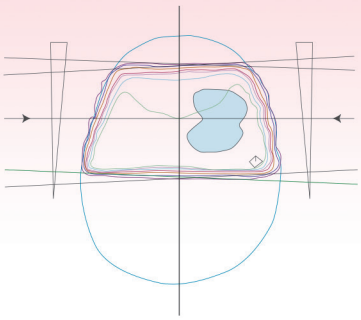
CHAPTER

3 Dose-Response Modifiers in Radiation Therapy



Michael R. Horsman, Jacob C. Lindegaard, Cai Grau, Marianne Nordsmark, Jan Alsner, and Jens Overgaard

When cancer patients undergo radiation therapy there is a clear dose-response relationship between the dose delivered and the response of the tumor to the radiation. This is illustrated in Figure 3-1. Unfortunately, there is also an increase in normal tissue damage with increasing radiation dose, and it is this complication that limits the total radiation dose that can be given. Substantial effort has been made to try and modify these dose-response relationships and to thus increase the separation between the tumor and normal tissue dose-response curves. The approach has been either to selectively increase the radiation damage in tumors without affecting the normal tissues or by protecting the normal tissues without having a similar protective effect in tumors.

Agents capable of enhancing radiation response include certain conventional chemotherapeutic agents, the halogenated pyrimidines, and treatments that specifically overcome radioresistance resulting from the presence of hypoxic cells that occur as a result of the environmental conditions within most solid tumors. The most widely investigated method applied to the hypoxia problem is radiosensitization of the hypoxic cells with either electron-affinic sensitizing drugs or hyperthermia. Another approach often used to reduce hypoxia—especially in experimental systems—involves increasing oxygen availability (1) by having patients breathe high-oxygen-content gas, (2) introducing perfluorochemical emulsions into the vascular system to increase the oxygen carrying capacity of the blood, (3) modifying oxygen transport or delivery by using agents that affect hemoglobin, (4) using drugs that increase tumor blood perfusion, or (5) a more recent approach of decreasing the oxygen consumption rate of the "nonhypoxic" cell population, thereby increasing the oxygen diffusion distance. Many experimental studies have also demonstrated that hypoxic cells can be preferentially destroyed by bioreductive drugs that are active under reduced oxygen conditions, or again using hyperthermia; each of these hypoxic-cell cytotoxins improve the radiation response of tumors. Another group of agents, which preliminary data suggests have the potential to enhance radiation damage, are the so-called vascular targeting agents. These include drugs that inhibit angiogenesis, the process by which tumors develop their own vascular supply, or agents that preferentially damage the already established tumor vessels.

Radiation protectors fall into several categories based on the timing of their administration in relation to radiotherapy. There are the true "radiation protectors," in particular sulfhydryl compounds, which are used as a prophylactic strategy and administered before radiotherapy and primarily appear to interact with radicals that are formed as a result of radiation exposure. Another group consists of "radiomitigators" that reduce the effects on normal tissues before the emergence of symptoms if given during or shortly after radiotherapy. Finally, there are "therapeutic agents," which are administered after radiotherapy to treat symptoms that have already developed, especially fibrosis.

Radiosensitization by conventional chemotherapeutic agents (e.g., cisplatin, 5-fluorouracil, and mitomycin C), halogenated pyrimidines (e.g., 5-bromodeoxyuridine and 5-iododeoxyuridine), and hyperthermia are discussed in detail elsewhere in this book. In this chapter, the focus will be on hypoxic cell modifiers, vascular targeting drugs, and radioprotectors.

THE HYPOXIA PROBLEM

Importance of Oxygen

In 1909 Gottwald Schwarz,[1] in a simple but elegant experiment, demonstrated that the radiation response of skin was markedly decreased if the blood flow in the irradiated area was reduced by compression. Although he did not acknowledge that the phenomenon was the result of a lack of oxygen, his study was probably the first radiobiologically oriented clinical study implicating the importance of environmental parameters in the outcome of radiotherapy. This finding was used to introduce the concept of "kompressionsanämie" by which the skin was made anemic, thereby allowing a higher dose to be given to deeply situated tumors. Following the work of Schwarz, in 1910 Müller[2] reported that tissues in which the blood flow was stimulated by diathermia showed a more prominent response to radiation. This early study not only demonstrated the importance of oxygen supply in radiotherapy, but it was also the first clinical approach showing how resistance could be overcome by using hyperthermia. Subsequently, sporadic clinical and experimental observations indicated the importance of sufficient blood supply to secure an adequate radiation response. These observations led Gray et al[3] in the early 1950s to postulate that oxygen deficiency or hypoxia was a major source of radiation resistance.

