalize our therapeutic approaches and this information may be valuable in finding the optimal agents to combine with radiation within a specific disease site.**TABLE 5-1FDA-Approved Biologic Modifiers8,9**RTConcurrent trials with radiation are ongoing or have been completed.*Initial approval by FDA Breakthrough Therapies program for expedited drug development under the FDA Safety and Innovation Act of 2012.*ALL,* Acute lymphocytic leukemia; *ALK,* anaplastic lymphoma kinas; *CLL,* chronic lymphocytic leukemia; *CML,* chronic myelogenous leukemia; *CRC,* colorectal cancer; *EGFR,* epidermal growth factor receptor; *FGFR,* fibroblast growth factor receptor; *GI,* gastrointestinal; *GIST,* gastrointestinal stromal tumor; *HDAC,* histone deacetylase; *HNSCC,* head and neck squamous cell carcinoma; *HTN,* hypertension; *mTKI,* multiple tyrosine kinase inhibitor; *NHL,* non-Hodgkin lymphoma; *NSCLC,* non–small cell lung cancer; *PDGFR,* platelet-derived growth factor receptor; *Ph(+)*, Philadelphia chromosome positive mutation; *PML,* progressive multifocal leukoencephalopathy; *RCC,* renal cell carcinoma; *RIT,* radioimmunotherapy; *SEGA,* subependymal giant cell astrocytoma; *TKI,* tyrosine kinase inhibitor; *VEGFR,* vascular endothelial growth factor receptor.Epidermal Growth Factor Receptor Family Inhibitors**Epidermal Growth Factor Receptor Family Biology**The epidermal growth factor receptor (EGFR) family signaling process has captured significant attention clinically over the past 15 years as a targetable pathway that could be used in conjunction with radiation. Why is it important and how does it work? EGFR signaling regulates mesenchymal-epithelial interactions during growth and development, transmitting extracellular cues to intracellular signaling cascades.10-12 The family has four known members: EGFR, HER2 (erbB2), HER3 (erbB3), and HER4 (erbB4). These membrane-spanning tyrosine kinase receptors contain an extracellular ligand-binding domain, a transmembrane domain, and an intracellular tyrosine kinase domain. These normally quiescent receptors are activated when ligand binds to the extracellular domain of a receptor monomer. Ligand binding induces a structural change that favors dimerization with the same member (homodimer) or a different member (heterodimer) of the family. When dimerized, the tyrosine kinase domains are activated and they phosphorylate key tyrosine residues in the intracellular domain, resulting in activation of several downstream signaling cascades.The end result of receptor activation, proliferation, differentiation, migration, or survival signaling depends on many factors, including which receptor pairs are

formed and for how long they are activated.13 This in turn depends on which receptors are predominantly present in the cell and which ligand is involved in activation. There are two ligand families that activate the EGFR family receptors, the EGF-like and heregulin families.14,15 The EGF-like family includes EGF, TGF-α, amphiregulin, betacellulin, and HB-EGF. The heregulin (neuregulin) family includes many proteins resulting from splice variations of two different genes, all designated as heregulin with different subtypes. The ligands exhibit preference for particular receptors and induce different receptor combinations. HER2 has no known ligand. Instead, HER2 is the favored partner of the other receptors when ligand binds to EGFR, HER3, or HER4.16 This complex interplay of receptors is important for understanding and interpreting the effect of an inhibitor of a single member of the EGF family.The effect of receptor activation also depends on which downstream signals are activated involving DNA synthesis and repair, apoptosis evasion, growth factor signaling, and proliferation. EGFR family members signal via a diverse network of signal transduction pathways, including the protein kinase C (PKC), Ras-Raf-ERK, PI3K-Akt, and STAT pathways17 (Figure 5-1). Furthermore, various receptor pairs recruit different downstream effectors. For instance, HER3 contains multiple PI3K-binding motifs, resulting in strong signaling via PI3K, which plays a role in cell survival, invasion, and proliferation. Interestingly, HER3 alone among the receptors has an inefficient kinase domain, requiring heterodimerization with other family members to become phosphorylated. The need for heterodimerization juxtaposes the PI3K signal emanating from HER3 with the Ras-Raf-ERK or STAT signal emanating from EGFR, HER2, or HER4.

Figure 5-1 General overview of major signaling pathways involved in oncogenesis (many of the molecular targets emphasized in this chapter are outlined in yellow). Cell surface receptors (e.g., EGFR, VEGFR, cMET) normally bind various growth factors and small molecules initiating cell signaling pathways, which lead to maintenance of various cell processes. Mutations involved in up-regulated oncogene or down-regulated tumor suppressor activity lead to inhibition of apoptosis, cell proliferation, epithelial mesenchymal transition (EMT), angiogenesis, migration/invasion, and a variety of additional pro-oncogenic processes.EGFR Family and Tumor PathogenesisIn 1986, the Nobel Prize was awarded to Stanley Cohen for the discovery of growth factors, resulting from his work in identifying EGF and its receptor.18 EGFR was first identified as a proto-oncogene because of its homology to the avian erythroblastosis (v-erb) oncogene.19 Aberrant function of EGFR or HER2 occurs frequently in human tumors; gene amplification results in massive overexpression in a proportion of gliomas and breast cancers.20-22 Alternatively, dysregulation occurs at more modest levels of expression when the receptor is activated as the result of autocrine stimulation, in which the tumor produces its own ligand to activate the receptor. This type of dysregulation occurs frequently in cancers of the head and neck, gastrointestinal system, and prostate gland.23-26 Another mechanism of dysregulation is the development of mutations in the kinase domain that render the kinase activity more potent, most clearly demonstrated in lung cancer.27 Likewise, mutations in the ligand-binding domain can cause the receptor to be constitutively active even in the absence of ligand, as occurs in a significant proportion of gliomas.28 Dysregulation via mechanisms other than amplification may not always result in overexpression as detected by standard immunohistochemical techniques, raising the issue of how best to identify all tumors in which EGFR dysregulation promotes tumor proliferation and resistance to therapy.EGFR Family InhibitorsThe frequent dysregulation of the EGFR family in tumors makes the family an attractive target for exploitation. Remarkable progress has been made in the development of EGFR family inhibitors.29 Many antibodies directed against the extracellular domains of EGFR and HER2 and small-molecule tyrosine kinase inhibitors have now been approved by the FDA for clinical application, and many more are in various stages of development.8,30The first EGFR family–targeted agent to be approved with radiation was cetuximab (Erbitux), an anti-EGFR monoclonal antibody that binds to the extracellular domain of EGFR, interferes with ligand binding and, hence, dimerization and activation.31 Cetuximab has modest activity as a single agent but gives more encouraging results when it is combined with cytotoxic therapy. Cetuximab is typically given intravenously on a weekly schedule. When combined with radiation therapy, a loading dose is given the week before initiation of radiation

