

## Chapter 2

# Biology of Large Dose per Fraction Irradiation

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**Keywords** Radiobiology of IORT • Tumor oxygenation • Hypoxic radiation sensitization

### Introduction

Experimental radiobiology has by happenstance focused on the implications of intraoperative and high-dose-per-fraction radiotherapy in more detail than it has standard fractionated radiotherapy. This is because the majority of radiobiological literature of tumor and normal tissue features in vivo and in vitro studies in which the radiation was administered in a single fraction. Similarly, when fractionation is used experimentally, fraction sizes near the clinical 1.8–2 Gy size used for most external beam irradiation therapy (EBRT) are rarely utilized. As a result, much of our radiobiological understanding of tumor and normal tissue response should and does relate well to that observed clinically for intraoperative irradiation therapy (IORT).

The first and most important implication of single, large-fraction irradiation is the clear disadvantage it gives to tumor kill compared with sparing of normal tissue. The majority of radiosensitive organs, including the lung, kidney, small bowel, and brain, have substantial ability to recover between daily radiation treatments [1], whereas the ability of the tumor is typically much less pronounced [2]. Thus, on first principle, intraoperative radiation places normal tissues at a disadvantage if they remain in the IORT field (Fig. 2.1). Other classical advantages of fractionation, including reoxygenation and redistribution of the cell cycle, must be considered and it is difficult to justify single-fraction intraoperative radiation as the sole method of irradiation on radiobiological grounds. In particular, the dose required to control 50% of tumors is on average only minimally changed with fractionation because of reoxygenation, redistribution, and repopulation (Fig. 2.2).

The principal advantage of IORT is the ability to exclude nontarget normal tissues from the radiation field. The success of IORT, therefore, requires full knowledge of the partial organ tolerances of normal tissues. However, the radiobiological literature falls short with respect to fully characterizing the toxicity

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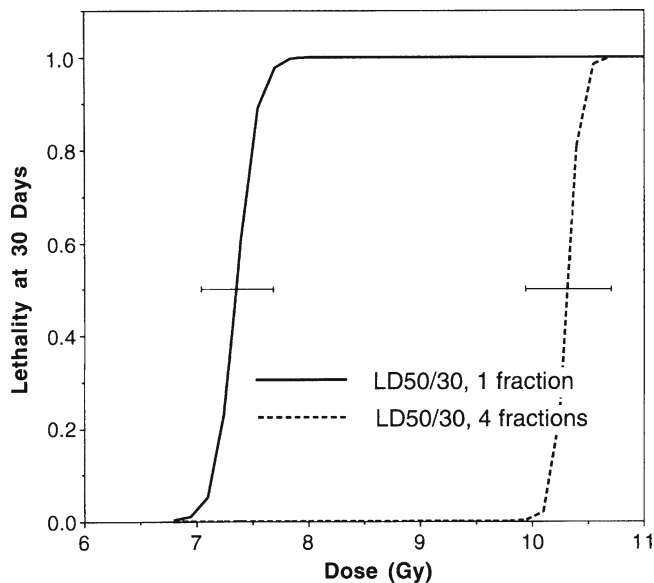
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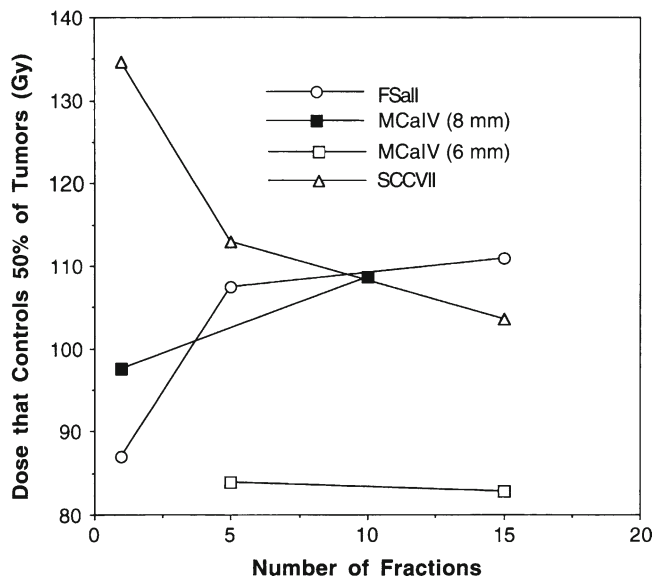
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**Fig. 2.1** Normal tissues benefit greatly from fractionation. The greatest benefit to fractionation is found in late reacting tissues like the lung, but even acutely reacting normal tissues benefit from fractionation. Bone marrow, for example, is an acutely reacting tissue. If whole-body irradiation is administered to C3H mice in a single fraction, the LD<sub>50/30</sub> is  $7.4 \pm 0.2$  Gy versus  $10.3 \pm 0.3$  Gy if the treatment is given in four fractions over two days. The calculated dose modifying factor of 1.4 is significant (95% CI 1.29...1.51) [105]. The error bars represent the 95% CI of LD<sub>50/30</sub>. Late-reacting tissues have larger dose-modifying factors with fractionation compared with a single fraction, usually greater than 2.



**Fig. 2.2** On average, tumor benefits little from fractionation due to competing effects of reoxygenation and cell cycle redistribution between fractions. Data from three different C3H tumor models and the dose that controls 50% of tumors (TCD<sub>50</sub>) are shown. Tumors are the FSaII fibrosarcoma, the MCalV mammary carcinoma, and the SCCVII squamous cell carcinoma. The therapeutic gain factors with fractionation were not significantly different from 1, and ranged from 0.77 to 1.28, with an average of  $1.05 \pm 0.23$  [64, 65, 106]. The absence of a clear increase in TCD<sub>50</sub> is remarkable considering that there can be substantial tumor growth between fractions, if the interfraction interval is long [107].

modification due to partial organ radiation. Lastly, delivering large-fraction radiation dose is appealing in view of the recent discovery that large, single doses of radiation may produce tumor autoimmunity.

This chapter reviews the classical radiobiological principles and some of the experimental and clinical data to help better understand the tolerances of normal tissues and tumor to large radiation doses.

### *Model Used to Predict Radiation Effects*

Several models have been used over the years to understand and quantify the radiation tolerances of tumor and normal tissues [3]. Perhaps the most successful and useful models are the clonogenic cell survival models. Based on these models, successful treatment results if all tumor clonogenic cells are killed by the treatment. By the same model, normal organ damage results if a regenerative unit is not preserved. For modeling tumor response, the clonogenic model has withstood extensive experimental scrutiny and has generally performed well. Use of the clonogenic model has been less successful in predicting normal tissue tolerance. The normal tissue model predicts tolerance best when the whole organ is treated. A limitation of the normal tissue model is the invention of a regenerative unit of tissue [1, 4]. This tissue unit is difficult to define based upon known organ physiologic and proliferative function.