The first clinical indication that hypoxia existed in tumors was made around the same time by Thomlinson and Gray[4] when, from histological observations in carcinoma of the bronchus, they reported seeing viable tumor regions surrounded by vascular stroma from which the tumor cells obtained their nutrient and oxygen. As the tumors grew, the viable regions expanded and areas of necrosis appeared at the center. The thickness of the resulting shell of viable tissue was found to be between 100 and 180 μm, which was within the same range as the calculated diffusion distance for oxygen in respiring tissues. It was thus suggestive that as oxygen diffused from the stroma, it was consumed by the cells and, although those beyond the diffusion distance were unable to survive, the cells immediately bordering the necrotic area might be viable yet hypoxic. In 1968, Tannock[5] described an inverted version of the Thomlinson and Gray picture, with functional blood vessels surrounded by cords of viable tumor cells outside of which were areas of necrosis. This "corded" structure, illustrated in Figure 3-2, is the more typical picture found in most solid tumors.[6] It arises because the tumor blood vessels, which are derived from the normal tissue vessels by a process of angiogenesis, are inadequate to meet the needs of the rapidly growing tumor cells. This hypoxia is more commonly called *chronic hypoxia*.

It was also suggested that hypoxia in tumors could be acute in nature.[7] However, it was not until later that Chaplin et al[8] were able to confirm the existence of acutely hypoxic cells in tumors and demonstrate that these cells were the result of

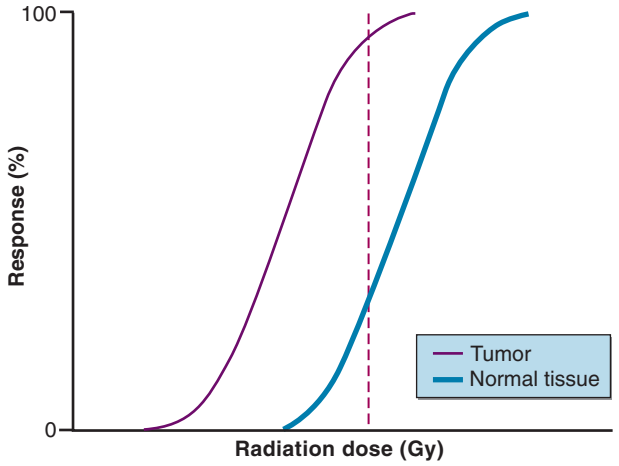


Figure 3-1 Schematic illustration of the proportion of patients cured and patients with normal tissue complications as a function of the total radiation dose received.

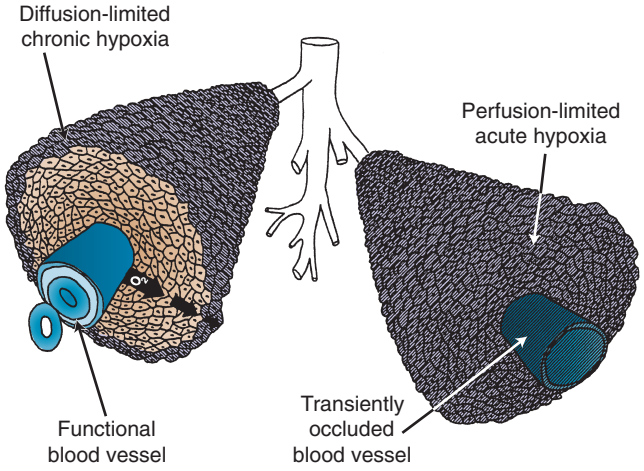


Figure 3-2 Schematic representation of the interrelationship between tumor cells and the vascular supply. On the left, cells are seen growing as a "corded" structure around a functional vessel from which the cells receive their oxygen supply. As oxygen diffuses out from the vessel it is used up, thus the outermost viable cells (shown by the shading) are oxygen deprived or chronically hypoxic. A similar arrangement is seen on the right, but here flow through the vessel is transiently stopped, thus making all the cells oxygen deprived.
Reprinted from Horsman MR: Measurement of tumor oxygenation. Int J Radiat Oncol Biol Phys 42:701–714, 1998. Copyright 1998, with permission from Elsevier Science.