treatment. Cetuximab has gained FDA approval for use in treating metastatic colorectal cancer and in locally advanced head and neck cancers. Initial approval for cetuximab in treating metastatic colorectal cancer was based largely on the results of a positive trial including 329 patients randomized to receive either cetuximab and irinotecan or cetuximab alone.[32] Further molecular analysis demonstrated that patients with KRAS mutations in codons 12 or 13 did not respond to cetuximab with survival benefits seen only in patients with wild type KRAS; this is presumably related to alternate activation of signal transduction pathways.[33,34] In head and neck cancers, we have yet to determine biomarkers that predict response to anti-EGFR therapy. This finding underscores the complexity of cancer biogenetics and marks an important turning point toward an era of personalized cancer therapy.The anti-Her2 antibody trastuzumab and more recently pertuzumab, an antibody that inhibits Her2 heterodimerization, have been shown to improve outcomes for patients with Her2 overexpressing breast cancer.[35-37] These agents are currently recommended for combined use by the National Comprehensive Cancer Network (NCCN) in a variety of stages and settings. Additional investigations are ongoing in other cancers with Her2 overexpressing, including esophageal and salivary duct tumors.Further discussions regarding the use of EGFR inhibitors alone or with chemotherapy are beyond the scope of this chapter, however, we provide references related to these avenues.[38-44] Our focus is a review of the use of these agents with radiation preclinically and the successes and failures in the clinical arena. One of the most frustrating aspects of this combination is the fact that we still struggle with a lack of predictive biomarkers related to response to EGFR inhibitors. Is it the ligand presence that predicts response? Does the presence of an EGFR mutation always predict response to EGFR inhibitors in diseases like lung cancer, or do we need to dig deeper?[45] Is it based on gene amplification or high gene copy numbers in EGFR wild type cancers?[46]Several small-molecule tyrosine kinase inhibitors targeting EGFR have gained FDA approval: gefitinib (Iressa), erlotinib (Tarceva), afatinib (Gilotrif), and lapatinib (Tykerb). These compounds specifically inhibit the tyrosine kinase activity of an EGFR family receptor while relatively sparing the other EGFR family members and related tyrosine kinases. Gefitinib and erlotinib act on EGFR; lapatinib is active against HER2; and afatinib targets both EGFR and HER2. These small-molecule agents have shown modest benefits in patients with advanced malignancies (primarily in patients with EGFR mutations); however, they have yet to demonstrate any significant benefits in phase II/III clinical trials with irradiation. One phase II trial of erlotinib with temozolomide and radiation in patients with newly diagnosed glioblastoma multiforme (GBM) exhibited unacceptable toxicity with multiple treatment related deaths and no evidence of increased efficacy.[47]The primary toxicity of both gefitinib and erlotinib occurs in the skin, similar to cetuximab, and as diarrhea. However, infrequent cases of serious, life-threatening interstitial lung disease have also been reported for both agents, as well as anaphylactic reactions in approximately 3% of patients treated with cetuximab. Because these reactions can be life threatening, careful monitoring is required with these agents.EGFR Family and Radiation ResponseEGFR family members play an important role in radiation response. Preclinical studies showed that cells made to express v-erb were rendered radioresistant.[48] Similarly, breast cancer cell lines become more radioresistant when made to overexpress HER2, and head and neck cancer cell radioresistance correlates with EGFR expression levels.[49-52]Clinical studies also suggest that EGFR family dysregulation influences radiation response. A study of 170 gliomas treated with primary radiotherapy demonstrated lower response rates in tumors that overexpressed EGFR; the response rate was 33% in EGFR-negative tumors, 18% in EGFR-intermediate tumors, and 9% in EGFR-positive tumors.[53] In smaller series of patients with head and neck cancers, locoregional recurrence after radiotherapy was associated with EGFR overexpression.[54,55] In breast cancer, a case-control series of patients with in-breast tumor recurrence after breast-conserving surgery and radiotherapy found that the proportion of patients with HER2 overexpression was higher in the recurrence group than in the controls.[56]**Preclinical Studies of EGFR Family Inhibitors as Radiosensitizers**The role of EGFR family members in radiation response was further clarified by studies using newly developed EGFR family inhibitors. In virtually every study, EGFR or HER2 inhibitors demonstrated modest radiosensitization.[57-59] Radiosensitization is more pronounced in vivo than in vitro and with fractionated-dose than with single-dose irradiation. The understanding

of the mechanisms underlying enhanced radiosensitization is evolving.[60-62] In every case, the combination of EGFR or HER2 inhibitors and radiation resulted in increased cell cycle arrest, predominately in the G1 phase, but with a substantial decrease in S phase, which translates in vivo to decreased proliferation. Radiosensitization with EGFR family inhibitors also causes decreased angiogenesis. It is not yet clear whether this is an additive result of combined antiangiogenesis effects from both radiation and EGFR inhibitors, or whether the EGFR inhibitors further increase the susceptibility of the vascular elements of the tumor to radiation. The combination of EGFR inhibitors and radiation increases apoptosis in some, but not all, models. Finally, EGFR inhibitors appear to directly interfere with EGFR induced DNA-PK-dependent nonhomologous end joining repair of radiation-induced DNA damage.[63,64] What is lacking in past studies were designs that actually mimicked what we do in the clinic, including comparing chemoradiation to chemoradiation plus an EGFR inhibitor for example in the disease setting to be clinically studied rather than extrapolating from another tumor type. These types of studies, albeit performed in somewhat artificial systems, might have provided valuable information as to the best way to move forward in a clinical trial.**Clinical Studies of EGFR Family Inhibitors as Radiosensitizers**
Promising preclinical studies with EGFR family inhibitors have translated into improved patient outcomes in randomized controlled trials when added to radiation alone primarily in patients with locally advanced head and neck squamous cell carcinoma (LA-HNSCC). The most mature of these is a phase III trial that compared the efficacy of standard radiotherapy with standard radiotherapy plus the anti-EGFR antibody cetuximab. In this study, 424 patients with LA-HNSCC of the oropharynx, hypopharynx, or larynx were stratified by T stage, nodal status, and performance status and then were randomly assigned to receive radiotherapy alone or radiotherapy plus weekly concurrent cetuximab. Radiotherapy was delivered using one of three fractionation regimens (stratified): a once-daily regimen (2 Gy × 35 fractions over 7 weeks), a twice-daily regimen (1.2 Gy × 60 to 64 fractions over 5 to 5.5 weeks), or a concomitant-boost regimen (1.8 Gy × 30 fractions with a second daily fraction of 1.5 Gy for the last 12 treatment days over 6 weeks). Concurrent chemotherapy was not allowed. Two-year local control was 56% in the radiotherapy-plus-cetuximab arm versus 48% in the radiotherapy-alone arm, with a median duration of local control of 36 months versus 19 months, respectively ($p = 0.02$).[65] Overall survival was also significantly enhanced with combined therapy. A recent update reported 45.6% 5-year overall survival in the radiotherapy-plus-cetuximab arm versus 36.4% in the radiotherapy-alone arm, with median survival of 49 months versus 29 months, respectively ($p = 0.018$).[3]Although concern for unwanted normal tissue effects when combining targeted drugs against EGFR and radiation therapy alone is justifiable, the additional morbidity of this approach appears minimal based on the experiences in the clinic to date. In the study by Bonner et al,[65] the improvement in outcome was associated with an increase in acute skin, but not mucosal, toxicity. Furthermore, there were no differences between the groups on standardized quality-of-life assessment scores. Interestingly, a grade 2 or greater acneiform rash correlated with better survival rates (HR 0.49, $p = 0.002$). This correlation, seen in other studies in multiple disease sites, has been hypothesized to be reflective of an anticancer immune response.[66,67] In a separate trial, concurrent administration of adjuvant radiotherapy with trastuzumab in patients with early-stage breast cancer did not increase the incidence of acute radiation toxicity.[68] Results have been mixed in trials of EGFR inhibitors added to combination chemotherapy and radiation. RTOG 0234, a phase II randomized trial comparing radiation plus cetuximab and either weekly cisplatin or weekly docetaxel in patients with LA-HNSCC, suggest that both regimens are feasible with outcomes superior to results from RTOG 9501 that used high-dose cisplatin on days 1, 22, and 43.[69] Grade 3 to 4 myelosuppression was observed in 28% (cisplatin) and 14% (docetaxel) of patients. Dermatitis was seen in 39% of patients in each group. The rates of grade 3 or higher mucositis were 37% and 33% in the cisplatin and docetaxel arms, respectively; these rates seem low but are encouraging compared with historical controls. The 2-year distant metastasis rate was 13% in the group that received docetaxel and cetuximab versus 26% with cisplatin and cetuximab. One of the conclusions from this trial is that perturbing growth factor signaling may allow us, under the right circumstances, to reduce administration of high doses of standard chemotherapy

