Quantitative Cell Kill of Radio- and Chemotherapy

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Michael Molls, Carsten Nieder, Claus Belka, and Jan Norum

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KEY POINTS

- Many anticancer drugs are cell-cycle specific and therefore most active against cells that are proliferating. Thus, the non-proliferating fraction is difficult to eradicate. Tumor regrowth in between cycles of therapy (repopulation) also contributes to limited efficacy.
- Experimental evidence suggests that single radiation doses result in 1% or less cell survival compared with 10–50% with cytotoxic drugs. Although clinically impressive remissions of solid tumors might occur after chemotherapy, the underlying cell kill is often not larger than 1–2 log and pathological examination of tissue specimens reveals residual viable tumor cells.
- The two Stockholm breast cancer trials in women treated with modified radical mastectomy provide a comparison of postoperative radiotherapy and chemotherapy with a median follow-up of 18 years. Locoregional recurrence was observed in 14% after radiotherapy and 24% after chemotherapy in premenopausal patients (hazard ratio 0.67, $p = 0.048$) and in 12% after radiotherapy and 26% after chemotherapy in postmenopausal patients (hazard ratio 0.43, $p < 0.001$).

M. Molls, MD
Professor, Director, Clinic of Radiation Oncology, Klinikum rechts der Isar, Technische Universität München, Ismaninger Straße 22, 81675 Munich, Germany

C. Belka, MD
Professor, Director, Clinic of Radiation Oncology, Ludwigs-Maximilians-Universität München, Marchioninistraße 15, 81377 Munich, Germany

C. Nieder, MD
Professor, University of Tromsø, Department of Internal Medicine – Oncology, Nordlandssykehuset HF Hospital, Prinsensgate 164, 8092 Bodø, Norway

J. Norum, MD
Professor, Department of Oncology, University Hospital Tromsø, Postboks, 9038 Tromsø, Norway

- The curative potential of chemotherapy alone has remained low in most solid tumors. Obviously chemotherapy or medical treatment alone is unable to control definitively macroscopic solid tumors in adults (either metastases or primary tumors) with the exception of testicular carcinomas. As a result of the limited efficacy, current studies are trying to enrich the patient population that is likely to respond, based, for example, on gene signatures or different pathology features that might predict the outcome.
- The introduction of combined modality approaches was a highly significant step in the evolution of curative cancer treatment. The most pronounced increase in therapeutic gain was probably seen by combining surgery and/or radiation with chemotherapy. As recently suggested from the data of patients with glioblastoma, head and neck, and esophageal cancer who received radiotherapy alone or radio- and chemotherapy, the effect of the drugs in combined modality treatment corresponds to the equivalent of 9–12 Gy in 2-Gy fractions. In many clinical situations, radiation dose escalation by 9–12 Gy would result in increased late toxicity risks. Under these circumstances, combining radio- and chemotherapy increases the therapeutic window.
- In practice, the efficacy of radiotherapy might be reduced by limitations in imaging/detection of malignant cells (target volume definition), precision of treatment delivery (intra- and interfraction motion), and various factors related to tumor biology (oxygenation, cell cycle distribution, etc.).
- Both experimental and clinical observations have repeatedly confirmed the influence of initial tumor volume or cell number on local control and the need for administration of higher radiation doses in large-volume disease.

Abstract

This chapter contains a review of the potential of radio- and chemotherapy to eradicate tumor cells. With regard to the amount of quantitative cell kill, important differences exist between ionizing radiation and chemotherapy. In principle, radiation treatment can be designed to cover the whole tumor with a homogeneously distributed full radiation dose, capable of inactivation of all tumor cells. In contrast, pharmacotherapy is limited by the fact that the dose of the active, cell-killing form of the compound is variable within the tumor and its cells. This results from problems in the delivery of drugs (perfusion, interstitial fluid pressure, tissue pH, protein binding, etc.), cellular uptake, efflux, inactivation, and other mechanisms of resistance. In many instances, the agent does not reach the relevant therapeutic targets in the required concentration and for a sufficient time period. In fact, the pharmacokinetic profile of anticancer drugs is characterized by substantial interpatient variability where two- to threefold variation is not uncommon. These issues even gain complexity with simultaneous administration of two or more drugs. Such multiagent regimens with different modes of action might be valuable when each agent kills different tumor cells, which would not become inactivated by the other agent. Depending on variations in actual drug concentration, a fixed combination of two drugs might either show additivity or antagonism in the same tumor cells. Both preclinical and clinical data confirm that rationally designed drug combinations often lead to improved results. Several studies support the superior quantitative cell kill of radiotherapy and suggest that simultaneous application of radio- and chemotherapy is an important measure to increase the efficacy of non-surgical cancer treatment.

10.1 Introduction

10.1.1 Clinical Relevance of Radio- and Chemotherapy

The curative potential of radiotherapy, for example, for limited-stage malignancies of the skin and other organs that could be treated to high doses with the technology available at that time, was explored very soon after the landmark discoveries by Wilhelm Conrad Roentgen and many other enthusiastic pioneers in the newly emerging field of radiation medicine. As early as 1912, the German journal *Strahlentherapie & Onkologie* was published for the first time. The elegant work on dose-effect relationships of, for example, Magnus Strandqvist, which was published in 1944, has been summarized in one of the early issues of the *Interna-*

tional Journal of Radiation Oncology Biology and Physics (del Regato 1989). Driven by rapid progress in both machine development, discovery of new isotopes, and understanding of the basic biological principles, the number of indications and successful treatment strategies has increased tremendously during the twentieth century. Eventually, the basis for high-precision proton and heavy ion beam application has been established (Lawrence et al. 1963). Today, patients with early-stage solid tumors (T1, T2; N0; M0), such as prostate cancer or non-small cell lung cancer (NSCLC), are cured by linear accelerator photon radiation treatment alone [prostate: brachytherapy or intensity modulated radiation treatment (IMRT) (Nguyen and Zietman 2007); lung: stereotactic fractionated radiation treatment (Zimmermann et al. 2006)].

Later during that century, the first encouraging efforts in systemic chemotherapy with cytotoxic drugs, in particular in patients with leukemias, malignant lymphomas, and testicular cancer, contributed to a continuous increase and refinement of cancer treatment approaches (Ben-Asher 1949; Scott 1970). More and more specific drug targets have been discovered, rational drug combinations have been designed, and, thus, an unprecedented number of clinically established neoadjuvant, adjuvant, and palliative regimens have become available today. However, the curative potential of chemotherapy alone has remained low in most solid tumors. The introduction of combined modality approaches was a highly significant step in the evolution of curative cancer treatment. Parallel to refinements of each single modality, combined treatment has actively been investigated in recent decades in both preclinical and clinical studies around the world. When judged at this time, the most pronounced increase in therapeutic gain was probably seen by combining surgery and/or radiation with chemotherapy.

Meanwhile a huge body of evidence supports the use of combined modality approaches based on the combination of ionizing radiation with cytostatic and cytotoxic drugs. In this regard, several randomized phase III trials for many relevant cancer sites provide a sound basis for level-one evidence-based decisions. This holds true especially for glioblastoma multiforme (Stupp et al. 2005), head and neck cancers including nasopharyngeal cancer and laryngeal cancer (Brizel et al. 1998; Forastiere et al. 2003; Budach et al. 2005), esophageal cancer (Minsky et al. 2002; Siewert et al. 2007), colorectal and anal cancer (Bartelink et al. 1997; Sauer et al. 2004), cervical cancer (Green et al. 2001), as well as lung cancer (Schaake-Koning et al. 1992).