transient stoppages in tumor blood flow (see Figure 3-2). To date, these temporary cessations in blood flow have been observed in mouse and rat tumors, as well as human tumor xenografts, with anywhere from around 4% to 8% of the total functional vessels involved,[9] although the exact causes of these stoppages are not known. The current use of chronic or acute to explain hypoxia in tumors is probably an oversimplification of the real situation. Chronic hypoxia generally refers to prolonged and reduced oxygen concentrations that influence radiation response, but there is evidence that oxygen concentrations that are higher, yet below normal physiological levels, are often found.[10] Furthermore, reduced perfusion can be both partial as well as total,[11] and while cells under the former condition would be oxygen deprived, with the latter they would be starved of oxygen and nutrients, and as such, their survival and response to therapy would be expected to be different.

Evidence for Hypoxia in Tumors

In experimental tumors it is not only relatively easy to identify hypoxia, but one can also quantitatively estimate the percentage of cells that are hypoxic. Three major techniques are routinely used.[12] These are the paired survival curve, the clamped tumor growth delay, and the clamped tumor control assays. All involve a comparison of the response of tumors when irradiated under either normal air-breathing conditions or when tumors are artificially made hypoxic by clamping. Using these procedures hypoxia has been directly identified in most animal solid tumors, with the values ranging from less than 1% to well more than 50% of the total viable cell population.[12] Unfortunately, none of these procedures can be applied to the clinical situation. One therefore must rely on indirect techniques.

Estimating hypoxia in human tumors has generally involved the use of indirect methods.[13] Some of the earliest attempts focused on the vascular supply because it was only via the tumor vasculature that oxygen could be delivered. The endpoints included immunohistochemical estimates of intercapillary distance, vascular density, and distance from tumor cells to the nearest blood vessel[14-16]; oxyhemoglobin saturation determined using cryophotometry, or noninvasively with near-infrared spectroscopy or magnetic resonance imaging (MRI)[17-19]; or measurements of tumor perfusion using MRI, computed tomography (CT), or positron emission tomography (PET).[20-22] With the finding that hypoxia could up-regulate gene/protein expression, it was suggested that endogenous markers could be used to identify hypoxia.[23] The principal markers have included hypoxia inducible factor 1 (HIF-1), carbonic anhydrase IX (CAIX), the glucose transporters GLUT-1 and GLUT-3, and osteopontin (OPN).[24-27] These have been applied either individually or combined with other endogenous markers in gene signatures.[13] More popular techniques involve measurements of the binding of exogenous markers. This can be achieved following immunohistological analysis of biopsied sections, using, for example, pimonidazole or EF5.[28,29] It can also be done noninvasively with PET, single-photon emission computed tomography (SPECT), or MRI analysis of radioactively labeled nitroimidazoles (i.e., [^{18}F] labeled misonidazole or FAZA; [^{123}I] labeled azomycin arabinoside), or PET imaging of [$^{60\text{-}64}$Cu]-ATSM.[30-33]

The most direct method involves determining oxygen partial pressure (PO_2) distributions with polarographic electrodes.[34-39] How this approach can be used to detect hypoxia and relate the measurements to radiotherapy outcome is illustrated in Figure 3-3. In this international multicenter study in head and neck cancer patients, their tumor's pO_2 was measured before radiation therapy and was found to correlate with overall survival, in that those patients with lower tumor oxygenation status, did significantly worse.[40]

Probably the best evidence for the existence of hypoxia in human tumors comes from the large number of clinical trials in which hypoxic modification has shown some benefit.[41] The latter situation constitutes a circular argument: if hypoxic modification shows an improvement then hypoxic clonogenic cells must have been present in tumors. It is, however, likely that even tumors with the same histological makeup and of the same type have substantial heterogeneity with respect to the extent of hypoxia. It must be admitted that today, a century after the first clinical description, the importance of hypoxia and its influence on the outcome of radiotherapy is still the subject of substantial debate. However, we will now discuss in detail how the different hypoxic modifiers have been used to modify the radiation dose response of tumors.