and reduce patient morbidity. The addition of cetuximab to the current standard of cisplatin and radiotherapy for LA-HNSCC was evaluated in the phase III RTOG 0522 with abstract only results thus far showing no improvement in progression-free survival (PFS) or overall survival (OS), equivalent overall grade 3 to 5 toxicities, and increased grade 3 or higher mucositis and skin reactions in patients receiving chemoradiation with cetuximab versus chemoradiation alone.1 In the phase II trial ACOSOG Z4051, 70 patients with locally advanced esophageal adenocarcinoma received preoperative therapy with the EGFR monoclonal antibody panitumumab added to docetaxel, cisplatin, and radiation.7 Despite 54% of patients showing at least a near pathologic complete response, nearly half of all patients had grade 4 or higher toxicities making this regimen unsuitable for further study. To our knowledge, this type of combination was not studied preclinically to assess its efficacy and safety before going forward into a clinical trial.An alternative strategy is to evaluate the use of induction chemotherapy before combination EGFR inhibition and radiation. The TREMPLIN study, a phase II randomized trial, directly compared the addition of cisplatin versus cetuximab to radiation for 116 patients with LA-HNSCC of the larynx/hypopharynx with a greater than 50% response following three cycles of traditional induction chemotherapy.70 Larynx preservation at 3 months, larynx function at 18 months, and overall survival at 3 years demonstrated equivalent outcomes. Local failures were slightly higher in the cetuximab arm (8 vs. 5), but only the patients in the cetuximab arm eventually underwent salvage surgery (7 vs. 0). Grade 3 or higher rates of mucositis were similar between arms, and skin toxicity was roughly doubled in the cetuximab arm; however, acute renal toxicity was substantial in the cisplatin plus radiotherapy arm. This suggests that perhaps by using induction chemotherapy we may be able to study combinations of biologically targeted agents without the added toxicity of conventional chemotherapy agents, assuming the preclinical studies support this approach. An additional trial, the Gruppo di Studio Tumori della Testa e del Collo (GSTCC) trial, employed a 2 × 2 factorial design for 421 patients with LA-HNSCC randomized with and without induction chemotherapy and randomized to concurrent radiation with either cetuximab or cisplatin.71 Abstract results showed similar response rates, PFS, and OS between patients receiving cetuximab versus cisplatin. Toxicity rates were also similar between the two groups and patients receiving cetuximab actually required more treatment interruptions with a median radiation therapy (RT) duration of 8 weeks versus 7 weeks in the cisplatin arms. Additional retrospective analyses examining cetuximab versus cisplatin have suggested that cisplatin provides better local control but similar overall survival outcomes.72,73Why the failures to date? As mentioned at the beginning of this chapter, perhaps the optimal preclinical studies were never performed. Often, biologic agents like EGFR inhibitors are combined with radiation in the laboratory and the assumption is made that this will be just as effective, if not more so, when combined clinically with chemoradiation. Preclinical studies must seek to optimize clinically relevant standards to truly understand the optimal sequencing and combinations for integrating EGFR inhibitors with, and perhaps without, radiation, chemotherapy, and additional biologics. Currently, multiple RTOG trials continue to address these issues in head and neck cancers in the setting of intermediate or high-risk postoperative settings (i.e., 0920, 1216) and oropharynx cancers that are positive for human papillomavirus (HPV) (i.e., 1016). Additional RTOG trials examining EGFR targeting concurrently with radiation include RTOG 0839 using panitumumab in locally advanced NSCLC, RTOG 0974 with trastuzumab for Her2 positive breast ductal carcinoma in situ, and RTOG 1010 employing trastuzumab for locally advanced esophageal adenocarcinoma. These are a handful of examples of the ongoing efforts needed to cull out which patients benefit from targeting a particular pathway such as epidermal growth factor signaling and which patients, based on specific mutations, may need interference with Akt, mTOR, or DNA repair pathways in addition to or in lieu of traditional chemotherapy approaches.
Angiogenesis Inhibitors**Angiogenesis and Tumor Pathogenesis**All tumors require development (or expansion) of blood vessels to promote further tumor growth and nutritional support beyond a 2-mm diameter.74 Molecules such as vascular endothelial growth factor (VEGF) mediate stimulation of angiogenic signaling and neovascularization. Elevated levels of specific isoforms of VEGF and other indirect markers predict for a worse prognosis in many types of cancer, including those of the gastrointestinal tract, such as pancreatic and esophageal cancers.75,76 VEGF

expression is affected by both the genetic aberrancies of the particular cancer as well as the microenvironmental changes, including hypoxia.Once VEGF binding activates VEGF-receptor signaling, a cascade of transcriptional signals to promote blood vessel formation is set in motion (see Figure 5-1). Tumors develop a nutritional support system by borrowing existing blood vessels, growing new vessels from surrounding endothelium, and entrapping circulating endothelial stem cells. In contrast to mature vessels, developing tumors display vessels that are immature and chaotic, with a resultant lack of cohesion within the vessel matrix. As a result of increased permeability, blood perfusion through the tumor can be heterogeneous. This can lend itself to areas of hypoxia, which in turn results in activation of pro-angiogenic molecules such as HIF1 and nuclear factor kappa B (NFκB). Transcriptional activation occurs with further production of VEGF and additional pro-angiogenic proteins such as cyclooxygenase (COX)–2, Tie-2, osteopontin, histone deacetylase, and hepatocyte growth factor. An autocrine and paracrine cascade is created to further tumor growth and invasion.Angiogenesis InhibitorsThree main strategies under preclinical or clinical investigation exemplify the important concepts underlying angiogenic inhibition: (1) small-molecule tyrosine kinase inhibitors (TKIs) that target vascular endothelial growth factor receptor (VEGFR) signaling, (2) agents that directly target the tumor vasculature, or vascular targeting agents (VTAs), and (3) agents that inhibit VEGF.Multiple VEGFR-TKIs have now gained FDA approval including sorafenib (Nexavar), sunitinib (Sutent), axitinib (Inlyta), pazopanib (Votrient), regorafenib (Stivarga), vandetanib (Caprelsa), and cabozantinib (Cometriq). Many of these biologics target multiple TKIs in addition to VEGFR signaling. They are indicated for treatment of a variety of cancers including unresectable hepatocellular carcinoma, advanced or metastatic renal cell carcinoma, metastatic colorectal cancer, gastrointestinal stromal tumors, advanced soft-tissue sarcomas, and metastatic medullary thyroid cancer based on positive results of phase III randomized studies. Vandetanib and cabozantinib are active against VEGFR, EGFR, and more specifically RET kinase, a mutation present in the majority of patients with sporadic or inherited medullary thyroid cancer. These drugs have now been FDA approved for advanced or metastatic medullary thyroid carcinoma following phase III trial results with each significantly prolonging PFS.76-80Agents that target the microtubule formation of intratumoral vasculature, thus destabilizing vessels and causing rapid necrosis within the central part of the tumor, might be effective compounds to combine with radiation. The rationale for pursuing agents that attack intratumoral vessels is based on the premise that endothelial cells in tumors display a different growth pattern than those in normal tissues; it is a chaotic, rapidly expanding pattern. VTAs were developed to take advantage of this differential to selectively occlude or destroy tumor blood vessels. Little progress has been achieved in clinical trials with VTAs, in part because of issues related to cardiac toxicity; however, this remains a potentially promising approach.An alternative approach, inhibiting VEGF using an anti-VEGF monoclonal antibody is akin to scooping up the keys rather than blocking the lock. Antibodies against VEGF have shown anticancer activity in a variety of preclinical models.81 Bevacizamab (Avastin) is a recombinant humanized version of the murine antihuman VEGF monoclonal antibody rhuMAb VEGF. Bevacizumab is FDA approved for use in patients with metastatic colorectal cancer when used in combination with 5-fluorouracil (5-FU). Two separate clinical trials demonstrated superior response rates, PFS, and OS in patients treated with combined 5-FU–based therapy with bevacizumab compared with others treated with 5-FU–based therapy alone in either the first- or second-line setting.82,83 The addition of bevacizumab to carboplatin and paclitaxel also improved overall survival in chemotherapy-naïve patients with metastatic or recurrent, nonsquamous non–small cell lung cancer (NSCLC) and has been approved for use in patients with recurrent GBM, based on trials demonstrating good rates of radiographic response and stable to decreased corticosteroid requirement.84,85 Improvements in PFS have also been demonstrated in patients with renal cell carcinoma and breast cancer receiving combinations of traditional systemic therapy and bevacizumab.86 These promising clinical results in the advanced setting have prompted further investigations in the locally advanced setting with radiation.Preclinical Studies of Angiogenesis Inhibitors as RadiosensitizersAt first glance, one might hesitate to consider blocking angiogenesis from a radiation oncology perspective. If