10.2 Basic Considerations

10.2.1 Treatment Aims

The most important aim of curative cancer treatment is to eradicate all tumor cells. With regard to the amount of quantitative cell kill, it has to be emphasized that important differences exist between ionizing radiation and chemotherapy (Fig. 10.1). In principle, radiation treatment can be designed to cover the whole tumor with a homogeneously distributed full radiation dose, capable of inactivation of all tumor cells. In contrast, pharmacotherapy is limited by the fact that the dose of the active, cell-killing form of the compound is variable within the tumor and its cells (Fig. 10.2). This results from problems in the delivery of drugs (perfusion, interstitial fluid pressure, tissue pH, protein binding, etc.), cellular uptake, efflux, metabolization, inactivation, and other molecular and cellular mechanisms of resistance. In many instances, the agent does not reach the relevant therapeutic targets in the required concentration and for a sufficient time period (Tannock et al. 2002; Primeau et al. 2005; Minchinton and Tannock 2006). In fact, the pharmacokinetic profile of anticancer drugs is characterized by substantial interpatient variability where two- to threefold variation is not uncommon (Brunsvig et al. 2007). These issues even gain complexity with simultaneous administration of two or more drugs. Such multiagent regimens with different modes of action might be valuable when each agent kills different tumor cells, which would not become inactivated by the other agents; however, sometimes all agents might act on the same cell, causing much more damage than necessary for cell death. Depending on variations in actual drug concentration, a fixed combination of two drugs might either show additivity or antagonism in the same tumor cells (Lee et al. 2006). Cells surviving initial chemotherapy may upregulate active resistance mechanisms, which allows for growth despite therapy (Teicher et al. 1990; Graham et al. 1994). Furthermore, cells may survive until therapy cessation by downregulating metabolism/cycling, becoming temporarily quiescent (Stewart et al. 2007). Another factor that interferes with our ability to deliver tumor-eradicating treatment is toxicity/damage to normal tissues and organs. While such toxicity typically is limited to the tumor surroundings in the context of surgery and radiotherapy, more widespread effects limit the maximum tolerable doses of systemically administered agents (bone marrow toxicity, neuropathy, cardiac

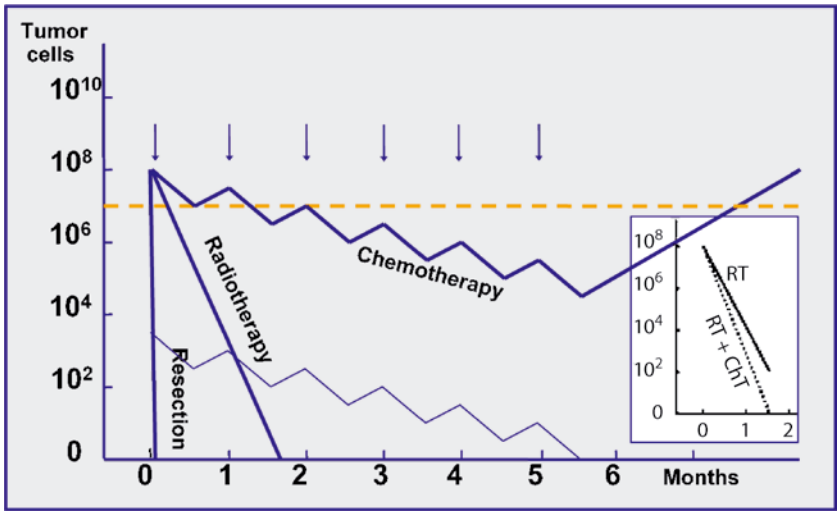

Fig. 10.1. Differences in quantitative cell kill and time course. Influence of different therapeutic modalities on number of tumor cells during a course of treatment, based on models (Tannock 1989, 1992; Minchinton and Tannock 2006). The *dashed line* represents the border between microscopic and macroscopic tumors, defined as a size of approximately 5 mm. Compared with surgical resection and fractionated radiotherapy, multiple courses of chemotherapy (in this case six, indicated by *arrows*) are less efficient in cell kill. While microscopic disease might be eradicated (*lower chemotherapy curve*), clinical evidence suggests that most macroscopic solid tumors (exception: more sensitive testicular cancers) will shrink temporarily but eventually regrow from surviving residues (*upper chemotherapy curve*). As shown in the *inset*, the strength of chemotherapy in combination with radiation treatment (in addition to spatial cooperation) is the modification of the slope of the curve

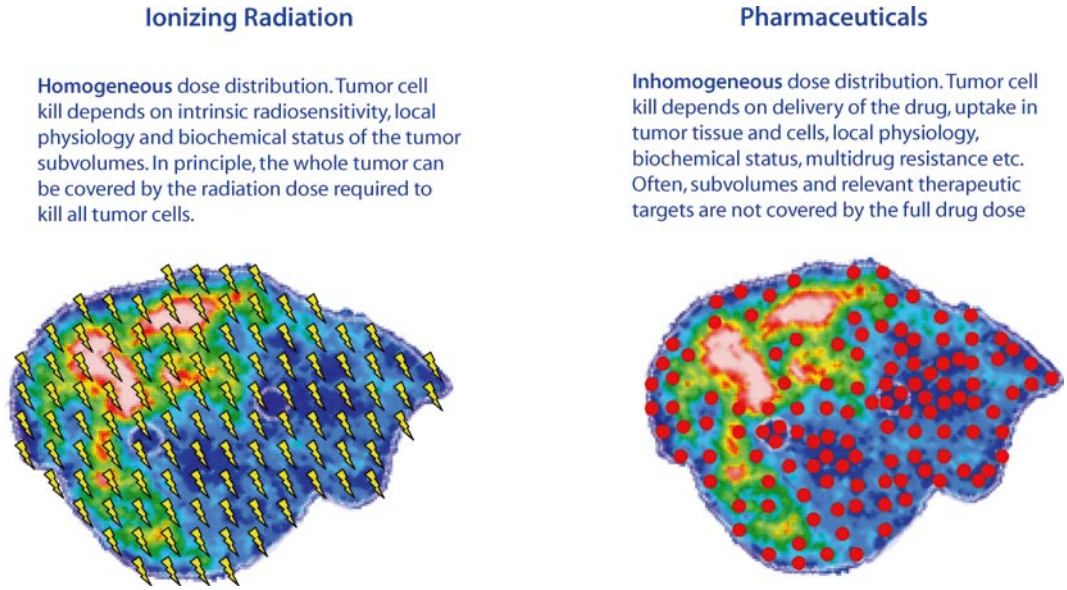

Fig. 10.2. Comparison between tumor dose distribution in radiation treatment and pharmaceutical treatment. Illustrative tumor sections from a squamous cell carcinoma demonstrate biological heterogeneity, reflected by the differently colored areas, within the tumor. Homogeneous radiation dose distribution within the tumor irrespective of differences in biology, physiology, functional factors, structure, and morphology. Heterogeneous dose distribution for drug treatment, related, for example, to regional differences in perfusion, pH, metabolism, etc. Drug molecules are shown as *red circles*. (The histological section is courtesy of W. Müller-Klieser, Johannes Gutenberg University, Mainz, Germany)

damage, kidney damage, infertility, etc.). Here it is also worth noting that in contrast to malignant tissues the normal tissue of the different organs does not develop resistance toward anticancer pharmaceuticals. Systemic radiotherapy such as radionuclides for the treatment of bone metastases or certain types of lymphomas will of course also be able to cause some of the systemic effects. This particular type of radiation treatment, however, will not be discussed in greater detail in this chapter.

10.2.2 Aspects Specific to Radiotherapy

As illustrated in Fig. 10.1, the quantitative cell kill of ionizing radiation is significantly larger than that of chemotherapy (Tannock 1992, 1998; Minchinton and Tannock 2006). The magnitude of the relatively low efficiency of chemotherapy might vary with cell type, culture conditions, drug, exposure time, etc. Experimental evidence suggests, however, that single radiation doses result in 1% or less cell survival compared with 10–50% with cytotoxic drugs (Epstein 1990; Kim et al. 1992; Simoens et al. 2003; Eliaz et al. 2004). Although clinically impressive remissions of solid tumors might occur after chemotherapy, the underlying cell kill is often not larger than 1–2 log and pathological examination of tissue specimens reveals residual viable tumor cells. From these cells local, regional, and distant failure can eventually emerge.

In practice, the efficacy of radiotherapy might be reduced by limitations in imaging/detection of malignant cells (target volume definition), precision of treatment delivery (intra- and interfraction motion), and various factors related to tumor biology, which will be discussed later in this chapter. However, the experience with image-guided high-precision radiotherapy based on combined biological and anatomical imaging suggests that the magnitude of such limitations is likely to diminish (Grosu et al. 2006).

Extensive discussion of radiobiological principles is beyond the scope of this chapter, yet a few definitions will be mentioned. The response of tumors to radiotherapy is determined by several factors such as repopulation, reoxygenation, number of clonogenic cells, and their intrinsic radiosensitivity. Since the introduction of mammalian cell survival curves, the parameters D_0 and N have been used as quantitative measures of inherent radiation sensitivity, as was the shoulder width Dq (Thames and Suit 1986). Today the ratio alpha/beta is the most common parameter for characterization of cell survival curves. It is also a measure of fractionation sensitivity.

When combining two treatment modalities, the resulting net effect on cell killing is mainly described by the terms "additivity, synergism, and subadditivity," which are derived from experimental investigations. They are not applicable to the clinical situation and do not reflect the results of clinical trials, where changes from radiation as a monotherapy to multimodal treatment usually do not result in extraordinarily favorable cure rates (or supra-additivity), although they have led to important gradual improvement. It appears prudent to refer to the term "enhancement of radiation effect" within a clinical context.