Two clonogenic survival models are commonly used: the linear-quadratic model and the multi-target model. The former model predicts that survival of clonogenic units follows the shape of a parabola on log-linear coordinates while the latter model predicts that low doses of radiation kill few clonogenic units, and at higher doses the survival curve becomes linear on log-linear coordinates. The formulae for each of these survival curves are:

$$\text{Linear-quadratic surviving fraction} = S / S_0 = \left[ e^{(-\alpha d - \beta d^2)} \right]^n$$

$$\text{Multi-target surviving fraction} = S / S_0 = \left[ 1 - \left( 1 - e^{-d/d_0} \right)^N \right]^n$$

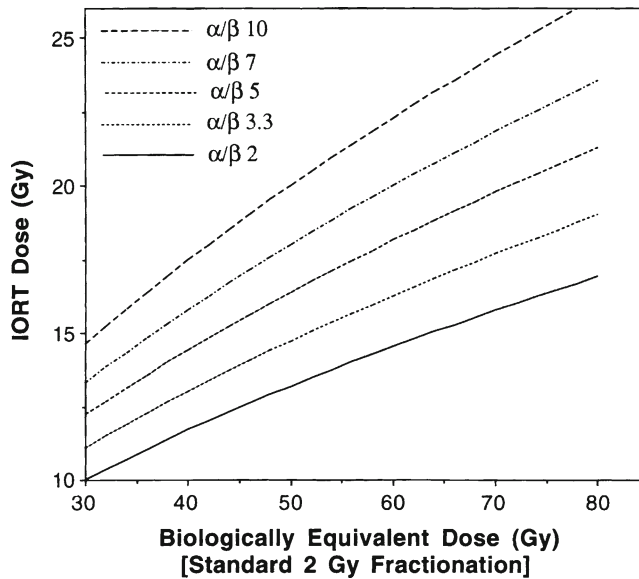
where  $d$  is the fraction dose,  $n$  is the number of fractions, and the remaining variables ( $N$ ,  $d_0$ ,  $\alpha$ ,  $\beta$ ) are fit parameters for the two models. In general, the linear-quadratic formula fits experimental data better at low doses (e.g., under 3 Gy), whereas the multitarget model better explains results at survivals under  $\approx 10^{-3}$  (e.g., above 10–15 Gy). In the dose range typically used for IORT (10–20 Gy), both models perform comparably.

Using the linear-quadratic model, the shape of the survival curve is determined by the  $\alpha/\beta$  ratio. This ratio has units of radiation dose. A low  $\alpha/\beta$  ratio is typical of late-reacting normal tissues. Most late-reacting tissues have  $\alpha/\beta$  ratios less than 5 Gy while acute-reacting tissues and tumor often have  $\alpha/\beta$  ratios of over 7 Gy. The simple, exponential mathematics make for convenient estimations of equivalent doses using the linear-quadratic model. Equivalent doses to compare IORT with standard 2-Gy fractionation can be estimated using the equation:

$$D_{\text{IORT}} = (1/2)(([\alpha/\beta]^2 + 4D_{2\text{Gy}}[\alpha/\beta + 2])^{0.5} - \alpha/\beta)$$

A graphic comparison of estimated equivalent doses, based on the above equation, is given in Fig. 2.3. For example, if one estimates the EBRT dose required to control a squamous cell carcinoma at 60 Gy delivered at 2 Gy per daily dose ( $D_{2\text{Gy}}=60$ ), and the  $\alpha/\beta$  of a squamous cell tumor is 10 Gy, then the equivalent single fraction needed to control the tumor would be  $D_{\text{IORT}}=22.3$  Gy. This dose is in good agreement with the  $\approx 20$  Gy estimated by classical Strandqvist plots [5, 6]. Evidence that the formulations actually produce the expected response has been shown in several clinical studies wherein the local control rate was predicted in the study design and then achieved.

Calculating the tolerance of a peripheral nerve with a conservative  $\alpha/\beta$  of 2 Gy and a generous tolerance dose of 70 Gy at 2 Gy per fraction, yields an equivalent IORT tolerance dose of only



**Fig. 2.3** The estimated biological effect of a given IORT dose is compared with that of radiation given in standard 2 Gy daily fractionation.  $\alpha/\beta$  values are chosen for conservative late reacting normal tissue 2, brain 3.3 [108], acute-reacting normal tissue 7 [109], and tumor 10 [110, 111].

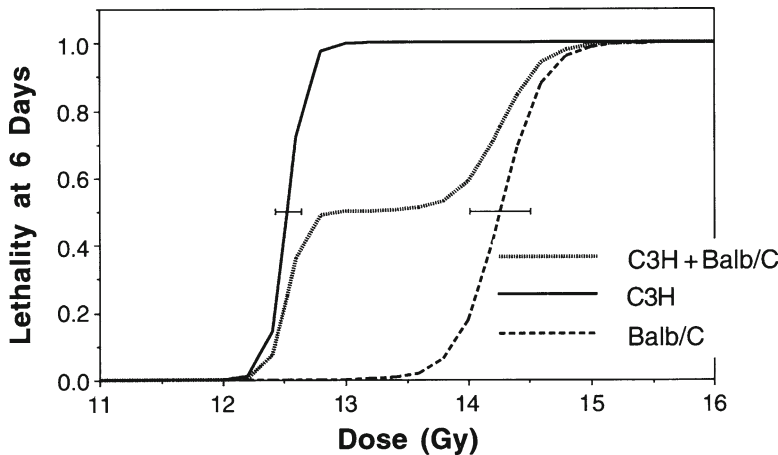
16 Gy. This number is similar to those obtained in canine and human studies. For the sacral plexus, the canine 5 year  $ED_{50}$  was 16.1–17.2 Gy, although the safe dose to nerve was 10 Gy and 25% of animals developed sacropathy at 15 Gy [7]. Sacral plexopathy in humans occurs at a slightly lower dose, with an estimated  $ED_{50}$  of 15 Gy at 2 years. The lower dose is probably related to the associated external beam, concurrent disease such as atherosclerosis and to chemotherapy [8].

With fractionation, the tolerance of peripheral nerve is higher than the tumor control dose. When radiation is administered in a single fraction, the tumor control dose becomes greater than the tolerance of the peripheral nerve. Thus, the normal tissue has a greater loss of tolerance due to the absence of fractionation. This phenomenon underscores the potential disadvantage inherent in any large hypofractionated radiation treatment approach. To be successful, therefore, IORT must take advantage of the surgical procedure to either exclude the nerve or other dose-limiting structure from the planned radiation field, or to accomplish a gross total resection of tumor so that lower IORT doses can be used. Since nerve rarely can be excluded from IORT fields, IORT should be used as a boost dose to supplement adjuvant EBRT (typically 45–50 Gy at 1.8–2.0 Gy fractions) and maximal resection as discussed in Chap. 10 of this text.

## Radiobiology of Normal Tissues

### *Dose Response of Normal Tissues*

Over the years, primarily based upon clinical studies with laboratory confirmation, the tolerance doses of normal tissues have been estimated and tabulated by several authors [9]. The dose-response curve of normal tissues is very steep. That is, small changes in dose near tolerance levels can result in large changes in the rate of complications [3]. For example, in estimating the whole body dose of radiation that causes half of C3H mice to die of gastrointestinal lethality (e.g.,  $LD_{50/6}$ ), none will die at doses under 11 Gy, and none will survive doses over 14 Gy (Fig. 2.4); at  $12.5 \pm 0.1$  Gy, half will survive the GI endpoint. Further, these tolerance doses can decrease 30% or more in animals that are not maintained in pathogen-free conditions.