sequencing is not optimal, blocking angiogenesis might to lead to increased hypoxia and reduced tumor control. In fact, the last 10 years have demonstrated the opposite effect: enhanced radiation sensitivity with anti-VEGF agents in the laboratory. Early work by Teicher et al[87-90] demonstrated enhanced radiation cytotoxic effects in a variety of antiangiogenic models. Antiangiogenic agents may enhance the effect of radiation by stabilizing tumor vasculature, thereby enhancing tumor oxygenation.[91] By inhibiting proangiogenic signaling, regulation of antiapoptotic proteins such as amplified NFκB may be improved. Many of these molecules, including VEGF, are activated by radiation, so reversing this process seems logical.In the clinical trial setting, we have typically combined antiangiogenic agents with chemoradiation; however, we struggle with determining the optimal sequencing. To this end, there have been preclinical studies looking at this issue. ZD6474, a dual VEGFR/EGFR inhibitor, was evaluated with radiation in a xenograft model bearing EGFR-TKI–insensitive NSCLC Calu-6 tumors.[92] Two combined treatment schedules were examined: (1) a concurrent schedule using ZD6474 (50 mg/kg) dosing given 2 hours before the first dose of radiation, and (2) a sequential schedule using ZD6474 dosing given 30 minutes after the last dose of radiotherapy. The sequential approach was superior in terms of the time for treated tumors to quadruple in volume (RTV4) from their pretreatment size ($p < 0.0001$). Importantly, the reduced RTV4 (30 ± 1 day) in the concurrent schedule was also significantly better than either ZD6474 or radiation alone ($p < 0.02$). Nevertheless, clinical trials tend to simply combine these agents concurrently with chemoradiation, ignoring preclinical results.Similar to studies showing enhanced radiation effects with VEGFR signaling interference, studies combining anti-VEGF antibodies with radiation confirmed that although exposure of human tumor xenografts to radiation promoted induction of VEGF expression, inhibiting VEGF with anti-VEGF antibodies supplanted this effect and resulted in increased endothelial cell killing and synergized antitumor effects in murine tumor model systems.[93]Clinical Studies of Angiogenesis Inhibitors as RadiosensitizersUnfortunately limited success has been realized in the clinic. In locally advanced rectal adenocarcinoma, initial phase I studies by Willett et al[94] in rectal cancer brought to light clinical evidence of the vascular normalization hypothesis in which regulation of tumor vasculature may impair entry of metastatic cells into circulation while improving drug delivery and tumor oxygenation for radiosensitization. Multiple subsequent trials have examined the efficacy of either anti-VEGF or anti-EGFR therapies added to standard preoperative chemoradiation regimens for rectal cancer with modest pathologic complete response (pCR) rates ranging from ~10% to 20%.[5,95,96] One recent phase I/II trial examined the combination of bevacizumab and erlotinib with 5-FU and radiation in this setting with an encouraging pCR rate of 33% (9/27) although ~47% of patients experienced at least one grade 3 to 4 toxicity.[5]In patients with GBM, two recently published major randomized phase III trials examined the addition of bevacizumab versus placebo to standard temozolomide and radiation in newly diagnosed patients.[97,98] Both RTOG 0825 ($n = 637$) and AVAglio ($n = 921$) showed a significant PFS improvement of ~4 months with bevacizumab, but similar overall survival outcomes between arms with a median survival of ~16 to 17 months. MGMT status did not influence response rates between arms in both trials. In the RTOG trial, patients receiving bevacizumab showed higher rates of decline in neurocognitive function and quality of life; whereas in the AVAglio trial, quality-of-life outcomes were improved in patients receiving bevacizumab. Adverse events were modestly increased in the bevacizumab arms of both trials. In the context of these results, the role of bevacizumab in upfront GBM treatment remains unclear.Results have been more cautionary in combining bevacizumab with EGFR targeting and radiation in lung cancer. Based in part on promising results with bevacizumab and erlotinib in patients with metastatic NCSCL, several phase I/II trials in locally advanced lung cancer have been completed. Bevacizumab and chemoradiotherapy were associated with a concerning incidence of tracheoesophageal fistula and aerodigestive hemorrhage in 3 of 29 patients with small cell lung cancer and NSCLC.[99] A more recent phase I/II trial treated 45 patients with stage III NSCLC with induction, concurrent, and consolidative regimens of bevacizumab and erlotinib with radiation and chemotherapy.[4] Both PFS and OS results were not significantly different from current published results with standard chemoradiation, and on the downside, a significantly higher than expected 29% of patients on this

trial had grade 3 to 4 esophagitis. Of note, the radiation in this trial treated elective mediastinal lymph nodes and used three-dimensional conformal radiotherapy to a total dose of 74 Gy (a dose recently shown to have inferior OS when compared to the standard 60 Gy by RTOG 0617); these combined factors likely contributed at least partially to the increased esophagitis rate.[100] The SWOG trial S0533 also attempted to integrate bevacizumab with chemoradiation in a three-step design with administration with docetaxel in the consolidation phase after cisplatin-etoposide and radiation.[101] Because of poor accrual and Cancer Therapy Evaluation Program (CTEP) warnings regarding toxicity, the trial was stopped early. Two of seven patients in the high risk cohort experienced grade 5 hemoptysis.Bevacizumab as a radiosensitizer has also been studied in cervical cancer with a recently completed RTOG 0417 trial giving the agent every 2 weeks for three cycles during concurrent cisplatin and radiotherapy in 49 patients with stage IB-III disease.[102] With primary endpoints focused on adverse events, this trial noted minimal protocol-defined toxicity with 13 patients (26.5%) having grade 3 toxicities; 5 patients (10.2%) experiencing grade 4 toxicity; and no grade 5 toxicities. The majority of all toxicities were hematologic. In terms of efficacy, 3-year rates showed an 81% OS, 69% disease free-survival, and 23% loco-regional failure.[103] These outcomes are on par with published major trials using standard chemoradiation in cervical cancer.[104]Thus, in 2014, we are still searching for successful clinical combinations of antiangiogenic agents with radiation in a multitude of disease sites with no positive phase III trials to demonstrate that this strategy is working. Jackson et al[105] further discuss the current use of bevacizumab in combined modality settings with an emphasis on cervical cancer in their recent review, providing the reader with a comprehensive discussion of current and future avenues.PI3K/Akt/mTOR Pathway, Inhibitors, and RadiosensitizationOne of the most important pathways related to cancer cell survival, the PI3K/Akt/mTOR kinase pathway (see Figure 5-1), is a central regulator of cell metabolism, proliferation, and survival by preventing apoptosis. Furthermore, PI3K/Akt/mTOR is up-regulated in many tumors and can also be up-regulated with radiation.[106] Selective and pan-PI3K inhibitors, PI3K and mTOR dual inhibitors, Akt inhibitors, and mTOR inhibitors are all being developed. Given the variety of targets and specificities of these agents, current research has focused on their efficacy, resistance, and toxicity profiles in a wide range of tumors, especially those with pathway alterations (e.g., loss of PTEN, KRAS mutations, and TSC1/2 alterations).[107]The agents furthest along in development to date are mTOR inhibitors, primarily rapamycin analogs. These include temsirolimus and everolimus, which block signal transduction downstream of mTOR and facilitate apoptosis, suggesting effectiveness as radiosensitizing agents. Currently these two drugs are approved for clinical use in advanced renal cell carcinoma, and everolimus is also approved for pancreatic neuroendocrine tumors, subependymal giant-cell astrocytomas, and advanced breast cancer with exemestane.[108-110] Their clinical role as radiosensitizers is less established but is an active area of research.To date, preclinical cancer cell models have demonstrated radiosensitizing effects of mTOR inhibitors.[111, 112] Temsirolimus is as effective as cisplatin in radiosensitizing HNSCC lines, and triple-combination therapy did not provide additional cooperative effects over temsirolimus with radiation.[112] The in vivo effects were more dramatic partly because of an antiangiogenic byproduct of mTOR inhibition.The radiosensitizing role of mTOR inhibitors continues to be investigated through clinical studies with mixed results. In a phase I trial of temsirolimus combined with palliative thoracic radiation in patients with NSCLC, dose-limiting toxicities included sudden death, pneumonitis, and pulmonary hemorrhage; however a safe maximum tolerable dose level was achieved.[113] Three separate phase I trials have examined the combination of radiation, temozolomide, and an mTOR inhibitor (i.e., temsirolimus or everolimus) in patients with GBMs, and infectious complications and stomatitis were the primary toxicities.[114-116] These trials have provided the basis for the phase I/II RTOG 0913 trial (i.e., everolimus with radiation and temozolomide up front and in combination with adjuvant temozolomide in GBMs) and the phase II EORTC 26082 (i.e., temsirolimus versus temozolomide in both the concurrent radiation and adjuvant settings for patients lacking methylation of the MGMT promoter with gliomas). In LA-HNSCC, the mTOR pathway has been shown to mediate expression of eukaryotic protein synthesis initiation factor 4E (eIF4E), with elevated eIF4E expression in histologically cancer-free margins associated with increased risk for