The smaller the tumor, the higher is the success rate of radiation treatment, as illustrated in the Japanese study of carbon ion therapy for stage I NSCLC (Miyamoto et al. 2007). For T1 disease, the local control rate was 98% at a median follow-up of 39 months, while it was 80% for T2 tumors. With the same modality, 97% of choroidal melanoma were locally controlled at 3 years (Tsuji et al. 2007). For skull base chondrosarcomas, local control was achieved in 90% of the cases at 4 years (Schulz-Ertner et al. 2007). In small early-stage NSCLC, comparable local control data were published for stereotactic radiosurgery with photon beams (Zimmermann et al. 2006; Hof et al. 2007). In early, stage Ib squamous cell carcinoma of the uterine cervix, radiation therapy alone resulted in 5-year survival of 93.5% and local control of 92% (Ota et al. 2007). Radiation doses that control early stage T1 prostate cancer result in less favorable outcome when administered to advanced T3 disease (Zelefsky et al. 2008). With higher doses and/or combined radiation and androgen ablation, however, high 5- and 10-year local control rates can be achieved even in T3 tumors (Zelefsky et al. 2008). Both experimental and clinical observations have repeatedly confirmed the influence of initial tumor volume or cell number on local control (Khalil et al. 1997; Zhao et al. 2007) and the need for administration of higher radiation doses in large-volume disease. The preclinical data of radiotherapy under hypoxic and ambient conditions also suggest that the dose-volume relationship is present under both conditions, i.e., not just related to increasing hypoxia in larger tumors.

Whether surgery and radiotherapy are equally effective in small-volume disease is difficult to judge as very few direct randomized comparisons with sufficient sample size have been published. One of the best examples is probably the French trial comparing 658 breast cancer patients with clinically uninvolved lymph nodes, which were treated with lumpectomy plus axillary dissection or axillary radiotherapy (Louis-Sylvestre et al. 2004). In the group with dissected axilla, 21% of the patients were node positive. The median follow-up was 180 months.

Recurrence in the axillary nodes was less frequent in the surgery arm (1% versus 3%, $p = 0.04$); however, distant metastases rates and overall survival were not significantly different, suggesting that the small difference in axillary control is not clinically meaningful. Different non-randomized studies, for example, in patients with inflammatory breast cancer initially treated with induction chemotherapy at the University of Texas M. D. Anderson Cancer Center in Houston, Texas, USA, also suggest that local treatment with either surgery or radiotherapy is equally effective (Ueno et al. 1997). Comparing urological and radiotherapeutic literature one can state that in early prostate cancer (up to T2a category, cN0, cM0) the cure rates of radiation treatment and prostatectomy do not differ in a significant manner. A similar situation exists also for other tumor entities, especially, for example, for head and neck cancers.

10.2.3 Aspects Specific to Chemotherapy

Many anticancer drugs are cell-cycle specific and therefore most active against cells that are proliferating. Thus, the non-proliferating fraction is difficult to eradicate. Tumor regrowth (repopulation) in between cycles of therapy also contributes to limited efficacy. Typically, one tries to administer the highest possible dose of drugs in the shortest possible time intervals. Even the use of dose-dense regimens, high-dose treatment with bone marrow or hematopoietic stem cell transplantation, and the development of non-cross-resistant regimens has not yet resulted in cure of the most common solid tumors with chemotherapy.

In earlier studies of neoadjuvant chemotherapy for locally advanced breast cancer, pathological complete remission (pCR) at surgery was seen in 5–15% of patients (typically anthracycline-based regimens) and it was found that pCR patients had better long-term outcomes (Ferriere et al. 1998; Karlsson et al. 1998). Even with modern drug combinations, pCR after neoadjuvant chemotherapy (for breast cancer with or without trastuzumab) is seen in only 15–38% of breast cancer patients (Deo et al. 2003; Smith et al. 2004; Evans et al. 2005; Reitsamer et al. 2005; Von Minckwitz et al. 2005; Ardavanis et al. 2006; Hurley et al. 2006; Veyret et al. 2006; Arnould et al. 2007) and 9–20% of cervical cancer patients (Buda et al. 2005; Modarress et al. 2005). In a randomized setting, the pCR rate in cervical cancer was much lower after neoadjuvant chemotherapy alone than after radiochemotherapy (10% versus 43%; $p < 0.05$; Modarress et al. 2005). The definitive cure rates with chemotherapy alone would certainly be lower than the pCR rates, because some surviving clonogenic tumor cells, which are not readily detectable, are still present in the histopathological specimen. As mentioned above, the curves shown in Fig. 10.1 depend on several variables related to patient selection, tumor microenvironment and sensitivity, agent and dose, etc. They are meant to illustrate the principle; however, the results of some neoadjuvant chemotherapy trials demonstrate the variability in the steepness of these curves. As a result of the limited efficacy of chemotherapy, current studies are trying to enrich the patient population that is likely to respond, based, for example, on gene signatures or different pathology features that might predict the outcome (Minna et al. 2007).

As recently demonstrated from an exploratory analysis of data from two parallel phase III chemotherapy studies in metastatic colorectal cancer, even non-responders, despite a poorer prognosis than responders, achieved extended progression-free and overall survival from more effective drug combinations, which were tested against older standards (Grothey et al. 2008). One of the trials examined IFL (irinotecan, 5-fluorouracil, leucovorin) versus IFL plus the angiogenesis inhibitor bevacizumab, and the other trial compared IFL to oxaliplatin, 5-fluorouracil, and leucovorin. The hazard ratios for the different study endpoints and drug regimens ranged from 0.63 to 0.76 in responders and non-responders. In a large analysis of 1,508 patients with advanced or metastatic colorectal cancer treated in a phase III study, 4% had complete remission after chemotherapy alone (Dy et al. 2007). The three treatment arms of the study consisted of IFL, oxaliplatin plus 5-fluorouracil/leucovorin, and irinotecan plus oxaliplatin. The highest rate of complete remissions was 6%, observed in the oxaliplatin plus 5-fluorouracil/leucovorin arm. Size of the metastases significantly influenced the likelihood of complete remission. Of the patients with initial complete remission to chemotherapy, 84% developed progression within 5 years. The median time to progression was 15 months. With second-line chemotherapy, complete remission is even more unlikely in this disease.

10.3 Attempts to Compare the Efficacy of Radio- and Chemotherapy

10.3.1 Animal Studies

In this section, examples are discussed that are focused on the undifferentiated human hypopharyngeal cell line

FaDu. This cell line was first described in 1972 and has a doubling time in vitro of about 1.2–2.8 days. Extensive experiments by the group from Dresden, Germany, are summarized in Table 10.1 and compared to data from other groups. Among different, but equally sized human head and neck squamous cell carcinomas growing in nude mice, which received total body irradiation before tumor transplantation, the radiation dose to control 50% of the tumors (TCD_{50}) after a sufficient follow-up of 120 days varied tremendously (Yaromina et al. 2007). After local radiation treatment with 30 fractions over 6 weeks, the TCD_{50} was, for example, 45 Gy for UT-SCC-8 cells, 85 Gy for FaDu cells, and 127 Gy for SAS cells. Thus, FaDu represents a cell line that is neither particularly sensitive nor resistant. Another reason for focusing on this cell line is the number of data available for review. Some in vitro data from experiments with FaDu are shown in Table 10.2. Comparable variations in sensitivity across a panel of cell lines were made for different pharmacological agents and tumor cell lines, emphasizing the role of intrinsic sensitivity. As shown in Table 10.1, FaDu tumors can be controlled with clinically readily achievable doses of radiation, while the results of chemotherapy at the maximum tolerated dose vary tremendously. Some drug combinations achieve better results than the respective single treatments. As previously described by other authors, reduction of the tumor volume before the start of radiotherapy, in this example by the use of epidermal growth factor receptor-tyrosine kinase inhibition, failed to translate into improved local tumor control after sufficient follow-up (Krause et al. 2007). All these observations question the value of clinical strategies where radiotherapy or simultaneous radiochemotherapy is preceded by induction chemotherapy or tyrosine kinase inhibitors. Summarizing this paragraph it is also very important to note that one has to be very cautious when transferring experimental in vitro and in vivo results into the clinical

Table 10.1. Overview of animal experiments with subcutaneously implanted FaDu tumor cells