**Fig. 2.4** Radiation toxicity to normal tissues typically occurs with a steep dose response. Figure 2.1 shows the steep dose response of bone marrow. In this figure, gastrointestinal toxicity is measured using the lethal dose at 6 days ( $LD_{50/6}$ ) following irradiation. For C3H mice, gastrointestinal death is rare below 11 Gy, and survival is rare above 14 Gy. A steep increase in lethality occurs between 11 and 14 Gy, with half the animals dying at a dose of  $12.5 \pm 0.1$  Gy. Gastrointestinal death occurs with a similarly steep dose response in BALB/c mice, but at a much higher dose. The effect of gastrointestinal irradiation of human subjects is likely to be just as steep for any individual. When populations of patients, each with individual genetic predispositions to gastrointestinal complications, are treated, the dose-response curve appears to be less steep. In the example, this is illustrated by the dose-response curve that might have been obtained had half the animals been C3H and half BALB/c. Also, note that if the C3H + BALB/c combinations model human population studies, one might conclude that mortality was 50% at 13 Gy, a dose at which no gastrointestinal deaths are expected in the BALB/c component of the population.

Clinically, the steepness of the response curve and the impact of fraction size can be seen easily. Two patients treated a few months apart with mantle irradiation fields are shown in Fig. 2.5. The first (left) was treated using single daily fields, anterior or posterior, using  $^{60}\text{Co}$  at 80 cm. The second (right) was treated with opposed fields. Both had a fraction size at midplane near 2 Gy; however, the second patient also had MOPP chemotherapy. The prescribed dose to the first patient was 40 Gy, but the effective fractionation at maximum, due to the inhomogeneous technique, was  $3.5 \text{ Gy} \times 10 \text{ fractions (anterior field)} + 1 \text{ Gy} \times 10 \text{ fractions (posterior field)} = 45 \text{ Gy}$ . The second had  $1.8 \text{ Gy} \times 25 \text{ fractions} = 45 \text{ Gy}$ . Despite the added chemotherapy, the late effects, including muscle wasting and permanent hair loss, are evident in the patient treated with a large fraction size. Hence, the dose response was steep enough that the change in fraction size had severe impact on late effects despite the similarity in total dose. Rib fragility, pulmonary fibrosis, pericardial constriction, and myocardial ischemia are other risks of altered-fractionation schemes.

The steepness of the dose-response curve aids in the selection of dose and of targets in IORT. If the radiation oncologist can maintain the IORT dose below the threshold dose for complications, then the risk of complication is expected to be minimal. Alternatively, if the radiation dose is above the tolerance range, then the oncologist can expect that the organ will be damaged and must assess the consequences of losing the function of the organ. When organ function is critical, the oncologist must either choose to omit the IORT or lower the dose delivered.

### *Vascular Effects of Single-Fraction Irradiation*

Radiation has a number of effects on vascular healing and angiogenesis. Vascular damage due to radiation is greatest for the smallest vessels, and is more pronounced in arteries compared to veins [10, 11]. Capillaries are typically the most severely affected by radiation, in part because of their



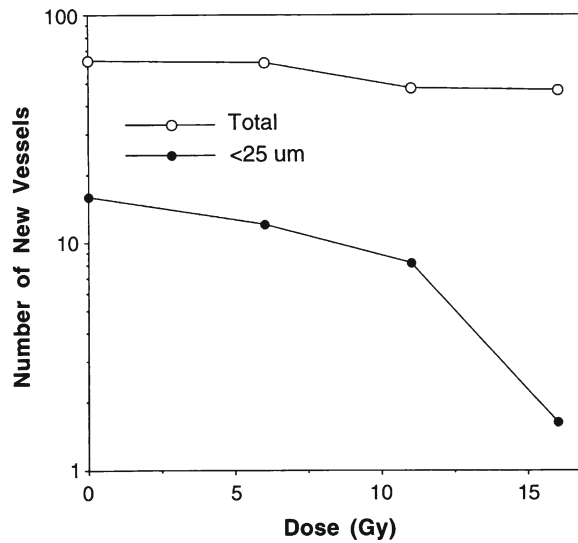
**Fig. 2.5** Clinically, the steepness of the response curve and the impact of fraction size can be seen easily. Two patients treated a few months apart are shown. The first (*on left*) was treated using single daily fields, anterior or posterior, using  $^{60}\text{Co}$  at 80 cm. The second (*on right*) was treated with opposed fields using 4 MV X-rays at 100 SAD. Both had a fraction size at midplane near 2 Gy. The prescribed dose to the first patient was 40 Gy, but the effective fractionation at maximum, due to the inhomogeneous technique, was  $3.5 \text{ Gy} \times 10 \text{ fractions}$  (anterior field) +  $1 \text{ Gy} \times 10 \text{ fractions}$  (posterior field) = 45 Gy. The second had  $1.8 \text{ Gy} \times 25 \text{ fractions}$  = 45 Gy. The late effects, including muscle wasting and permanent hair loss, are dramatically evident with the larger fraction size.

natural fragility, and in part because antiangiogenic effects of radiation can prevent their regeneration [11]. As with other late-responding tissues, damage to blood vessels is dependent upon both total dose and the dose of each fraction. It is already possible to detect differences in angiogenesis in skin after 6 Gy in mice, and after 11–16 Gy given in a single fraction there is a vast decrease in the capacity of mouse skin to generate microvasculature (Fig. 2.6). Likewise, in response to radiation-induced antiangiogenesis, angiogenic factors are among the early genes activated in irradiated connective tissue. These cytokines are, however, unable to correct completely the antiangiogenic deficit induced by radiation. Interestingly, large vessels have a complex response to irradiation that is incompletely understood. In the case of angioplasty damage to pig coronary arteries, low doses of radiation appear to increase intimal proliferation compared with angioplasty alone while intermediate doses of radiation reduce the natural, intimal proliferation seen 1–6 months after angioplasty [12]. In contrast to the beneficial prevention of endothelial proliferation at lower doses, fractionated irradiation taken to a total dose over 40 Gy is associated with a detectable increase in ischemic heart disease in pediatric lymphoma patients followed for over 5 years [13]. Hence, radiation can both increase (Fig. 2.7) and decrease hyperplasia of larger arteries, each with a different time course and dose response.

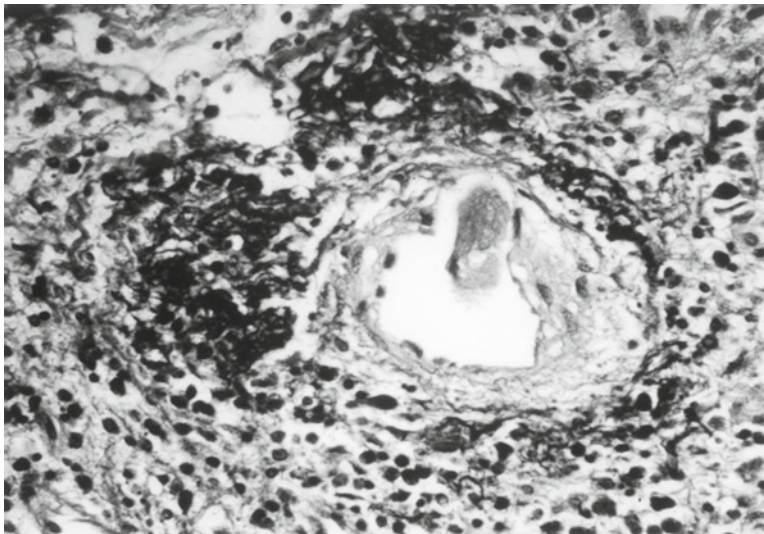
Studies of vascular tolerance in IORT of canine and human subjects appear to reproduce this complex dose and time response. Most vascular complications, like many of the neurological complications, are associated with fibrovascular proliferation and stenosis. In contrast, some data suggest that at the highest IORT doses (e.g.,  $\geq 25 \text{ Gy}$ ) radiation may actually decrease the natural intimal proliferation after vascular anastomosis [14]. Vascular rupture and aneurysm have also been described when large arteries must be taken to full dose. In this case, it appears that the vasa vasorum that feed the arterial wall have been damaged, with the small vessel disease then precipitating the large vessel complication [15].