recurrence. Based on this evidence, Fury et al117 performed a phase I trial adding everolimus to standard of care concurrent cisplatin and radiation therapy in 13 patients with LA-HNSCC. Three patients experienced dose-limiting toxicities (i.e., two with mucositis, one with failure to thrive), and lymphopenia was the most common grade ≥3 adverse event seen in 12 patients (92%). Overall, a tolerable everolimus dose of 5 mg daily was found, and at 19.4 months follow-up, only 2 patients (15%) had experienced recurrent disease. Translation of these results into phase II and III trials and beyond is anticipated.The remaining classes of PI3K/Akt/mTOR pathway inhibitors are still in their infancy with many additional phase I/II trials under way.118 Several investigations are specifically examining their potential as radiosensitizers in both curative and palliative settings including LA-HNSCC, NSCLC, and malignant gliomas. Emerging data in HPV-positive head and neck cancers suggest an increased prevalence of PI3 kinase (PI3K) pathway mutation and copy number alterations.119 In HPV-positive tumors harboring PI3K mutations mTOR appears activated rather than AKT downstream and thus may be a reasonable target to combine with radiation for this subgroup of patients.120 A phase I trial of BKM120 or buparlisib, an oral PI3K inhibitor with cisplatin-radiation, is in the process of opening in patients who are HPV positive with locally advanced head and neck cancers.8 Next-generation sequencing is a powerful tool that continues to deliver new information on the genomic landscape of many cancers including head and neck and should lead to more personalized cancer care.121
DNA Repair Inhibitors: Focus on PARP**PARP and DNA Repair**Up-regulated DNA repair within cancers contributes to radioresistance and is a concept across all histologies. DNA damage from radiation or chemotherapy results in a variety of mechanisms that attempt to fix both single- and double-stranded breaks quickly, so cancer cells can continue to replicate and grow. To counteract this repair, one promising strategy incorporates the use of poly(ADP-ribose) polymerase (PARP) inhibitors. The PARP family consists of 17 proteins with PARP-1, 2, 3, and 4 and Tankerase 1 and 2 playing key roles in posttranslational modification of proteins involved in multiple pathways.122, 123 PARP-1 is the most heavily studied of these enzymes and is activated by base damage, single-stranded DNA breaks, and double-stranded DNA breaks caused by insults, including chemotherapy and ionizing radiation.124 Activation leads to poly(ADP-ribosyl)ation of PARP-1, nicotinamide adenine dinucleotide (NAD+) consumption, a localized negative charge, and direct enzyme interactions with subsequent interaction of multiple pathways involving DNA repair (especially XRCC1), chromatin restructuring, and cell-cycle check points (Figure 5-2).125
Figure 5-2 Overview of the role of poly(ADP-ribose) polymerase (PARP) activation in response to DNA damage from chemotherapy or irradiation. Activation of PARP triggers various DNA repair pathways, cell cycle regulation, and regulation of gene expression facilitated by chromatin restructuring.Initial interest in PARP inhibition in oncology stemmed from the concept of synthetic lethality in which cancer cells with preexisting deficiencies in homologous-recombination pathways (e.g., BRCA mutations) exhibit selective cytotoxicity to single agent PARP inhibitors. Although many complex interactions occur with PARP inhibition, these cancer cells' susceptibility is in part attributed to their reliance on PARP-dependent DNA repair pathways such as base excision repair.126 Remarkable activity has been observed in patients with BRCA1/2 mutations in a phase I study using olaparib (AZD2281), an orally bioavailable PARP inhibitor.127 Importantly, minimal toxicity was observed in doses up to 600 mg twice a day. Objective antitumor activity was reported only in mutation carriers (22/60 patients entered, all of whom had refractory ovarian, breast, or prostate cancer). Two subsequent international phase II trials in patients with metastatic BRCA1/2 mutated breast (*n* = 57) and ovarian cancers (*n* = 54), respectively, treated two cohorts with the oral PARP inhibitor olaparib at doses of 400 mg twice daily (i.e., the maximum tolerated dose) and 100 mg twice daily.128,129 These trials saw objective response rates of ~30% to 40% in the high-dose groups and ~10% to 20% in the low-dose groups. Both trials reported mostly low grade toxicities with grade 3 to 4 toxicities limited to fatigue, nausea, vomiting, and anemia.Beyond synthetic lethality, PARP inhibitors show promise as chemosensitizers and radiosensitizers by directly preventing cancer cells from repairing induced DNA damage. In the preclinical setting, many groups have shown the ability of PARP inhibitors to sensitize a variety of histologies, both p53 wild type and null, to radiation in both in

vitro and in vivo settings.130-136 These results are rapidly being translated into the clinic with open phase I and II trial using a variety of PARP inhibitors with radiation and additional systemic agents in sites including central nervous system (CNS), head and neck, breast, lung, esophagus, pancreas, and rectum (Table 5-2).8 Additional trials have focused on PARP inhibitors with DNA damaging chemotherapies, particularly temozolomide. Specifically, the current RTOG 0929 trial is exploring the use of the oral PARP inhibitor ABT-888 with temozolomide for patients with recurrent GBMs.137 In development through the NRG (National Surgical Adjuvant Breast and Bowel Project (NSABP), the Radiation Therapy Oncology Group (RTOG), and the Gynecologic Oncology Group (GOG)) cooperative group mechanism is a trial that will evaluate this compound with radiation in patients with newly diagnosed GBM. Of note is the fact that PI3K inhibitors can down-regulate BRCA1/2, suggesting a possible dual targeting approach with these agents and PARP inhibitors to prevent homologous recombination and DNA damage repair.138**TABLE 5-2Select PARP Inhibitors Currently Undergoing Clinical Trials8,9**

Agent	**Manufacturer**	**Sites**
BMN-673	Biomarin	BRCA(+) breast, solid tumors, hematologic malignancies
CEP-9722	Cephalon	NSCLC
E7449	Eisai Incorporated	Advanced solid tumors or B-cell malignancies
Iniparib	Sanofi	Breast, NSCLC
Niraparib	Tesaro	Ovarian, BRCA(+) breast
Olaparib	AstraZeneca	BRCA(+) ovarian, gastric, NSCLC, glioblastoma, esophagus, HNSCC, CRC
Rucaparib	Clovis Oncology	Ovarian, fallopian tube, peritoneal,
Veliparib	Abbott Labs	Pancreatic, ovarian, cervical, breast, NSCLC, DPG, SCLC, liver, prostate, melanoma, metastatic solid tumors, leukemia, myeloma,