Reference	Model	Treatment details	Results
Schütze et al. (2007a)	Nude mice having received 4 Gy TBI	Ambient conditions without anesthesia, air-breathing animals	Single dose 25 Gy controlled 29% at day 120 Single dose 35 Gy controlled 57% at day 120
Krause et al. (2007)	Nude mice having received 4 Gy TBI	Ambient conditions without anesthesia, air-breathing animals	Total doses 8–60 Gy, 5 fractions in 5 days Evaluation of tumor control at day 120 Up to 32 Gy: <25% of tumors are controlled TCD_{50} = 41 Gy TCD_{100} = 60 Gy
Cao et al. (2005)	Nude mice	Maximum tolerable dose	10–20% cure with capecitabine or irinotecan 80–90% cure with combination of both agents
Azrak et al. (2004)	Nude mice	Maximum tolerable dose	<30% cure with irinotecan 0% cure with 5-fluorouracil 60–100% cure with combination of both agents
Joschko et al. (1997)	Nude mice having received 5 Gy TBI	Maximum tolerable dose	Daily gemcitabine results in a median time to regrowth to 200% of the initial volume of 5 days Once weekly gemcitabine results in a median time to regrowth to 200% of the initial volume of 13 days Twice weekly gemcitabine results in a median time to regrowth to 200% of the initial volume of 16 days
Joschko et al. (1997)	Nude mice having received 5 Gy TBI	Ambient conditions without anesthesia, air-breathing animals	40 Gy in 20 fractions in 2 weeks results in a median time to regrowth to 200% of the initial volume of 43 days

TBI Total body irradiation

Note that the sensitivity to drug treatment might change with tumor location within the host animal, as described by (Holden et al. 1997). In general, time to regrowth is a less valuable and accepted endpoint than local tumor control

Table 10.2. Overview of in vitro experiments with the FaDu cell line (cell culture conditions varied between the individual reports)

Agent	IC_{50} (µmol/l)	Resistance fraction	Radiation therapy parameters	Reference
Cisplatin	1.02 ± .15	5.3 ± 1.5		Lee et al. (2006)
5-Fluorouracil	7.59 ± 1.15	25.4 ± 1.1		
Paclitaxel	1.25 ± 0.57	11.8 ± 2.3		
			SF 0.1 4.1–4.5 Gy SF 0.01 7.3–7.7 Gy	Schütze et al. (2007b)

IC_{50} Inhibitory concentration at 50% survival, *SF 0.1* radiation dose reducing the survival fraction to 1%

IC_{50} values for FaDu are within the range of those reported for other squamous cell carcinoma, for example, Raitanen et al. (2002)

IC_{50} values for different cell lines treated with the same agent are variable: 3–35 nM, for example, for paclitaxel (Gorodetsky et al. 1998) and 5–50 nM for docetaxel (Clarke and Rivory 1999)

situation. Often one must conclude that experimental treatments especially with pharmacological substances are highly efficient whereas with corresponding treatments in patients the efficiency cannot be reproduced.

10.3.2 Clinical Data

Direct randomized comparisons unfortunately are very rare. However, the two Stockholm breast cancer trials in women treated with modified radical mastectomy provide a comparison of postoperative radiotherapy and chemotherapy with a median follow-up of 18 years (Rutqvist and Johansson 2006). All patients had node-positive disease or a tumor diameter exceeding 30 mm. The radiation dose was 46 Gy in 2-Gy fractions to the chest wall, axilla, supraclavicular fossa, and the ipsilateral internal mammary nodes. Chemotherapy initially consisted of 12 cycles (later 6 cycles) of cyclophosphamide 100 mg/m² orally on days 1–14, methotrexate 40 mg/m² i.v. on days 1 and 8, and 5-fluorouracil 600 mg/m² i.v. on days 1 and 8 (CMF). In the trial that included premenopausal patients, 291 were allocated to CMF and 256 to radiotherapy. In each arm, 12% were node negative. Sixty-two and 64% were estrogen-receptor positive, respectively. Locoregional recurrence was observed in 14% after radiotherapy and 24% after chemotherapy (hazard ratio 0.67, $p = 0.048$). The absolute benefit increased with the number of positive lymph nodes. As might be expected, fewer patients developed distant recurrence after CMF and the eventual difference in breast cancer deaths was 50% versus 56%. This difference in favor of CMF was not statistically significant ($p = 0.12$), but the sample size was very limited. In the trial that included postmenopausal patients, 182 were allocated to CMF and 148 to radiotherapy. Ten and 12% were node negative, respectively. Sixty-seven and 68% were estrogen-receptor positive, respectively. Locoregional recurrence was observed in 12% after radiotherapy and 26% after chemotherapy (hazard ratio 0.43, $p<0.001$). Again, distant recurrence was reduced by treatment with CMF, as were breast cancer deaths ($p = 0.07$). While treatment of breast cancer has changed to a greater extent after the initiation of these two trials, their results add to the evidence of increased local cell kill after radiotherapy compared to systemic chemotherapy. Data from a subgroup of patients from the Stockholm trials suggest that the magnitude of expression of certain DNA repair proteins (Mre11, Rad50, Nbs1) is associated with the favorable response to radiotherapy (Söderlund et al. 2007).

In an observational study in patients with metastatic melanoma, local treatment with fractionated radiotherapy, single-fraction radiosurgery, or hyperthermia each was superior to systemic treatment (dacarbazine, fotemustine, carboplatin, temozolomide) with regard to local response rates (Richtig et al. 2005). Another study describes the response rate and time to progression in patients with metastatic esophageal cancer treated with chemotherapy alone or combined chemo- and radiotherapy (Lee et al. 2007). All 74 patients initially received two cycles of capecitabine/cisplatin chemotherapy. Patients with distant lymph node metastases continued with lower doses of the same two drugs plus radiotherapy to 54 Gy, while patients with non-lymph

node distant metastases continued on full-dose chemotherapy. Partial response to the first two cycles was observed in 20% and 15%, respectively (not significantly different). After treatment completion, a significant difference in favor of radiotherapy-containing treatment was observed (36% versus 63%). Median time to progression also was longer, 5.9 versus 8.4 months ($p = 0.03$).

Interesting data can also be derived from various recently published randomized studies in stage IIIB/IV NSCLC. Some of these studies used chemotherapy combinations, while one focused on palliative radiotherapy to the chest (different fractionation regimens) with only a few of the patients receiving additional chemotherapy (Sundstrom et al. 2004). With the lowest radiation dose of 17 Gy in 2 fractions, 2-year survival was 8%. With 15 fractions of 2.8 Gy, 13% was achieved. These figures are very close to those reported by the same group with carboplatin/vinorelbine or carboplatin/gemcitabine, i.e., 7% (Helbekkmo et al. 2007), and those from studies of cisplatin/vinorelbine (Yasuda et al. 2006) or carboplatin/paclitaxel (Paccagnella et al. 2006). Although various types of imbalances between the study populations might exist and some chemotherapy patients likely will also have received radiotherapy, the data are compatible with the hypothesis that the cell kill induced by commonly used cytostatic regimens can only be compared to that of palliative radiotherapy with low to moderate total doses.

While radiotherapy with or without androgen deprivation has long been accepted as the primary curative treatment modality in patients with prostate cancer, the limited experience with chemotherapy before prostatectomy (docetaxel or epirubicin) suggests that pCR is very unlikely. In fact, it was not observed at all in the studies by Dreicer et al. (2004), Febbo et al. (2005), and Francini et al. (2008). Assuming that surviving cancer cells will ultimately result in treatment failure, current cytotoxic drugs are not suitable for curative treatment in this disease, although their palliative role in hormone-refractory disease clearly has been established in recent years (Berthold et al. 2008).

In most clinical situations, chemotherapy augments the radiation-induced cell kill within the irradiated volume and may improve distant control. To maximize augmentation of cell kill, optimization of parameters of drug exposure is necessary. It has been shown, for example, that continuous infusion is better than bolus administration of 5-fluorouracil. The following example illustrates the efficacy of chemotherapy as a radiation enhancer. In the large randomized FFCD 9203 trial in rectal cancer preoperative radiotherapy (45 Gy in 25 fractions) resulted in a pCR in 4%, whereas the addition of 5-fluorouracil and folinic acid improved this figure to 12% (Gerard et al. 2005). As recently suggested from the data of patients with glioblastoma who received radiotherapy alone or radiotherapy plus temozolomide (Stupp et al. 2005), the effect of the drug in combined modality treatment corresponds to the equivalent of 9.1 Gy in 2-Gy fractions (Jones and Sanghera 2007). In patients treated with neoadjuvant combined chemo- and radiotherapy for esophageal cancer (data from 26 trials combined), it was estimated that 1 g/m^2 of 5-fluorouracil was equivalent to a radiation dose of 1.9 Gy and that 100 mg/m^2 cisplatin was equivalent to a radiation dose of 7.2 Gy (Geh et al. 2006). A combined analysis of 14 head and neck cancer trials confirms these data (Kasibhatla et al. 2007). With 2–3 cycles of cisplatin, carboplatin, and/or 5-fluorouracil containing radiochemotherapy regimens, the additional dose corresponds to 12 Gy in 2 Gy per fraction daily. In many clinical situations, radiation dose escalation by 9–12 Gy would result in increased late toxicity risks. Under these circumstances, combining radio- and chemotherapy increases the therapeutic window.