The lack of a clear understanding of the dose-time effects of radiation on arteries limits our ability to fully understand the toxicity to any perfused tissue. Canine and clinical studies of radiation tolerance





**Fig. 2.6** Radiation doses of 0, 6, 11, or 16 Gy were given to the skin of C3H mice immediately prior to the injection of intradermal FSaII tumor cells. The tumor cells supply an angiogenic stimulus. Three days later, the angiogenesis was measured by a photographic technique [11]. Pre-irradiation of the skin results in a reduction of neovascular formation that is most severe as the dose exceeds 11 Gy in a single fraction. Large vessel number is well preserved at the full range of doses. Microvessels, however, were severely reduced, indicating that capillaries and nutritive vasculature are the most severely affected by irradiation of normal tissues. Conduit flow, which occurs in larger vessels, is better preserved.



**Fig. 2.7** The pulmonary arteries are normally thin-walled vessels. Four months after irradiation to a dose of 62 Gy at 2 Gy per fraction, there is substantial perivascular connective tissue proliferation, intimal proliferation, exposure of vascular basement membrane, and associated platelet thrombus. Vascular effects of large-dose-per-fraction irradiation are complex and can be difficult to predict; however, in most cases, the damage is more severe than with fractionated irradiation.

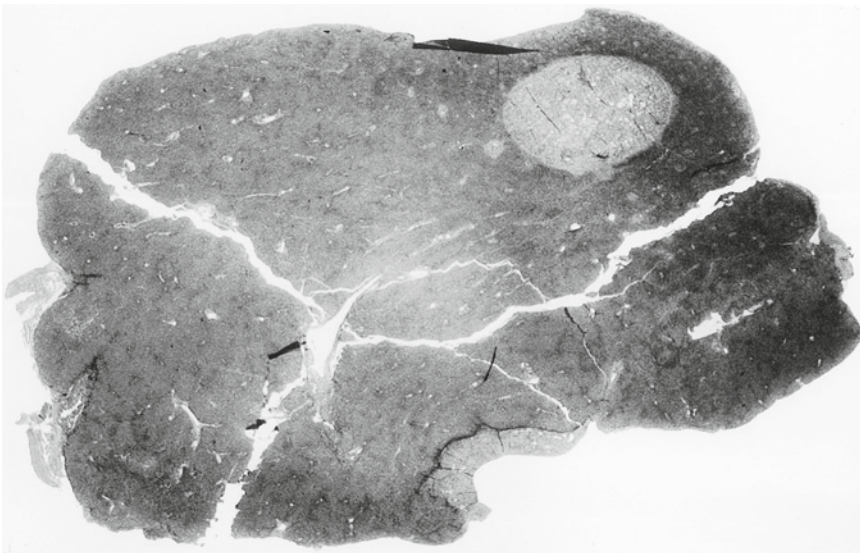
of large arteries, however, suggest that clinically significant complications are rare under 15–17 Gy and become common if circumferential irradiation over approximately 20 Gy is administered. In contrast, fractionated irradiation is usually safe even to coronary arteries at doses up to 40 Gy. Other large arteries are commonly given over 60 Gy safely when fractionation is employed. When fractionated

and single-fraction IORT irradiation are both given, the frequency of complication is similar to that expected from the IORT treatment alone. In either case, vascular ischemic complications increase with time, are dose dependent, and can take over a decade to occur.

### ***Partial Organ Tolerance***

The radiation dose safely tolerated by many critical organs is determined by the volume of tissue irradiated. For the central nervous system, the dose-volume relationship is well understood and can be easily quantified using several models [16–18]. The volume-response curve, like the dose-response curve, is steep. Namely, at a given radiation dose, the frequency of toxicity is low at small volume and, above a threshold volume frequency of complications rises quickly to near certainty. As an example, with a single dose treatment of the brain, the frequency of complication is minimal for targets under 3 ml (frequency under 3%) and rises to 40% for volumes over 10 ml [19]. Likewise, lung tolerance is generally quoted as less than 20 Gy with standard fractionation of the whole lung and under 16 Gy for total body irradiation [9]. In contrast, pulmonary dysfunction is rarely symptomatic even when doses of over 70 Gy are given to small lung volumes [20]. Similar observations have been made for partial organ treatment of the liver (Fig. 2.8) [21].

Unfortunately, more precise parameters for estimating partial organ tolerance are not available; however, certain rules apply. Circumferential treatment to a high dose is unwise for any hollow viscous organ or large vessel [14, 22]. Transmural treatment is tolerated less well than glancing treatment of hollow organs. Organs involved by tumor are at higher risk for fibrovascular complication. For example, ureteral and peripheral nerve tolerance appears to be lowered by tumor involvement [23]. Care should be made to limit irradiation of vascular grafts and bowel anastomoses, and all sutures should be placed securely and with some redundancy. Finally, portions of organs that can be sacrificed surgically can also often be safely treated to a high radiation dose (i.e., lung, liver).



**Fig. 2.8** A canine's liver was irradiated using a point source. At 1 month following irradiation, the liver shows a region of necrosis 3 cm in diameter, corresponding to the 15-Gy isodose volume. The animal had no detectable increase in liver function tests and no detectable hepatic dysfunction. Necrosis-inducing doses of radiation are well tolerated with no detectable metabolic abnormalities if only a small portion of the liver is irradiated [105].



Exceptions include the small bowel, which might perforate or obstruct if overdosed compared with benign resection of the same region of bowel [14, 22].

### ***Dose Rate Effects***

Dose rate effects rarely enter into IORT. This is because the surgical procedure must be completed in a timely manner. Dose rate effects do not become important clinically until rates under 5–10 cGy/min are achieved [24, 25]. Experimental models suggest that even lower dose rates are required to take full advantage of the dose rate effect [26]. In clinical practice, it is rarely, if ever, possible to slow dose rate to these levels when IORT is employed since the procedure duration would be lengthened by a minimum of 2–5 h.

### ***Clinical Modifiers of Normal Tissue Radiosensitivity***

Patients undergoing IORT have commonly undergone several surgical procedures, previous EBRT and multiple cycles of chemotherapy. Patients may also have other conditions, including cardiovascular disease, diabetes, collagen vascular disease, autoimmune disease, or undetected genetic instability syndromes (e.g., heterozygosity of ataxia telangiectasia, heterozygosity of Fanconi's anemia) [27–29].