CRC, Colorectal carcinoma; *DPG,* diffuse pontine glioma; *HNSCC,* head and neck squamous cell carcinoma; *NSCLC,* non–small cell lung cancer; *SCLC,* small-cell lung carcinoma.CHK1 and WEE1In addition to PARP inhibitors, recent investigations into targeting DNA repair related cell-cycle proteins CHK1 and WEE1 have begun to generate excitement for their role as radiosensitizers. In response to DNA damage, these proteins help mediate both the S and G2 phase checkpoints.139 Both CHK1 and WEE1 are up-regulated in P53 mutated cancers, which already bypass the G1 checkpoint.140 Theoretically, inhibition of either CHK1 or WEE1 would render P53 mutant cancer cells susceptible to DNA damaging treatments such as radiation resulting from critical checkpoint failures, while allowing normal tissues, with intact G1 checkpoints, to remain relatively unaffected. Although lacking preclinical data, several phase I trials are evaluating WEE1 inhibitors with concurrent radiation and chemotherapy in patients with recurrent GBMs, cervical cancer, and unresectable pancreatic adenocarcinomas.8Immune Targeted Biologics**Cancer and the Immune System**In the past decade, increasing research emphasis has been placed on understanding the role the immune system plays in preventing and controlling cancers and the system's response to radiation. Despite maintaining a multitude of distinctly nonself-antigens among clonal cell populations, many cancers still develop the capability to circumvent the immune system's surveillance of nonself-antigens. Recent research has elucidated that these cancers reach a state of immune tolerance through halting the immune system at immune checkpoints and preventing T-cell activation through alteration of both stimulatory and inhibitory signaling.141 Two clinically significant regulators of the immune checkpoint are cytotoxic T-lymphocyte associated antigen 4 (CTLA-4), which inhibits initial T-cell activation, and programmed cell death protein 1 (PD-1), which suppresses subsequent T-cell activity in peripheral tissues and tumors.142-144 Evolving quickly in many different disease sites, we may be entering a new frontier of durable responses never quite seen before in deadly diseases. Can the efficacy of immunologic strategy be amplified with radiation?CTLA-4 InhibitorsIpilimumab, a CTLA-4 antibody, is the first FDA-approved targeted therapy to specifically promote the body's antitumor immune response, rather than directly targeting cancer cells. This human monoclonal antibody's approval for use in unresectable or metastatic melanoma was based on results from two recent phase III trials.145,146 One trial in 676 patients with metastatic melanoma saw a 3.6-month improvement in median OS from 6.4 months with placebo to 10.0 months with ipilimumab, regardless of whether patients received an

additional vaccine that was also part of the study.145 The 2-year OS nearly doubled for patients on ipilimumab versus placebo (23.5% vs. 13.7%). Regarding toxicity, 14 treatment-related deaths occurred and 10% to 15% of patients had grade 3 to 4 immune-related adverse events. A subsequent trial tested the addition of ipilimumab versus placebo to dacarbazine in 502 patients with metastatic melanoma and again saw a significant improvement in OS.146 Patients receiving dacarbazine with ipilimumab versus placebo had median OS of 11.2 months versus 9.1 months and 3-year OS of 20.8% versus 12.2%. Although no drug-related deaths occurred, grade 3 to 4 adverse events occurred in 56% of patients in the ipilimumab arm versus 27.5% of those in the placebo arm. As previously seen, this difference was primarily as a result of increased immune-related events including skin, gastrointestinal, and hepatic toxicity. Another CTLA-4 inhibitor, tremelimumab, also underwent multiple phase I-III trials but was not approved because of dose levels inducing unacceptable toxicities and lack of a proven survival advantage.147Ipilimumab, Radiation, and the Abscopal EffectFirst proposed in 1953, the abscopal effect has since been elucidated as an immune-mediated systemic cancer response induced by localized radiotherapy.148,149 Since ipilimumab's FDA approval in 2011, at least four cases have been published linking its use with radiation to the abscopal effect.150-152 In each case, the patients received ipilimumab either before, concurrent with, or subsequent to stereotactic body radiotherapy or radiosurgery. The high doses of radiation given to each patient have been postulated as necessary to induce sufficient tumor necrosis to allow for an immune response.A group from Memorial Sloan-Kettering initially reported on a case of a woman with metastatic melanoma who received ipilimumab for four doses followed roughly 1 year later by palliative radiotherapy to a pleural-based spinal para mass to 2850 cGy in three fractions. Following one additional ipilimumab dose 2 months after radiation, the patient was noted to have radiographic regression of both the irradiated lesion and multiple additional distant metastases that were previously progressing.150 This same group subsequently reported on a patient with metastatic melanoma receiving radiation to an internal mammary lymph node with regression of distant, nonirradiated, left axillary lymph node metastases. In a separate report, a patient with metastatic melanoma received stereotactic radiosurgery to a brain metastasis with concurrent ipilimumab and had a complete clinical response of all metastatic lesions.151 Notably, his titers of melanoma autoantibodies to melanoma antigen A3 increased from 1 : 300 to 1 : 700 following radiation and ipilimumab, supporting a systemic immune response. A Stanford group noted a complete systemic response in a patient with metastatic melanoma receiving stereotactic radiotherapy to two of eight hepatic lesions sandwiched in the middle of four cycles of ipilimumab.152 Despite the promise of these cases and the growing use of radiation and ipilimumab in patients with melanoma, the abscopal effect remains a rare phenomenon. Ongoing research is investigating the optimal radiation and ipilimumab dosing and timing and the possibility of a biomarker indicating which patients will be most suitable for these treatments. Currently, the National Cancer Institute reports six clinical phase I or II trials hoping to further elucidate the underlying immune mechanisms of the abscopal effect and possibly finding a consistently reproducible method of inducing this phenomenon.118PD-1 and PDL-1 InhibitorsIn a similar but separate immune suppressing vein, the primary effect of PDL-1 binding of PD-1 is suppression of the cytotoxic T-cell response in peripheral tissues and tumors.153 This mechanism was initially elucidated in the setting of chronic viral infections and inflammatory states, but in the past decade, basic and translational research has shown that PD-1 pathway activation provides an alternative approach to CTLA4 through which cancers can achieve a durable state of immune tolerance.144,154,155 Notably, cancers have been shown to activate this pathway via two different mechanisms. In one method, a tumor develops constitutive overexpression of PDL-1 on its membrane providing immune suppression regardless of tumor microenvironment. Separately, tumor cells can be induced to express PDL-1 by cytokines (e.g., interferon-γ [IFN-γ]) in a local inflammatory state.156Several molecular therapeutics targeting PD-1 or its ligand PDL-1 are currently under investigation in multiple disease sites with published results appearing promising to date. Three separate phase I studies of more than 600 total patients with advanced cancers of eight separate disease sites receiving an anti-PD-1 or anti-PDL-1 antibody showed objective responses in ~10% to 50% of patients with

grade 3 to 4 toxicities in the range of 10% primarily involving immune-related complications.157-159 In each study, a subset of patients was noted to have durable response off therapy. A recent report notes two patients who achieved eventual durable complete responses lasting more than 3 years off therapy and one patient with 16 months of a stable off-therapy partial response, eventual relapse, and subsequent partial response for at least 16 months following reinduction of anti-PD-1 therapy.160 In a subset immunohistochemical analysis of tumors for one of these trials, only those tumors expressing PDL-1 by immunohistochemical analysis achieved a response.159 Based on results of one of these trials focusing on melanoma patients, pembrolizumab (previously lambrolizumab) has gained expedited FDA approval through the FDA Breakthrough Therapies program.9 In addition, many more trials are ongoing, and further research with these agents is needed to determine optimal dosing and timing; whether patients can be selected for these therapies based on molecular studies; whether these agents can be combined with complementary immunomodulatory biologics such as ipilimumab; and whether these agents have a role in conjunction with radiation including the interesting possibility of an alternative mechanism for generating an abscopal effect. A more in-depth review of immune-targeted biologics by Pardoll et al is provided for reference.141Future DirectionsSeveral other classes of agents have not yet received FDA approval but have significant potential as radiosensitizers. The central role of signal transduction, DNA repair, and cell cycle control in radiation response leads to obvious interest in investigating agents that selectively perturb these processes in tumor cells as radiosensitizers.161,162 Additionally, newer agents targeting cancer stem cells (CSCs) may help impair tumor resistance and relapse following radiation, surgery, and systemic therapies. Here we briefly discuss the bright future of several of these classes.c-METc-MET, another receptor tyrosine kinase, binds hepatocyte growth factor (HGF) and has been associated with a variety of oncogenic pathways including angiogenesis, cell proliferation, DNA damage repair, epithelial-mesenchymal transition (EMT), and the related metastatic capabilities of cell motility and invasion.163,164 Recent in vitro and in vivo studies have shown that c-MET is up-regulated in many cancer histologies and also up-regulated further in irradiated cells possibly also playing a role in radioresistance.165,166 At present a variety of these biologics are in varying stages of development as far as phase III trials; however, minimal clinical applications have attempted to use these in conjunction with radiation. Further preclinical investigations examining the radiosensitizing effects of these agents will be necessary before reaching the clinical stage as radiosensitizers.TGF-βAn intriguing area of anticancer research that is associated with chronic inflammation and potentially tied into the immune-modulating story is the transforming growth factor β (TGF-β) pathway.167 The TGF-β pathway is found in abundance in solid tumors and is associated with malignant progression through a variety of interactions on the tumor cell and in the surrounding microenvironment. What is its specific mechanism of action? TGF-β1 signaling occurs primarily through a heteromeric complex of type II and type I TGF-β receptors that activates the Smad pathway by TβRI-mediated phosphorylation of Smad2 and Smad3. Subsequent nuclear relocation of receptor-associated phosphorylated Smads bound to Smad4, results in activated TGF-β-driven transcriptional responses.168 The TGF-β receptor complex can also signal via non-Smad pathways to affect cell survival and EMT.The paradox lies in the fact that TGF-β actually demonstrates tumor suppressive effects that cancer cells attempt to get around; yet later, TGF-β promotes cancer cell proliferation and invasion once the suppressor activity is blocked (Figure 5-3). Evidence exists that radiation can activate the TGF-β signaling pathway with cross-talk activation of COX-2.169 This phenomenon has been observed in normal tissue injury in patients treated with radiation.170 For a wonderful overview of TGF-β signaling and its association with cancer progression as well as opportunities to exploit this pathway for improved anticancer effects we point you to an extensive review by Dancea et al.171