While radiation alone can be considered as a curative treatment in a variety of early-stage solid tumors (especially T1-2 N0 M0, for example, skin, anal, cervix, larynx, lung, and prostate cancers, see also above), long-term control with chemotherapy alone is rarely observed. Even in the adjuvant situation, chemotherapy often fails to control micrometastatic disease. Current concepts of cancer biology suggest that most traditional chemotherapy approaches fail to eradicate cancer stem cells, which are slow-cycling cells that often express multidrug resistance (MDR) proteins (Miller et al. 2005). It has been proposed that approaches targeting this subpopulation of cancer cells might increase the efficacy of drug treatment (Korkaya and Wicha 2007). Previous strategies of chemotherapy intensification, either by local delivery, systemic high-dose treatment, or simultaneous administration of several non-cross-resistant drugs, for example, 8-drugs-in-1-day, were mostly disappointing (Farquhar et al. 2005). Among newer concepts is the so-called metronomic chemotherapy, which refers to prolonged administration of comparatively low doses of cytotoxic drugs with minimal or no drug-free breaks. This strategy is thought to have an antiangiogenic basis and shows encouraging results in preclinical models (Shaked et al. 2005). It is now also combined with maximum-tolerated dose chemotherapy and targeted agents in vivo (Pietras and Hanahan 2005). Again we like to mention here that in summary one has to assume that especially in macroscopic but very often also in microscopic tumors the specific pathophysiology (vessel architecture, blood flow, inter-

stitial pressure, etc.) is the predominant biological factor minimizing drug efficiency due to an inhomogeneous drug distribution within the tumor tissue and leaving tumor subvolumes with inefficient drug concentrations (Minchinton and Tannock 2006).

10.4 Interaction of Radiation and Chemotherapy

Therapeutic gain is defined by an increase of tumor control and finally survival without a parallel increase in the severity of specific side effects (Fig. 10.3). Only a few reports are available proving that the combination of radiation and chemotherapy actually results in an increased therapeutic gain. A very nice preclinical example is the comprehensive studies with cisplatin and 5-fluorouracil in different tumors transplanted into mice, which were reported by Kallman et al. (1992). In our opinion, this group has demonstrated in an excellent fashion how clinically relevant experiments of radiochemotherapy can be designed. Also worth mentioning is a clinical example, a randomized German phase III trial (Budach et al. 2005), where a total of 384 stage III and stage IV head and neck cancer patients were randomly assigned to receive either 30 Gy (2 Gy/day) followed by 1.4 Gy b.i.d. (2 fractions per day) to a total dose of 70.6 Gy concurrently with 5-fluorouracil and mitomycin C (C-HART) or 14 Gy (2 Gy/day) followed by 1.4 Gy b.i.d. to a total dose of 77.6 Gy (HART). The overall treatment time was equal in both groups. At 5 years, the locoregional control and overall survival rates were significantly better in the radiochemotherapy arm compared with the radiation-only arm. Interestingly, the maximum acute reactions of mucositis, moist desquamation, and erythema were significantly lower in the radiochemotherapy arm compared with radiotherapy alone. No differences in late reactions and overall rates of secondary neoplasms were observed; thus, this trial impressively documents that the combination of radiotherapy with chemotherapy agents may effectively widen the therapeutic window; however, it is clear that although the specific toxicities may not be increased, new toxicities in terms of hemotoxicity will be added; thus, the net effect of radiochemotherapy results from a cooperation regarding tumor control and, in parallel, a diversification of toxicities. Independently of the term "therapeutic gain," the interaction of radiation with chemotherapy follows a precise nomenclature based on some groundbreaking theoretical considerations published in the late 1970s (Steel 1979; Steel and Peckham 1979). In every case of a scientific description and quantification of the effects of combined modality therapy in appropriate models, it is highly recommended to adhere to the proposed nomenclature. The complexity of effects increases with each step of investigation, i.e., from cell culture to tumor-bearing

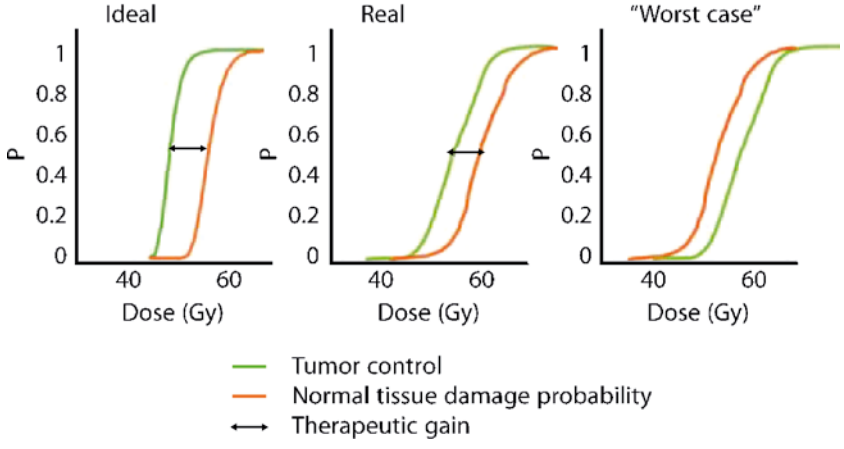

Fig. 10.3. Therapeutic gain. Therapeutic gain is defined as the resulting benefit when tumor control is weighted against the normal tissue damage. In an ideal setting (*left*) the probability of normal tissue damage is minimal at a dose level with a maximal probability of tumor control. More realistically (*middle*), doses required to achieve local control are associated with a certain, but low, probability of normal tissue damage. In situations where the doses required to control the tumor are continuously higher than the doses being toxic (*right*), treatment will be palliative in most cases ("worst case")

animal to cancer patient (Wurschmidt et al. 2000). A thorough examination of all possible treatment combinations and administration schedules for a given drug plus radiation is very challenging, as can be seen in the publication by Kallman et al. (1992), who studied in depth the radiosensitizing effects of cisplatin and other chemotherapeutic substances.

10.4.1 Spatial Interaction

On a large scale, chemotherapy and radiation may be effective on several levels. The concept of spatial interaction was devised to mean that chemotherapy and radiation act on spatially distinct compartments of the body, resulting in a net gain in tumor control. The concept of spatial interaction does not take into account any drug–radiation interaction on the level of the tumor itself, but rather assumes that radiation or chemotherapy would be active in different compartments, respectively. In a narrow sense, this concept describes the fact that chemotherapy would be employed for the sterilization of distant microscopic tumor seeding, whereas radiation would achieve local control (Fig. 10.4). Obviously, this is a theoretical consideration only, since chemotherapy also increases local control and radiotherapy reduces distant metastasis via increased local control rates; thus, when integrating the concept of spatial interaction into a more complete view on combined modality, spatial cooperation is still of major importance. In a more narrow sense, the aspect of spatial interaction is of major importance when one attempts to adequately cover sanctuary sites during multimodality approaches for certain types of leukemia and lymphomas. Next to spatial effects, several other important mechanisms may increase the efficacy of a combined treatment approach. In this regard, inhibition of repopulation and effective killing of hypoxic radioresistant cells by medical substances may contribute to the efficacy of a combined treatment.

10.4.2 Role of Repopulation

The fractionated treatment of tumors with ionizing radiation is associated with the phenomenon of repopulation (Kim and Tannock 2005). Speaking simply, a cer-