The interaction between standard radiation and surgery on the IORT site is usually limited to the specific anatomy or its physiology. Delayed treatment-induced fibrosis is known to be more pronounced in patients who undergo irradiation before, after, or concurrent with a surgical manipulation. Delayed fibrosis can also worsen with time. Acute surgical toxicities may be exacerbated by irradiation. Toxicities include impaired granulation of irradiated tissue, and wound strength can be reduced. In performing IORT, it is usually possible to avoid treatment of skin, making the frequency of wound closure complications low. The interaction of radiation and surgery, however, in the tumor bed cannot be avoided.

Radiation and surgery can sometimes interact in more complex ways. For example, in animal models, if the left kidney is removed, and the entire right kidney is irradiated 1 month later, the radiation tolerance of the right kidney increases substantially [30, 31]. The hypertrophic response apparently leads to radiation protection in this animal model. In contrast, if the entire left kidney is irradiated, and the right kidney is immediately nephrectomized, the left kidney develops nephritis at a reduced dose [30, 31]. Here, the induction of a proliferative response seems to result in a stress that is poorly handled by an irradiated kidney.

### ***Chemical Modifiers of Normal Tissue Radiosensitivity***

The impact of chemotherapy on radiosensitization of tumor and normal tissues is difficult to predict. The enhancement ratio is a measure of radiosensitization induced by combinations of drug and radiation. The enhancement ratio is the differential cell kill obtained by the combination of radiation and drug after correction for the independent cytotoxicity of the individual therapies. The enhancement ratio may increase either due to a steeper slope and/or reduction in the shoulder of the radiation dose-response curve.

In general, a therapeutic gain is only obtained if the normal tissues irradiated are not similarly sensitized by the combination of radiation and drug. If the enhancement ratio seen by the tumor is

also experienced by the normal tissue, and the normal tissues must be irradiated, radiosensitizing drugs are of no theoretical advantage. IORT can be advantageous from this perspective, since it is frequently possible to exclude sensitized organs from the IORT port.

Enhancement ratios for chemical sensitizers are almost always greater when given with large radiation doses, such as IORT, because the enhancement ratio is diluted in a fractionated course of radiotherapy. An enhancement ratio of 2 indicates that cell kills normally seen at a given dose are seen at one-half of that dose. If such effects were seen clinically, responses would be dramatic. However, fractionation severely attenuates the enhancement ratios that are observed when radiation is given in a single fraction. It is common for large enhancement ratios of 2 or 3 to decrease to 1.1 or less with fractionation. This dilution is probably due to redistribution of tumor cells in the cell cycle, repopulation of tumor between fractions, reoxygenation, and other modifiers of the radiation dose-response curve. Since IORT emulates the experimental model in which radiation is given in a single fraction, the utility of combining radiation and radiosensitizing drugs are expected to be significant. Thus, radiosensitizing drugs with enhancement ratios of 1.1–1.5 might still be expected to be important biologically when radiation is given in large single fractions.

The interaction between drugs and radiation is most pronounced when both are used simultaneously [32]. Some drugs interact with radiation even if separated substantially in time, a phenomenon termed recall (Table 2.1). The most well-known drug in this category is doxorubicin, and related intercalating drugs include bleomycin [33, 34]. For other chemotherapeutic drugs, the interaction seems to be more pronounced if the chemotherapy is given following radiation. The possibility of

**Table 2.1** Radiosensitizing drugs with potential application to IORT

| Drug  | Proposed mechanism   | Mode of radiosensitization   |
|---|--|--|
| Adriamycin, Bleomycin, Actinomycin D, and Mitomycin C | Antibiotics: intercalation into DNA where it can remain for long periods of time     | Greatest radiosensitizing effect if given concurrent with radiation<br>Radiosensitization sometimes seen when given months or years before or after irradiation. Commonly associated with pulmonary fibrosis or cardiac toxicity |
| Cis-Platinum  | Alkylating agent   | Sensitizer of hypoxic cells even at very low concentration   |
| Cyclophosphamide                                      | Alkylating agent   | Primarily interacts in lung and heart<br>Toxicity greatest when given in close proximity to radiation  |
| 5-FU and Gemcitabine                                  | Antimetabolite: primarily S-phase cytotoxin. Complex mechanism of action             | Sensitizer of cells in the most radioresistant portion of the cell cycle.<br>Particularly useful for gastrointestinal malignancies   |
| Methotrexate  | Antimetabolite   | Primarily interacts in CNS. Worse if given with or after irradiation   |
| Paclitaxel  | Tubulin binder. Synchronizes cells in G2/M   | Places cycling cells in the most radiation sensitive portion of the cell cycle.<br>Sensitization requires appropriate schedule of drug before irradiation  |
| Topotecan and Camptothecin                            | Topoisomerase inhibition   | Greatest effect when given concurrently or in close proximity. Believed to sensitize by unraveling DNA and contributing to double-strand breaks  |
| Misonidazole, SR2508                                  | Nitromidazole<br>Radiosensitizers<br>Oxygen mimetic, hypoxic cell radiosensitization | Typically neurotoxic at radiosensitizer dose levels  |
| IUDR and BUDR   | Halogenated pyrimidines<br>Thymidine replacement in DNA                              | Sensitizes only actively replicating cells   |

deleterious interaction is decreased if the drug administration schedule is completed well before radiation. An example of a drug in this category is high dose methotrexate. If high dose is given following whole brain radiation, the neurocognitive complications are substantially greater than if it is given before the radiation [35]. This is probably due to the chronic subclinical radiation effects interacting with a drug toxicity that would have otherwise been subclinical and temporary.

In animal models and probably humans, alkylating agents can worsen pulmonary toxicity if given in close sequence to irradiation [36, 37]. Cisplatin, a bifunctional alkylating agent, is a powerful radiosensitizer of both tumor and normal tissues [38–45]. The effects are most pronounced at low doses [46–49]. At higher doses, cisplatin kills tumor cells and thus cannot sensitize those cells (cells cannot die twice). At low drug doses, however, radiation appears to enhance the sublethal drug toxicity. Both oxic and hypoxic tumor cells are prone to cisplatin-induced radiosensitization [50, 51].

Other chemotherapy drugs with independent cytotoxicity have also been studied with clinical success. Perhaps the most important of these being 5-fluorouracil (5-FU) [52, 53]. This drug has a complex mechanism of action and is particularly cytotoxic to S-phase cells. For radiation, S-phase is the least sensitive portion of the cell cycle and killing of these cells is undoubtedly a component of the 5-FU-mediated synergistic effects. Another cell cycle active drug, paclitaxel, sensitizes cells by synchronizing them in G2/M, the most radiosensitive portion of the cell cycle [54–56].

A final category of radiosensitizing drugs worth discussing are the topoisomerase inhibitors [57–60]. Topoisomerases uncoil supercoiled DNA by nicking one strand and serving as a swivel to allow uncoiling without tearing of the remaining single strand of DNA. By inhibiting the swiveling of the DNA, topoisomerase inhibitors preserve the single-strand break. The effective single-strand break may allow for easier breakage of the remaining DNA strand, leading to a lethal double-strand break. The effects of topoisomerase inhibitors are primarily observed in cycling cells, but topoisomerase activity occurs in all cells.