Figure 5-3 The TGF-β pathway exhibits tumor suppressive effects through cell cycle regulation and apoptosis early on in carcinogenesis. Eventually, cancer cells bypass the inhibitory signaling, and TGF-β becomes tumor promoting via a variety of hallmark oncogenic pathways, while concurrently up-regulating TGF-β production.Targeting Cancer Stem CellsIn

the CSC model, a select group of CSCs drive the renewal, differentiation (including epithelial-to-mesenchymal transition), invasion, and often treatment resistance of an overall heterogeneous population of a cancer.172 Notch, WNT, and the hedgehog (HH) pathways play central roles in the livelihood of these CSCs, offering a handful of promising biologic targets. Multiple phase I and II clinical trials testing inhibitors of these pathways are under way in a variety of solid tumors with early results showing general tolerability but mixed efficacy.173-175 Furthermore, the potential to impair repopulation makes CSC pathway inhibitors attractive agents as radiosensitizers, especially in the setting of cancers known to exhibit radiation-induced accelerated repopulation such as HNSCC. An early in vivo study presented at ASCO 2014 found that the combination of a HH antibody with radiation in mice with primary cervical cancer xenografts led to better tumor growth delay, reduced lymph node metastasis, increased survival, and had no overt toxicity when compared to radiation alone.176The heterogeneous population of cancers and CSCs may be responsible for the mixed efficacy of biologics previously discussed in this chapter. Nevertheless, this challenge offers an opportunity founded in the hypothesis that cancers may be more vulnerable to combined modality therapies including combinations of complementary biologics, not necessarily limited to CSC pathway inhibitors.172 In the clinical setting, increased expression GLI-1, a zinc finger protein induced in HH pathway activation, was recently shown to be associated with increased metastasis, progression, and overall survival independent of stage and EGFR expression in a retrospective analysis of HNSCC tumors from patients treated on RTOG 9003.177 Subsequently, Keysar et al178 showed that in vitro and in vivo HH pathway inhibition drove HNSCC cells into an EGFR-dependent state thereby preventing cetuximab resistance and significantly impairing tumor growth when combined with cetuximab. A better understanding of such cross-talk signaling pathways among CSCs and the collective cancer cell population will be critical in determining the most clinically effective and tolerable combinations.Designing Clinical TrialsIf the past few years are any indication, we can expect continued exponential growth of targeted therapeutic agents in oncology. With endless permutations of dosing, timing, and combinations of these agents and modern radiation and chemotherapies, increasing emphasis must be placed on careful design and implementation of clinical trials. The NCI and RTOG recently released collaborative strategic guidelines for early stage development of radiosensitizers.179 The guidelines highlight multiple hurdles in the development of radiosensitizers including difficulties in translating preclinical studies, poor endpoints such as tumor response rates which are expected to occur with radiation alone, and attributing toxicity to an agent versus radiation in a single-arm phase I trial. Furthermore, they provide recommendations for optimizing radiosensitizer trials including rigorous evaluation of preclinical and single-agent clinical data; avoidance of redundant pharmacokinetic studies; efficient methods of dose-escalation; and accurate assessment of expected toxicities. Given the extensive costs of these modern targeted therapies, we must continue to investigate biomarkers for optimal selection of patients whose tumors are most likely to respond to a specific intervention. This sentiment has been echoed by ASCO's recent Choosing Wisely recommendations and recently pursued by large-scale screening efforts.180,181 Large-scale phase III trials in unselected patients are an inefficient and extremely costly means for scientific progress in our field. A notable alternative to the current trial design paradigm is the concept of the basket study design. In this setting, trials would test targeted therapies in patients with specific mutations regardless of cancer site or histology or within a specific disease site such as lung or rectal cancer with therapy directed based on the mutation status of each patient. Advances in next-generation sequencing will play a central role in making these types of trials feasible.In addition, and very exciting, is the recent discoveries using cancer personalized profiling by deep sequencing (CAPP-Seq), an efficient and relatively inexpensive ultrasensitive method for quantifying circulating tumor DNA (ctDNA). As an example, Diehn et al182 at Stanford applied CAPP-Seq for NSCLC with a design covering multiple classes of somatic alterations that identified mutations in >95% of tumors. Subsequently, they detected ctDNA in 100% of patients with stage II-IV NSCLC and in 50% of patients with stage I, with a remarkable 96% specificity for mutant allele fractions down to ∼0.02%. Importantly, the measured levels of ctDNA were highly correlated with tumor volume and were accurate in distinguishing between residual disease

after radiation and treatment-related imaging changes by computed tomography (CT) or positron emission tomography (PET). ctDNA levels also assisted in determining earlier response to therapy compared to radiographic measurements that can often be difficult to assess. This type of emerging technology offers a glimpse into future possibilities when combining novel agents with radiation and will help us determine if patients are developing resistance to a particular drug. Additionally, integrating radiation into this new framework would need to account for site-specific targeting and toxicity factors (Figure 5-4). With less than 15% of current phase I trials involving radiation, much work lies ahead for our field toward better understanding the role of targeted therapeutics as radiosensitizers, presenting exciting opportunities to improve treatment results. [118]

Figure 5-4 Preclinical studies leading to clinical translation using molecular discoveries such as next-generation sequencing to rationally tailor and sequence various biologics during and after radiation therapy for locally advanced disease—getting away from traditional chemoradiation approaches.References