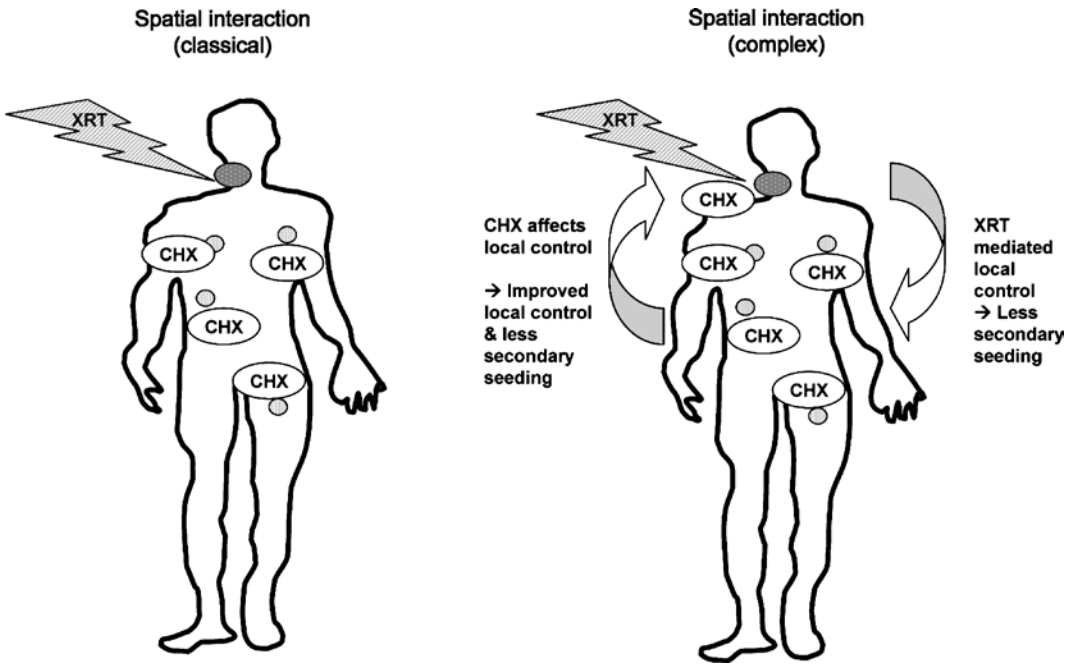

Fig. 10.4. Spatial interaction. In a classical interpretation (*left*) the term spatial interaction refers to the fact that chemotherapy (*CHX*) is effective on tumor compartments where radiation (*XRT*) has no efficacy, and vice versa, resulting in a generally increased control rate. In a more complex view (*right*), spatial interaction is relevant on multiple interacting levels: increased local control by radiation reduces the risk of a secondary seeding. Furthermore, the interaction of radiation with chemotherapy increases local control; thus, in addition to the classical spatial interaction, several levels of interacting feedback loops exist, which increase efficacy of spatial interactions

tain amount of tumor cells repair the induced damage in between two fractions and proliferate. Repopulation may neutralize around 0.5 Gy/day; however, the range of repopulation is considerably large and may reach higher levels (Trott 1990; Baumann et al. 1994; Budach et al. 1997). Based on these findings, radiation biologists advocated the use of accelerated radiation schedules; however, the acute and late effects of such approaches turned out to be more intense so that the final value of those approaches in terms of a real therapeutic gain remains unclear (Beck-Bornholdt et al. 1997; Dische et al. 1997; Horiot et al. 1997). The phenomenon of repopulation must also be taken into account when trying to design combined modality regimens. In theoretical models, cell loss from neoadjuvant chemotherapy preceding fractionated radiation treatment might trigger accelerated repopulation (Fig. 10.5). Then, a certain percentage of the daily radiation dose is wasted to counteract increased tumor cell proliferation. Under such conditions, despite a response to chemotherapy, cell survival after radiotherapy is no better than after the same course of radiotherapy alone (yet toxicity results from both modalities). Accelerated repopulation has also been described after treatment of murine breast tumors with sequential, weekly cycles of 5-fluorouracil and cyclophosphamide (Wu and Tannock 2003).

The clinical observation that the simultaneous combination of 5-fluorouracil, mitomycin C, or cisplatin with radiation is of value in rapidly proliferating squamous cell cancers has led to the assumption that the addition of drugs may influence the potential of cancer cells to repopulate. At least for mitomycin C this effect was documented precisely using a xenograft model (Budach et al. 2002). In this model, transplanted tumors were treated with 11 × 4.5 Gy fractionated radiation under ambient conditions with or without mitomycin C followed by a graded top-up dose on days 16, 23, 30, or 37 given under hypoxic conditions. Repopula-

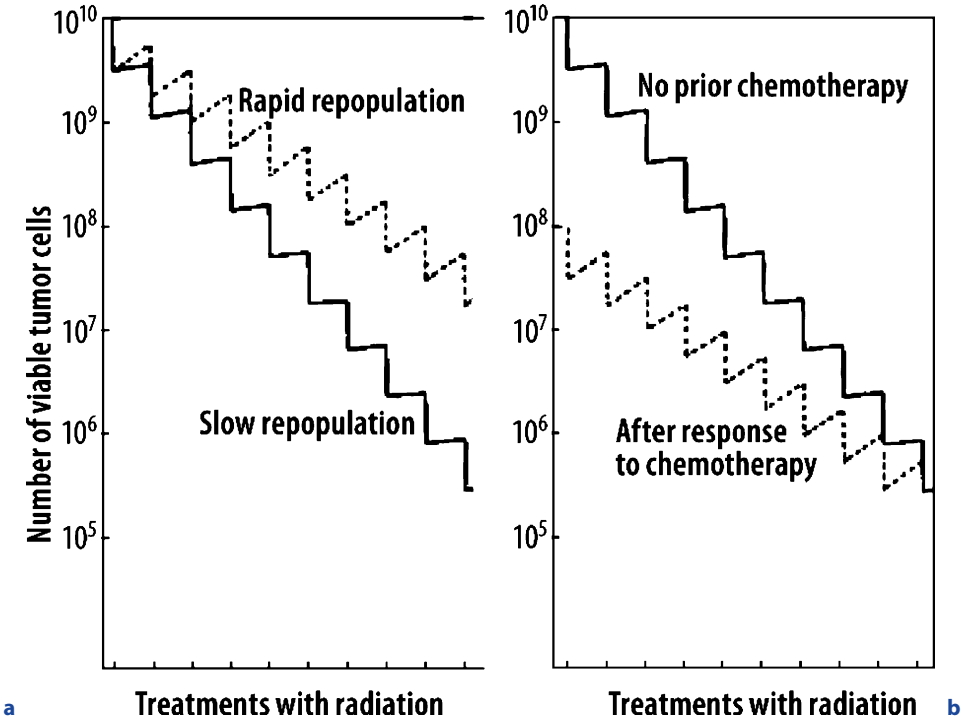

Fig. 10.5a,b. Influence of tumor cell repopulation on outcome. **a** Cell survival during a fractionated course of radiotherapy depends not only on the proportion of cells killed with each dose (which is equal for the two examples shown), but also on the rate of proliferation of surviving cells between the fractions, which differs between the two curves. **b** Hypothetical diagram to illustrate the number of surviving cells in a tumor during treatment with radiation alone, or during radiation treatment in a tumor that has responded to neoadjuvant chemotherapy (i.e., cell number reduced to 1% at start of radiotherapy) but where proliferation has been stimulated. Despite neoadjuvant chemotherapy, ultimate cell survival is similar. (From Tannock 1989, 1992)

tion in the interval between the fractionated treatment and the top-up dose accounted for 1.33 Gy top-up dose per day in animals not receiving mitomycin C, but only 0.68 Gy in animals receiving the drug; thus, at least mitomycin C may increase the efficacy of radiation by the inhibition of repopulation.

10.4.3 Role of Hypoxia

As known for years, radiation-induced cell kill is strongly dependent on the presence of adequate oxygen tensions. In larger tumors, for example, head and neck cancers, areas of hypoxia and even anoxia are present leading to an increased radiation resistance of clonogenic tumor cells within such areas (Molls and Vaupel 1998; Stadler et al. 1999; Nordsmark et al. 2005; Wouters et al. 2005). It has been speculated that chemotherapeutic agents, especially those killing even hypoxic cells, may overcome global radiation resistance simply by killing radioresistant hypoxic cells, thereby being of special value in highly hypoxic tumors (Teicher et al. 1981; Rockwell 1982).

Comparing the effects of several cytostatic drugs in combination with radiation on the growth of a C3H mammary carcinoma, it turned out that cyclophosphamide, adriamycin, and mitomycin C had the most significant effect on the proportional cell kill of hypoxic cells. In contrast, bleomycin and cisplatin did not exert strong effects on hypoxic cells (Grau and Overgaard 1988). In addition, it has clearly been shown that tumor blood flow in xenografts is increased after mitomycin C treatment (Durand and LePard 1994). Using two different squamous cell carcinomas, the latter authors tested the drug's influence on the outcome of radiation treatment with or without hypoxia (Durand and LePard 2000). The authors reported neither an increased killing of hypoxic cells by mitomycin C nor a consistent increase in tumor blood flow rates; however, mitomycin C in combination with radiation was associated with a slight increase in cell killing of hypoxic subpopulations of the xenograft system. Based on this observation it was concluded that the efficacy of a combined treatment with mitomycin C and radiation cannot be rationalized on either a complementary cytotoxicity or on drug-induced improvement in tumor oxygenation secondary to an increased blood flow.