## **Radiobiology of Tumor**

### ***Tumor Oxygenation and Hypoxic Radiation Sensitization***

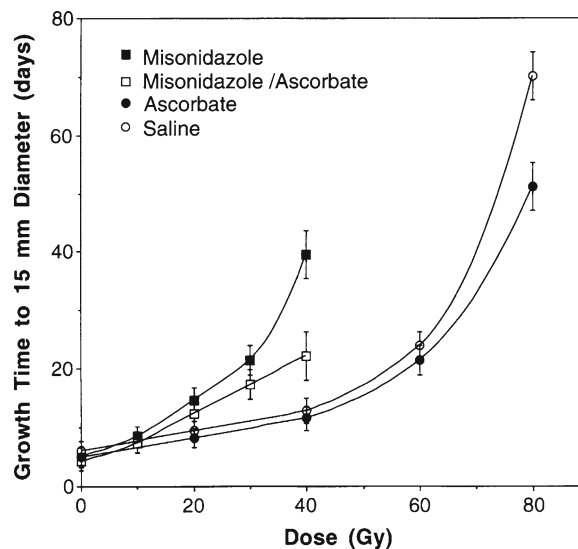
When experimental tumors are treated with a single fraction, tumor response is usually determined by the hypoxic fraction of cells. This is because well-oxygenated cells are far more sensitive to radiation than those with poor oxygenation. The differential sensitivity is exponential with dose. Hence, even if oxygenated cells outnumber the hypoxic cells by one or two orders of magnitude, the hypoxic cells can still dominate as the cause of treatment failure. When fractionation of the radiation dose is employed, the impact of hypoxia is diminished due to a spatial redistribution of the oxygenated cells between treatments, a process termed reoxygenation [61]. While in experimental animals the hypoxic fraction of tumor consistently increases with tumor size, in humans the relation is less consistent. Thus, even small tumors can be hypoxic in human subjects. Hypoxic fractions for human tumors are often similar to that of small murine tumors, and like small murine tumors, oxygenation is quite variable even among tumors of the same size and histologic type. Thus, many human tumors have no significant hypoxia, and in others, hypoxic cells can comprise more than half the tumor. The use of vascular ligation, clamps, and anesthesia during surgery add to the potential of increased tumor hypoxia during IORT.

Several clinical approaches have been taken to reduce hypoxia. First, patients are anesthetized and blood pressure is maintained by appropriate hydration and transfusion. Anesthesia can cause vasodilation, which, in the absence of concomitant hypotension, can actually improve tumor blood flow. During irradiation, patients can be ventilated with near pure oxygen. In this case, increasing

the oxygen partial pressure can improve the oxygen carrying capacity modestly and does appear to at least temporarily increase tumor oxygenation [62]. Interestingly, tumor metabolism is often oxygen limited, and when oxygen breathing is allowed to continue for over approximately 30 min, some tumors augment their metabolic rate and consume the added oxygen. Hence, the inspired oxygen should not be unnecessarily increased until just before radiation is to be delivered.

Hypoxic radiosensitizing drugs, and in particular the nitroimidazole drugs, have been used in combination with IORT. While the data is still inconclusive, this approach has much theoretical merit. The major toxicity of the nitroimidazole radiosensitizers is neurologic. The effect is cumulative with total drug dose. The concentrations of drug that are required to significantly sensitize tumor are often prohibitive in fractionated studies, given that humans appear to tolerate these drugs poorly compared with rodents. As a result, many clinical studies of fractionated irradiation have given the drug at doses that do not even sensitize animal tumors treated in a single fraction. Successful use of these drugs with fractionated irradiation, a condition where hypoxia is less important than single fraction irradiation, has thus been difficult to achieve. Single fraction therapies, like IORT, allow for a therapeutic dose of nitroimidazole radiosensitizer to be delivered. Only hypoxic cells would be sensitized by this therapy, and dose modifying factors over 2 are typically achieved with these drugs (Fig. 2.9) [63–65]. If a doubling of dose effect were to be observed in the clinic, it should have an important benefit to patients.

While most normal tissues are well oxygenated and are thus not expected to be sensitized by increased inspired oxygen or nitroimidazole drug, it is known that brain involved by tumor can be quite hypoxic [66, 67]. Skin and liver are two other organs that commonly have high natural hypoxia and would be sensitized by procedures aimed at hypoxic cells [68]. Normal tissue radiation toxicity, therefore, can sometimes occur when tissue oxygenation is increased. For example, augmenting the inspired oxygen in the case of IORT for brain tumors might be expected to increase the oxygenation



**Fig. 2.9** In single fraction irradiation treatment, the response of tumor is primarily determined by the fraction of tumor cells that are radiobiologically hypoxic. For example, using the hypoxic sensitizer misonidazole at a dose of 0.3 mg/g body weight, dose modifying factors of 1.5–2.5 are typically observed [64, 65]. Likewise, drugs that only radioprotect well-oxygenated cells, like ascorbate, do not protect tumor in single fraction studies. Ascorbate can reduce some of the sensitizing effects of oxygen mimetic drugs like misonidazole. The data shown were measured using FSaII fibrosarcoma tumors growing in C3H mice. Tumors were irradiated at 8 mm diameters and time to reach 15 mm was tabulated. Values are means  $\pm$  1 SE. At 40 Gy some misonidazole-treated tumors had permanent control. Likewise, at 80 Gy, the tumors in some ascorbate or control animals were permanently controlled.

of the normal brain and thus increase the risk of necrosis. Hence, an understanding of the actual physiology of the tissues being treated should be taken into consideration when any radiosensitizer is employed.

Oxygen diffusion distances change depending upon the metabolic rate of tumor, the cell density of tumor, and the availability of carbon substrate (e.g., sugars and protein). Over the years, several drugs have been developed for improving tissue hypoxic cell sensitivity. These drugs include the oxygen mimetic sensitizers, oxygen unloading drugs, vasodilatory drugs, hyperbaric and hyperoxic breathing, blood doping, hypo- and hyperthermia, drugs that alter the oxygen consumption rate, and therapies that alter tumor angiogenesis. The diffusion of oxygen is primarily limited by metabolism, and since the latter is rarely known, the oxygen status is rarely known. Hence, the ability to evaluate the benefit of employing these toxic drug therapies, aimed only at hypoxic cells, is plagued by the problem of identifying tumors that have substantial hypoxia [69]. Progress is being made in imaging hypoxia using PET and electrode technology, and this should ultimately impact the successful routine use of hypoxic radiosensitizers [70].