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182. Newman AM, Bratman SV, To J, et al. An ultrasensitive method for quantitating circulating tumor DNA with broad patient coverage. *Nat Med*. 2014;20(5):548–554. **Part B**Physics **Outline**Chapter 6 Radiation Oncology PhysicsChapter 7 Radiation Physics: Stereotactic **Chapter 6** Radiation Oncology Physics*J. Daniel Bourland*Radiation oncology is a physical medical modality by which radiative energy is delivered to a target volume to effect palliation or cure. An understanding of the particles and processes involved in imparting radiation energy to matter is fundamental to the clinical application of radiation to patients.In the irradiation of a biologic system, physical and biologic events occur in the following order:1. *Physical events:* Physical interactions (e.g., photoelectric, Compton, collisional) result in ionizations and radiation dose.2. *Chemical events:* Ionizations result in broken atomic and molecular bonds or chemical changes.3. *Biological events:* Changes in the chemistry of molecules mean that there are changes in biological function (i.e., cells have improper or changed function).4. *Clinical events:* Biologic alteration may result in clinical changes such as tumor regression, cancer induction, and tissue fibrosis.Matter and Physical Definitions**Atomic and Nuclear Structure, Particles, and Nomenclature**Matter is made up of atomic and nuclear particles that have interaction and binding energies from 1 to 10 million electron volts (MeV). The smallest structural subunit that retains the character of an element, the atom, has a nucleus consisting of one or more protons and zero or more neutrons and a surrounding cloud of orbiting electrons. In an atom with zero net electrical charge, the number of orbital electrons equals the number of protons. A simplistic representation shows the atom to be neatly arranged with neutrons and protons in the nucleus and electrons in uniform orbits (Figure 6-1). In reality, the atom is a dynamic structure with defined energy states for the electron orbits and the nucleus. Detailed atomic and nuclear models have been formalized.1,2 **Figure 6-1** The atom has a nucleus composed of protons and neutrons. The nucleus is surrounded by a cloud of electrons in distinct energy levels called *orbitals* named s, p, d, and so on by chemists or called *shells* named k, l, m, and so on by physicists.Neutrons and protons are the building blocks of the nucleus; hence, they are called *nucleons.* The two particles have similar rest masses (the mass of the particle at rest, when kinetic energy is zero) but different electrical charges. The neutron, symbolized by *n,* has no charge (neutron for neutral) and the proton, *p,* has a charge of +1. The electron, *e,* has an atomic mass about that of a proton or neutron and has a charge of −1. Besides the neutron, proton, and electron, other particles exist with unique masses and properties (e.g., spin). These include neutrinos, pions, muons, and others. The neutrino was first proposed as the neutral accompanying particle emitted in beta decay. There has been great interest in determining the mass of the neutrino because the cumulative neutrino mass may account for the “missing mass” in the universe, with great cosmologic implications. More recently it has been determined that the neutrino mass, inferred through observed oscillations in the neutrino state,3,4 is finite and also small (close to zero). However, even with nonzero mass, the total amount of neutrino mass is estimated to be insufficient to make up for the “missing” mass. The universe is now stated to be made up of matter (4.6% the material “stuff” we are familiar with), dark matter (23%), and dark energy (which comprises more than 70% of the total mass-energy content of the universe).5 These “dark” components are unknown entities.Particles are classified by their mass as leptons or hadrons. Leptons, which include the electron and neutrino, are “lightweight” particles with mass comparable to that of the electron and spin of . Hadrons are heavy particles with two subclasses called *mesons* (middleweight, spin 0 or 1) and *baryons* (heavyweight, spin or). Fundamental, or elementary, particles are those that have no subparts and cannot be divided. All leptons are elementary particles; however, the neutron and

proton are not and are instead made up of three fundamental particles each, called *quarks,* which have been observed through high-energy physics experiments. Quarks have positive or negative charge in integer increments of , spin of , and other properties with whimsical, quark-deserving names.1 Combinations of quarks yield the neutron, proton, and all other hadrons. For instance, a proton is made of two up quarks and one down quark, whereas a neutron is made of one up and two down quarks, which explains their similar mass but difference in charge. Antimatter is real, and an *antiparticle* is defined as a particle with identical mass but opposite charge to the particle. An antiparticle for a neutral particle has opposite spin or internal charge (i.e., quark) compared with the particle.Matter is held together by four fundamental forces that operate over certain ranges and with certain particles. They are the strong, electromagnetic (coulomb [C]), weak (there are two), and gravitational forces, with respective relative strengths of 101, 10−2, 10−13, and 10−42.1 Each force acts by exchange of its respective mediator particle: the gluon, the photon, the W and Z particles, and the graviton (which has not yet been discovered). Counting mediators, leptons, hadrons, and their antiparticles, there are about 170 fundamental and composite particles that make up matter.1 The model in which matter consists of fundamental particles as described previously is called the *standard model.*1 Table 6-1 summarizes the fundamental particles in the standard model, which are six quarks, six leptons, six antiquarks, six antileptons, and also the four known forces. Of conceptual importance is the 2012 discovery of the “Higgs boson,” predicted in 1964 by Higgs, which is a heavy particle (~125 GeV in energy) responsible for the character of matter we have named “mass.” The Higgs boson is placed by itself alongside the other entries to the standard model as the description of fundamental particles that make up matter (Table 6-1). How fundamental particles fit together to make up other particles continues to be studied. A relatively new theory called *string theory* is a descriptor of how quarks and other fundamental particles are assembled with vibration (energy) states for stringlike entities that give each fundamental particle its unique character.6 However “flexible” they may be, the strings still need additional dimensions called “branes” (after “membranes”) to enable their full character to be described.7,8 Clearly, the fundamental properties of the universe continue to be studied in basic physics research.**TABLE 6-1The Standard Model of Matter: Fundamental Particles and Mediators**Particle mass can be expressed as the atomic mass unit ([amu]; of the mass of the carbon nucleus) or in units of energy by conversion with Einstein's formula, E = mc2. Table 6-2 gives the symbol, charge, mass, and stability for the electron, proton, neutron, and other particles of interest.**TABLE 6-2Physical Characteristics of Selected Atomic and Nuclear Particles***amu,* Atomic mass unit.Combinations of nucleons form a variety of nuclei and determine the physical character of an atom. The number of protons in the nucleus, *Z,* is called the *atomic number* and determines the chemical properties of an atom and the atom's identity as an element. The atomic number also equals the number of electrons in the neutral atom, with one electron per proton. The number of neutrons in the nucleus, *N,* is called the *neutron number.* Whole protons and neutrons constitute the nucleus, and *Z* and *N* have integer values. An atom's mass number, *A,* is the sum of its neutrons and protons. The mass number (an integer) has a value close but not equal to the actual nuclear mass. Their values are similar but must not be confused. Nuclear mass is the noninteger sum of the masses of the individual particles minus their binding energies. Definitions for *Z, N,* and *A* are summarized in Table 6-3.**TABLE 6-3Atomic Nomenclature**
Nuclides and RadionuclidesNuclides are atomic species made of different combinations of nucleons, and they may be classified by their number of protons, neutrons, or nucleons (*Z*, *N*, or *A*) and by their energy state. Nuclei with the same *Z* but different *N* are called *isotopes* (p for proton), and they exhibit identical chemical characteristics (they are the same elements). Nuclei with the same *N* but different *Z* are called *isotones* (n for neutron). Nuclei with the same *A* but different *Z* and *N* are called *isobars*. In a last category, nuclei with the same *Z* and *N,* and therefore *A,* but different nuclear energy states (i.e., excited versus ground) are called *isomers.* Table 6-3 shows these classifications and example nuclides. A nuclide, or nuclear species, *X,* is denoted asin which *X* is the chemical symbol for the element with atomic number *Z*, *N* is the neutron number, and *A* is the mass number. Because *A* = *Z* + *N*, the *N* value is often dropped to give the following form:

Because the atomic number determines the element's name, represented by the chemical symbol *X*, the *Z* is also dropped to give the form *AX*. An alternative nomenclature uses the nuclide's name followed by the mass number, such as hydrogen-3 and iridium-192.Not all combinations of *Z* and *N* exist in nature or can be manufactured. Instead, certain combinations are possible, whereas other combinations cannot occur. Figure 6-2 shows the distribution of stable nuclides as a function of the number of neutrons and protons. Notice that at low *Z,* the ratio of neutrons to protons (*N *: *Z*) is about 1.0. Above *Z* = 20, stable nuclides have more neutrons than protons (*N*/*Z* > 1.0). At higher *Z*s, the stability of nuclei tends toward neutron-rich nuclides. It has been observed that nuclei with 2, 8, 20, 28, 50, 82, or 126 nucleons (protons and neutrons combined) are stable. These stability “magic numbers” relate to the filling of nuclear energy levels, similar to the complete filling of electron shells. Pairing of like nucleons also results in increased nuclear stability. There are 165 stable nuclei with an even number of both protons and neutrons, 57 stable nuclei with an even number of protons and odd number of neutrons, 53 stable nuclei with an odd number of protons and even number of neutrons, but only 6 stable nuclei with an odd number of both protons and neutrons.
Figure 6-2 Distribution of stable and naturally radioactive nuclides. Data from Radiological health handbook. Bethesda, MD, U.S. Department of Health, Education, and Welfare, 1970.

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