In the case of paclitaxel it has been tested whether the enhanced killing by the combination of paclitaxel and radiation is connected to the presence of oxygen. Using an MCA-4 xenograft system, the authors could show that in the absence of oxygen the paclitaxel-mediated change of the TCD_{50} value is strikingly less prominent (Milas et al. 1994, 1995); thus, it can be concluded that at least in part the influence of paclitaxel on the radiation response is mediated via an optimized oxygenation. In a clinical trial of neoadjuvant chemotherapy in breast cancer, paclitaxel significantly decreased the mean interstitial fluid pressure and improved oxygenation, effects which were not observed in a randomized control group receiving doxorubicin (Taghian et al. 2005).

In conclusion, several sets of data indicate that the efficacy of chemotherapy in combination with radiation may be related to an increased oxygenation of hypoxic tumors; however, it still remains speculative whether or to what amount the efficacy of a combined treatment is strictly related to specific influences on the hypoxic cell compartment (Fig. 10.6).

10.5 Molecular Interactions

10.5.1 DNA Damage

One of the underlying molecular aspects of the efficacy of the combination of radiation and chemotherapy, which has been understood in more detail, is the influence on DNA repair. The induction of DNA damage is probably one of the most crucial events after irradiation of cells. In this regard, ionizing radiation triggers a wide array of lesions including base damage, single-strand breaks, and notably, double-strand breaks (DSB). After irradiation, different molecular systems are involved in recognition and repair of the damage. Whereas most of the induced damage is quickly repaired, DSB repair is slow and unrepaired DSBs are considerably important for the final induction of cell death.

Many chemotherapeutic agents, especially those known to be of value in combination with radiation, also induce considerable DNA damage or interfere with effective DNA repair; therefore, two general patterns of interactions may be separated: (1) the combination of the drug with radiation directly leads to more damage and (2) the drug may interact with the DNA repair pathway thus increasing the level of DNA damage more indirectly; however, one has to assume that none of the potential mechanisms acts without the other in real settings.

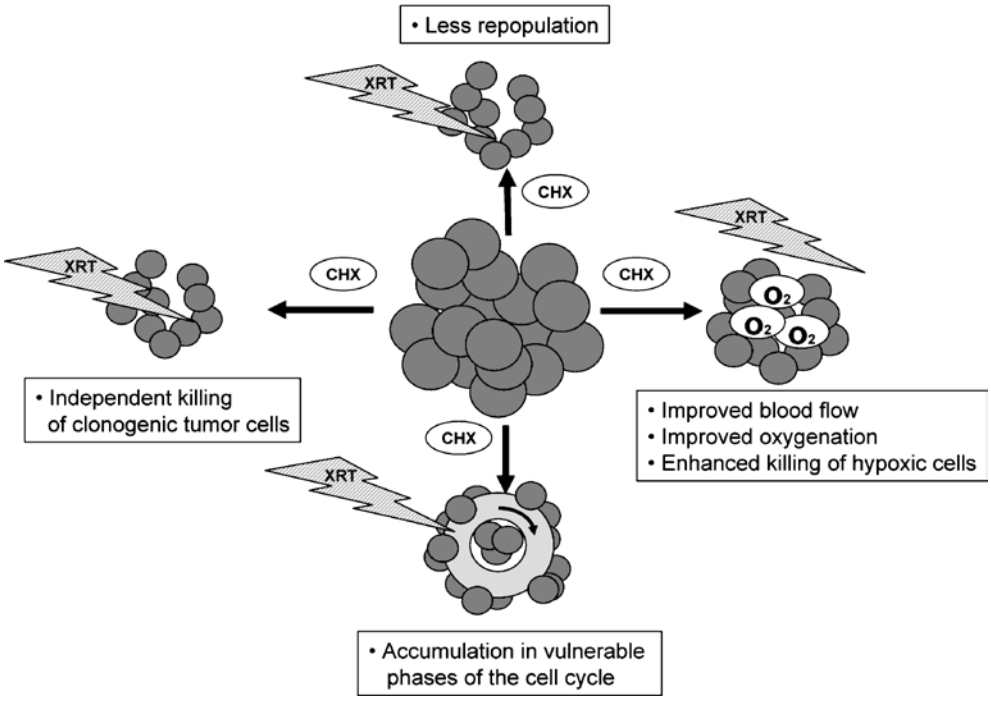

Fig. 10.6. Mechanisms of chemoradiation on a cellular level. At least four major mechanisms contribute to the efficacy of the combination of radiation with chemotherapy. In general, the addition of chemotherapy adds to the combined effect simply by an additional independent killing of clonogenic tumor cells. This mechanism is backed up by several other more interactive pathways: chemotherapy may induce a certain reassortment of tumor cells in more vulnerable phases of the cell cycle, chemotherapy may reduce the level of repopulation during a course of fractionated radiotherapy, and, finally, chemotherapy may partially overcome hypoxia-mediated radiation resistance

Cisplatin, for example, acts by complex formation with guanosine residues and subsequent adduct formation ultimately resulting in intra- and interstrand crosslinks. This type of damage is mostly removed by base excision repair and mismatch repair. Several sets of data suggest that single-strand damage induced by radiation in close vicinity to DNA damage triggered by cisplatin results in a mutual inhibition of the damage-specific repair system; thus, the amount of resulting damage leads to an increased net cell kill (Begg 1990; Yang et al. 1995).

Similarly, etoposide, which is a strong topoisomerase IIa-directed toxin, induces DSBs mostly during the S-phase of the cell cycle (Berrios et al. 1985; Earnshaw and Heck 1985). Again, several lines of evidence show that the combination of both agents results in a strongly increased level of damage (Giocanti et al. 1993; Yu et al. 2000).

The biochemical pathways involved in DNA repair and DNA synthesis overlap in several regards; thus, drugs acting on the synthesis of DNA putatively also interfere with the repair of DNA damage after application of ionizing radiation. Several prototypical radiation sensitizers may act via these mechanisms. Besides cisplatin, 5-fluorouracil is probably the most commonly employed drug in clinical combined modality settings. Basically, 5-fluorouracil inhibits thymidylate synthase thereby reducing the intracellular pool of nucleoside triphosphates (Pinedo and Peters 1988; Miller and Kinsella 1992). In addition, the drug is integrated into DNA via fluorodeoxyuridine, also contributing to its antineoplastic effects. Several lines of evidence suggest that the amount of 5-fluorouracil integrated into DNA directly correlates with the radiosensitizing effect. In addition, the complementation of the cell culture medium with higher levels of thymidine reverses the effects of 5-fluorouracil on the radiation sensitivity (Lawrence et al. 1994; McGinn et al. 1996).

Gemcitabine, which is another radiation sensitizer, was also shown to deplete the pool of deoxynucleosides and is integrated into DNA. The drug is known to exert a pronounced radiosensitizing effect in squamous cancer cells, as well as adenocarcinoma cells from pancreatic

cancer. In vitro this effect was especially pronounced during the S-phase passage (Robertson et al. 1996; Lawrence et al. 1997; Rosier et al. 1999). Although few data regarding the mechanistic basis of the interaction between radiation and gemcitabine are available, the exact mechanism remains elusive. The radiation-sensitizing effect was seen over a prolonged time period (~48 h) after incubation of HT29 cells with low doses of gemcitabine (100 nm). During the first 48 h the level of S-phase cells increased, whereas the amount of deoxynucleosides remained low even up to 72 h (Shewach et al. 1994; Lawrence et al. 1997); thus, it seems likely that the depletion of the deoxynucleoside pools in combination with an increased killing of cells in S-phase is a mechanism responsible for an enhanced radiation susceptibility mediated by gemcitabine.

10.5.2 Radiation Sensitization Via Cell Cycle Synchronization

The fact that striking differences in the radiation sensitivity occur as cells move through the different phases of the cell cycle has stimulated the speculation that the efficacy of a combined treatment may also be related to possible effects on the reassortment of cells in more vulnerable cell cycle phases.

Several experimental settings provide evidence that cell cycle effects are involved in the modulation of the efficacy of combined modality approaches. In this regard the use of a temperature-sensitive p53 mutant allows the analysis of cell cycle effects. The underlying hypothesis was that fluoropyrimidine-mediated radiosensitization occurs only in tumor cells that inappropriately enter S-phase in the presence of drug resulting in a subsequent repair defect of the radiation-induced damage. The use of the mutated p53 allowed p21-mediated arrest prior to S-phase entry when cells are grown under 32°C, in contrast to no arrest in cells grown at the non-permissive temperatures of 38°C. The radiation-sensitizing effect of fluoropyrimidine was directly connected to the lacking G1 arrest when cells were grown under non-permissive temperatures; thus, the fluoropyrimidine-mediated radiosensitization clearly requires progression into S-phase (Naida et al. 1998).