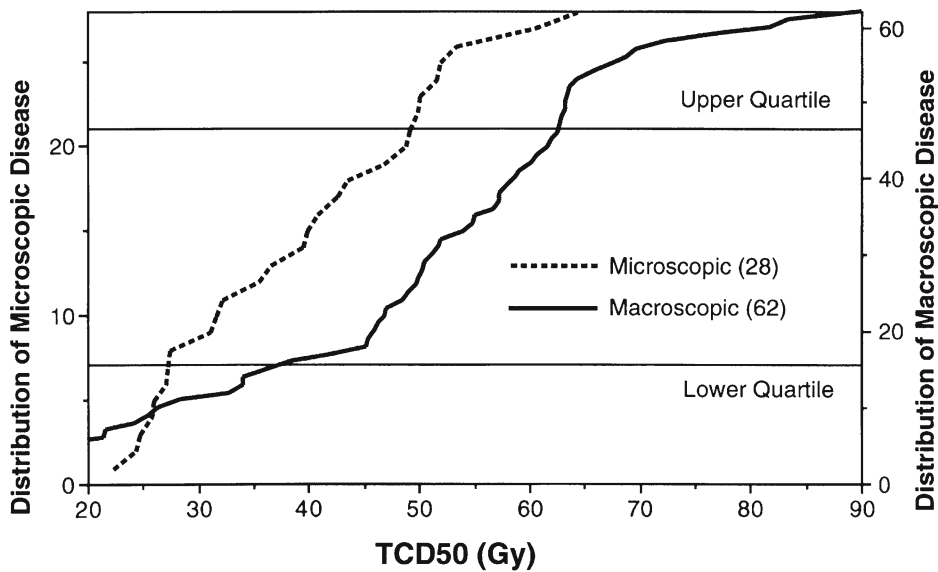
Many drugs with independent tumor cytotoxicity are known to function as radiosensitizers, and some are routinely used clinically. These drugs are of obvious interest as an adjunct to IORT. Some chemotherapy, interestingly, is more effective at killing hypoxic cells and thus might synergize with radiation given during IORT [71–74].

### ***Dose Response of Human Tumors and Implications for IORT Dose***

There is little discussion of dose response for tumor control in the IORT literature despite the large range of doses used in various studies. In contrast, there is a more comprehensive discussion of the correlation between dose response and complications. Thus, it appears that the heterogeneity of tumor response to IORT may be more determined by the ability to safely encompass the tumor and less by the selected dose. Radiobiologically, this can be explained if even the lowest IORT doses are already sufficient for in-field control of most tumors.

The dose response of human tumors had been published in multiple clinical series and organized by several authors [75–78]. The median dose range that locally controls 50% of adult solid tumors ( $\text{TCD}_{50}$ ) is approximately 45–65 Gy in standard fractionation (Fig. 2.10). The  $\text{TCD}_{50}$  for microscopic residual disease is closer to 25–50 Gy for typical adult solid tumors [75]. As previously discussed, the dose response curve is steep. The  $\gamma_{50}$  factor was defined to estimate the steepness of the dose response curve [4, 75]. It has units of percent change in local control divided by percent change in dose measured at the  $\text{TCD}_{50}$ . Thus, a  $\gamma_{50}$  of 1 to 2, which is typical of most tumors, suggests that a 1% increase in dose near the  $\text{TCD}_{50}$  level increases control by 1–2%. As an example, if the  $\gamma_{50}$  is 1–2%/percent change in dose, and the  $\text{TCD}_{50}$  is 50 Gy, then 55 Gy (10% increase in dose) would increase local control 10–20% (i.e., 60–70% local control).

No detailed analyses are possible for IORT because of the complexity of cases treated, and the routine combination of EBRT and IORT. As an estimate, however, an IORT boost of 10, 15, or 20 Gy, using data in Fig. 2.3, preceded or followed by 45 Gy fractionated EBRT, would have a theoretical biological effect equivalent to 61, 76, or 95 Gy, assuming an  $\alpha/\beta$  of 10. Since these doses exceed the expected  $\text{TCD}_{50}$  for most solid tumors, there is little radiobiological justification to ever exceed total IORT doses of 15–20 Gy, if EBRT is also delivered. Perhaps the only exception to this rule would be in the case of a known severely hypoxic tumor. The experience with stereotactic radiosurgery of brain metastases supports the conclusion that tumor can be controlled locally with radiation doses  $\approx 15$  Gy when combined with external beam radiation. For example, fractionated whole brain radiation doses of 30 Gy, combined with 10–15 Gy stereotactic boost, yields local control in  $\approx 90\%$  of patients [79].



**Fig. 2.10** The distribution of 100 dose response curves for human malignancies were collected based upon single and multi-institutional studies [75]. Sixty-two calculations of  $TCD_{50}$  were made for unresectable tumor, and 28 calculations for patients at high risk of recurrence or with positive margins. The calculated  $TCD_{50}$ s are displayed as a cumulative histogram. Typical tumors, based on the middle quartiles, had  $TCD_{50}$ s of 45–65 Gy for gross tumor and 25–50 Gy for resected tumor. In most studies, the radiation was administered with standard fractionation using external beam. The lowest  $TCD_{50}$ s occurred in hematopoietic and pediatric malignancies, the highest  $TCD_{50}$ s were for unresectable esophageal cancer. In the context of steep dose response curves, these data suggest that total effective doses over 70 Gy rarely are indicated for control of macroscopic disease and that doses over 60 Gy should obtain in-field control for microscopic disease.

## ***Radiobiological Benefits of Low-Dose IORT When a Full Dose Cannot Be Delivered Safely***

### **Rationale for Field Within a Field**

Considering the steepness of the radiation dose response curve, one might predict that if doses of radiation near the tumor control dose cannot be administered safely or are not delivered for other reasons, the efficacy of IORT is in question. This concept, however, is true only when IORT is the only therapy being delivered. If the patient has received preoperative EBRT, is planning to receive postoperative EBRT, or has had a gross total resection, then IORT could yield tumor control even if the dose of IORT is not optimal. The rationale for this stems from the expectation that the largest number of potentially surviving clonogenic tumor cells are in the primary mass or surgical bed, and that potential disease outside the field can be controlled by EBRT, chemotherapy, or surgical excision. Under these circumstances, even a low boost dose of radiation given by IORT may improve control rates [77, 80]. Theoretical estimations of improved control rates have been proposed by several authors. The concept of partial tumor boost is still controversial, but the conditions of IORT make its consideration particularly important. Some estimates suggest that, for many tumors, as much as 10% of the tumor can be excluded from the IORT boost field and still yield 10–20% improvements in local control [4, 81]. Theoretically, therefore, if the entire tumor cannot be safely taken to full dose, it is still worth considering giving the safe dose to the entire tumor and an additional intraoperative dose (field within a field) to the volume which excludes the dose-limiting sensitive tissue.



## Future of Radiobiology and Relevance to IORT

### *New Biological Parameters of Consideration*

It has been established that patients with certain inherited abnormalities are substantially more radiation sensitive than “normal” patients [28, 82]. It is hypothesized that many apparently normal people are more radiosensitive than true “normals” due to undetected heterozygosity for an inheritable disorder [27]. With recent advances in molecular technique, it is becoming possible to test for disorders of DNA repair. Interestingly, deficiencies in any DNA repair pathways cause increased radiation sensitivity, although double-strand break repair mechanisms appear most important. Radiation may be unique in this ubiquitous effect; cytotoxic drugs typically affect only one or two DNA repair pathways. As the enzymes and genes are sequenced and mutant patterns identified, ultimately it might be possible to identify patients with increased risk for complications from IORT. Gene array profiling is already available and is being used for clinical investigations [83, 84].

Recent studies of cytokine expression also suggest that toxicities to normal tissues resulting from IORT may be predicted. Rubin et al. showed that transforming growth factor  $\beta$  (TGF $\beta$ ) was elevated preceding the development of radiation pneumonitis [85]. TGF $\beta$  is one of many fibrogenic and proinflammatory cytokines induced to different degrees in animal and human following radiation. Experimentally, the levels of cytokine expression appear to depend on animal species and strain, the type of tumor growing in the animal, and the type of therapy delivered. As with different mouse strains, the levels of expression in different human subjects are highly variable [86, 87]. Correlative studies in humans confirm that many tumors produce TGF $\beta$  and that individuals who chronically have elevated levels of these cytokines, whether endogenous, disease-induced, or therapy-induced, are at increased risk for late radiation complications. TGF $\beta$  and tumor necrosis factor (TNF), for example, have been associated with pulmonary and/or hepatic fibrosis following radiation or chemotherapy [86, 87]. These cytokines can be readily measured by ELISA, paving the way for predictive assays. It is interesting to speculate that medications designed to alter the chronic expression of these cytokines may prevent some complications of IORT.

### *Oncogenesis*

The oncogenic potential of radiation is well known. In general, as with other complications of radiation, the frequency of late radiation-induced cancers are related to fraction size, total dose, and field size. Oncogenesis in the IORT field is common in canines [88], although not yet reported in human subjects. Malignancies attributed to IORT, however, must originate in the IORT field, must be of a different histology than the original primary, and must occur after a significant time lag (usually, over 6 months and often years or decades). Radiation-induced oncogenesis can include leukemias, carcinomas, and sarcomas [89]. IORT-induced malignancies in canines, however, are most commonly sarcomas of bone or soft tissue. The type of cancer induced by treatment is related to the form of radiation used, the target tissue irradiated, and the size of the radiation dose used for the treatment. For example, orthovoltage techniques have the disadvantage of severe dose inhomogeneity; thus, high dose regions can occur in nonmalignant tissues included in the IORT field. Murine models suggest that single doses of  $\geq 35$  Gy are associated with near certain sarcomatous degeneration [90]. Estimates of carcinogenesis in canine models are typically not actuarially corrected, resulting in an underestimation of long-term oncogenic risk. In two canine studies from the National Institute of Health, one found that animals that received over 20 Gy have a crude long-term malignancy rate of

1/8 (12%), and, in a shorter analysis, 10/46 (22%) developed sarcomas, all but one of which received over 20 Gy [88]. None of the sham irradiated animals in either study developed cancers.

The frequency of malignancy due to IORT is difficult to discern in patients. Unlike the animal models, most patients treated with IORT already have aggressive tumors and a high rate of death from other causes. Therefore, follow-up is often short due to high mortality. Further, the usual IORT dose of 10–20 Gy is lower than the reported doses needed for inducing a second malignancy. Perhaps for these reasons, oncogenesis is and will be rare in patients treated with IORT.

The mechanism of radiation-induced oncogenesis is unknown in most cases since few cancers occur within the first years after irradiation. However, it is unlikely that direct DNA damage from the irradiation is the primary cause of most cancers. In some cases, the mechanism of radiation-induced oncogenesis is well-defined. For example, patients with hereditary retinoblastoma are at high risk of developing multiple malignancies, including sarcomas of bone and soft tissue. Cancers in these patients develop due to radiation-induced mutation of the remaining normal Rb gene [91–93]. Recently, it was discovered that cycles of hypoxia and reoxygenation can select for cells with p53 mutations [94]. As previously discussed, radiation causes a prolonged antiangiogenic effect that includes intimal proliferation, thrombosis, and intermittent vascular occlusion [11]. An important function of p53 is the promotion of apoptosis in cells which have incurred genetic damage [95]. p53 mutant cells selected by years of impaired blood flow would fail to undergo apoptosis and could, therefore, accumulate genetic damage [96, 97]. If this proves to be an important mechanism of oncogenesis, strategies aimed at preventing the vascular effects of radiation might also reduce the incidence of radiation-induced malignancy.

Finally, as previously described, the normal tissues in the IORT bed can develop chronically elevated proliferative and fibrogenic cytokine levels. It is now known that elevated levels of many cytokines inhibit apoptosis [98]. As with mutations in the p53 pathway, this process could predispose to oncogenesis, and might be preventable using anticytokine therapies.

### ***Radiation-Induced Tumor Autoimmunity***

A holy grail of cancer therapy has been the development of tools that can help the body produce natural immunity to malignancy. Among the best documented is immune surveillance. There is substantial evidence that immune surveillance is an integral component of cancer prevention and contributes to tumor responses and possible reduction in the number of metastases. Fully satisfactory and ubiquitous antigens against a class of tumors are rare, an example being B1 for lymphoma or Her2/neu for breast cancers. Although patient-specific antigens can sometimes be employed, and there are some cytotoxic immune reactions documented for melanoma and renal cell carcinoma, producing and employing these antigens is technically demanding. Radiation is known to activate tumor specific immunity in animals [99, 100]. For example, inoculation of irradiated tumor cells in animal models, or curative treatment of animals with transplanted tumors, yields specific resistance to subsequent tumor challenge. Tools to detect similar effects in humans after irradiation are beginning to provide evidence that a similar effect may be observed in humans [101]. A mechanism of the possible effect has been attributed to the depletion of regulatory T cells (T(reg)) and myeloid-derived suppressor cells that otherwise limit the function and proliferation of autoimmune cells [102]. Older hypotheses include unrepaired radiation-induced cell membrane damage leading to prolonged antigen exposure. In animals, the tumor autoimmune phenomenon is dose-dependent and appears to require a large fraction size. Fortunately, it appears to be tumor histology-independent and might be augmented by appropriate systemic or locally administered cytokines that act as immune adjuvant [103]. IORT is uniquely positioned to create tumor “vaccines” of this sort given both the ability to directly inject the tumor with immune adjuvant and to administer the large single

doses of radiation. We suggest that vaccination conferred by IORT combined with an immune adjuvant might protect against the development of future micrometastases and/or cytoreduce existing metastatic or primary disease [104]. Reducing existing and future disease burden should prove to be clinically beneficial. More work is needed in this promising field.

## Conclusions

The most important advantage of IORT is the potential for high-dose irradiation of the tumor, while minimizing radiation to nontarget tissues. Another advantage of IORT is the potential for delivering concurrent radiosensitizing drugs under circumstances where a minimum of normal tissue experiences the sensitization effects. Finally, IORT offers the potential for optimizing the dose and dose distribution, thereby allowing us to test the hypothesis that radiation induces tumor autoimmunity. Since tumor response to a single fraction is predominantly determined by the hypoxic cell fraction, strategies aimed at this population of tumor cells should be pursued. Normal tissue complications are the main limitation of IORT, and they can be minimized by avoidance of full organ irradiation and by procedures designed to reduce dose to nontarget organs. Reducing dose is reasonable in many cases since there is experimental and theoretical evidence that even low-dose IORT can improve local control when employed in conjunction with other therapies. Late side effects of radiation are currently difficult to predict, and they occur with a steep dose and volume response. Ongoing research investigating the mechanisms and genetics of fibrosis, angiogenesis, and oncogenesis suggest that some of these effects eventually are alleviated or obviated by appropriate therapeutic interventions.

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(Eds.)

2011, XVII, 529 p., Hardcover

ISBN: 978-1-61779-014-0

A product of Humana Press