In an extension of these findings, Naida et al. (1998) analyzed the effects of fluorodeoxyuridine on the radiation sensitivity in HT29 and SW620 human colon cancer cells under nearly complete inhibition of thymidylate synthase (both cell lines harbor a similar p53 mutation). Interestingly, only the HT29 cells were sensitized. As an underlying feature, the authors found that only the HT29 cells progressed into S-phase and demonstrated increased cyclin E-dependent kinase activity. In contrast, SW620 cells were found to be arrested just past the G1-S boundary and an increase in kinase activity was not detectable; thus, the findings underline the requirement of an S-phase transition for the efficacy of halogenated fluoropyrimidines in combination with radiation. These findings also highlight the role of molecules involved in cell cycle regulation as key players for the modulation of a combined modality approach (McGinn et al. 1994; Lawrence et al. 1996a–c). In addition to the fact that the S-phase transition is required for the radiosensitization effect, it has also been shown that fluoropyrimidines under defined dosage conditions facilitate the accumulation of cells in S-phase (Miller and Kinsella 1992).

In addition to the findings on halogenated fluoropyrimidines, several other sets of data obtained with paclitaxel suggest that an increased radiation sensitivity occurred at the time of a taxane-induced G2-M block; however, the situation for taxane combinations is highly complex in so far as other data provide evidence that the mitotic arrest is not sufficient for the effects of paclitaxel (Geard and Jones 1994; Hennequin et al. 1996). The picture becomes even more complicated when taking into account that radiation was shown to decrease the net killing of taxanes (Sui et al. 2004). In this regard, it has been shown that the combination of paclitaxel and gamma radiation did not produce a synergistic or additive effect in a breast cancer and epidermoid cancer cell model. Instead, the overall cytotoxicity of the combination was lower than that of the drug treatment alone. In particular apoptosis induction was found to be strikingly reduced. A detailed analysis revealed that radiation resulted in cell cycle arrest at the G2 phase preventing the G1-M transition-dependent cytotoxic effects of paclitaxel. Furthermore, radiation inhibited paclitaxel-induced IκBα degradation and bcl-2 phosphorylation and increased the protein levels of cyclin B1 and inhibitory phosphorylation of p34(cdc2).

Taken together, the impact of chemotherapy-induced cell cycle alterations as a major mechanism for the efficacy of the combined action is still questionable. In clinical settings, the importance of an adequate cell cycle progression for the efficacy of radiochemotherapy approaches has been impressively documented. In the case of a neoadjuvant 5-fluorouracil-based radiochemotherapy for rectal cancer, it has been shown that a decrease of the cell cycle inhibitory protein p21 during neoadjuvant treatment is strongly associated with an improved disease-specific survival. This finding has been corroborated by the observation that a parallel increase of the expression level of the proliferation

marker ki-67 is similarly associated with an improved outcome (Rau et al. 2003); thus, preclinical findings on the action of 5-fluorouracil in combination with radiation are clearly reflected by clinical observations.

10.6 Potential Influences on Programmed Cell Death Pathways

In order to inactivate a tumor cell, several distinct yet overlapping pathways may be activated. Besides the induction of pure apoptosis, other cell inactivation modalities, including programmed necrosis, mitotic catastrophe, senescence, or terminal differentiation, may be triggered (Belka 2006). The influence of a combined modality treatment on any of these end points has never been analyzed in greater detail; thus, only very few data are available showing that the combination of paradigmatic radiation sensitizers with radiation quantitatively alters the induction of certain predefined mechanisms of cell death (Fig. 10.7).

In the case of gemcitabine, the efficacy of a combined treatment in terms of apoptosis induction has been analyzed in more detail using HT29 colon cancer cells, UMSCC-6 head and neck cancer cells, and A549 lung cancer cells. A key feature was that all cell systems differ substantially in the ability to undergo radiation-induced apoptosis, with HT29 being the most apoptosis-sensitive cell in this experimental setting. It turned out that the radiosensitization of HT29 cells was accompanied by an increase in apoptosis, whereas in UMSCC-6 cells and A549 cells, the radiosensitizing effect was mediated via non-apoptotic mechanisms; thus, this effect is rather a cell-type-specific feature than a general property of the drug.

In the case of definitive treatment approaches in esophageal or rectal cancer, the importance of apoptosis signaling has been documented. Esophageal cancer patients with lack of the proapoptotic Bax molecule have significantly reduced outcome rates (Sturm et al. 2001). Similar findings have been observed for neoadjuvant radiation or radiochemotherapy in patients with rectal tumors with a low expression of Bax (Chang et al. 2005; Nehls et al. 2005).

10.7 Effects of Protracted Drug Exposure

More than 30 years ago, in vitro studies demonstrated increased efficacy when tumor cells were exposed to mitomycin C or several other drugs for a prolonged time (Shimoyama 1975). This finding was confirmed

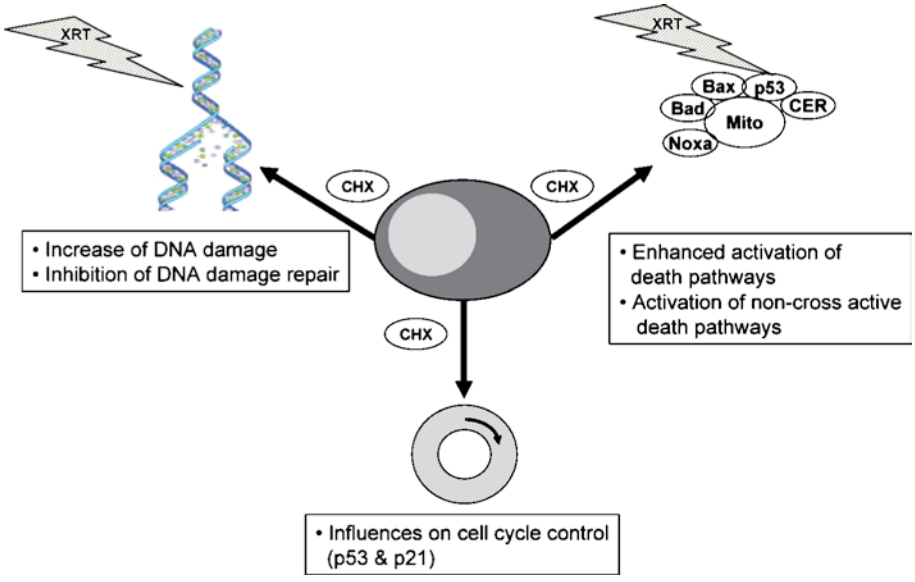

Fig. 10.7. Mechanisms of chemoradiation on a molecular level. The most prominent points of interaction of radiation with chemotherapy being of importance for the efficacy of a combined modality treatment are found on the level of DNA damage induction and repair, cell death induction, and cell cycle control

in clinical trials of continuous infusion versus bolus 5-fluorouracil (Seifert et al. 1975). Furthermore, and probably related to avoidance of peak concentrations, reduced normal tissue toxicity was observed. In principle, these divergent effects on tumor and normal tissues improve the therapeutic window. Considering tumors, longer exposure times of 5-fluorouracil result in enhanced cell killing also in the context of simultaneous radiation therapy (Moon et al. 2000). A combined analysis of more than 3,100 patients with rectal cancer treated with preoperative radiochemotherapy demonstrated that the pCR rate was significantly higher when continuous infusion 5-fluorouracil was used, as compared with other modes of delivery (Hartley et al. 2005). Protracted exposure is also currently being tested for other drugs such as temozolomide. Whether such regimens hold promise depends on the mode of action of the drug, cell-cycle specificity, pharmacokinetics, etc.

10.8 Conclusion

A large body of in vitro results and data from animal experiments and clinical trials show very clearly the high efficacy of radiotherapy and the fact that cell kill from chemotherapy is often comparable to that of rather low doses of radiation. The underlying principles are now better understood than in earlier decades. They provide the basis for development of improved methods of delivery, modification of blood flow and microenvironment, measures to counteract resistance and metabolization, and, maybe most importantly, rationally designed combination treatment. Compared with the relatively homogeneous models used for description of experimental end points, the clinical situation is complicated by a very complex tumor biology with changes in physiological and microenvironmental parameters over time, and even differences between the primary tumor itself and regional lymphatic metastases, which receive identical treatment. There has been a long-lasting interest in prediction of individual response, for example, by means of pretherapeutic ex vivo chemosensitivity testing in cell culture or determination of molecular marker genes (Shimizu et al. 2004; Staib et al. 2005). More recently, treatment monitoring early during a course of chemotherapy or radiochemotherapy by means of positron emission tomography, diffusion magnetic resonance imaging, and other biological imaging methods has shown promising results (Weber 2005). Nevertheless, treatment individualization, also with regard to normal tissue toxicity and drug metabolism, for example, based on single nucleotide polymorphisms (Efferth and Volm 2005; Robert et al. 2005), continues to be an area of active investigation.

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