TABLE 3-1 Multicenter Randomized Trials with Hyperbaric Oxygen (HBO)

Site and Study	No. of Patients	Endpoint*	HBO	Air
HEAD AND NECK CARCINOMA				
MRC 1st trial (1977)	294	Control (5 yrs)	53%	30% ($p < 0.01$)
MRC 2nd trial (1986)	106	Control (5 yrs)	60%	41% ($p < 0.05$)
UTERINE CERVIX CARCINOMA				
MRC (1978)	320	Control (5 yrs)	67%	47% ($p < 0.001$)
BRONCHOGENIC CARCINOMA				
MRC; 60 Gy/40 fx (1978)	51	Survival (2 yrs)	15%	8% (NS)
MRC; 36 Gy/6 fx (1978)	123	Survival (2 yrs)	25%	12% ($p < 0.05$)
CARCINOMA OF THE BLADDER				
MRC (1978)	241	Survival (5 yrs)	28%	30% (NS)

fx, *Fractions;* NS, *not significant.*
**Endpoints were control (locoregional control) or survival. See Overgaard[53] for additional information.*

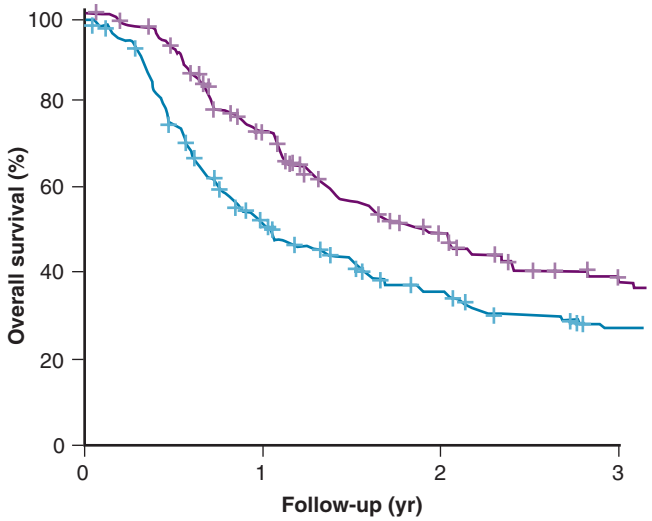


Figure 3-3 Oxygen levels were measured with Eppendorf electrodes before radiation therapy in 397 patients with squamous cell carcinomas of the head and neck. Tumors were stratified by whether the fraction of pO_2 values ≤ 2.5 mm Hg (HP2.5) were above or below the median value for the whole group (i.e., 19%). The lines show Kaplan Meier estimates of actuarial overall survival probability for patients with less hypoxic tumors (HP2.5 ≤ 19%; *red line*) compared with more hypoxic tumours (HP2.5 > 19%; *blue line*), $p = 0.006$.
From Nordsmark M, Bentzen SM, Rudat V, et al: Prognostic value of tumor oxygenation in 397 head and neck tumors after primary radiation therapy. An international multi-center study. Radiother Oncol 77:18–24, 2005, with permission.

OVERCOMING TUMOR HYPOXIA

High-Oxygen-Content Gas Breathing

Because the oxygen supply to tumors is insufficient to meet the needs of all the tumor cells, radiation-resistant hypoxia develops; therefore, an obvious solution to improving the tumor's radiation response would be to increase the oxygen supply. This has been tried, both experimentally and clinically, by simply allowing the tumor bearing host to breathe high-oxygen-content gas mixtures before and during irradiation.

Early experimental studies reported that breathing either oxygen and carbogen (95% O_2 + 5% CO_2) could substantially enhance the response of murine tumors to radiation and that the best effect was generally seen when the gasses were inspired under hyperbaric (typically 3 atmospheres [3 atm]) rather than normobaric conditions.[42,43] This is not surprising because hyperbaric conditions would be expected to saturate the blood with oxygen more than normobaric conditions. However, later studies indicated that the radiosensitizations produced by normabaric oxygen or carbogen were quite substantial;[44,45,46] because it is quicker and easier to breathe gas under normobaric conditions, the use of cumbersome, expensive, and complex hyperbaric chambers is probably not necessary.

Clinically, the use of high-oxygen-content gas breathing, specifically under hyperbaric conditions, was introduced relatively early by Churchill-Davidson et al.[47] Most trials were fairly small, and suffered from the applications of unconventional fractionation schemes, but it appeared that the effect of hyperbaric oxygen was superior to radiotherapy given in air, especially when few and large fractions were applied.[47-49] In the large, multicenter clinical trials conducted by the British Medical Research Council (Table 3-1), the results from both uterine cervix and advanced head and neck tumors showed a significant benefit in local tumor control and subsequent survival.[48,50-53] The same findings were not observed in bladder cancer nor were they seen in a number of smaller studies.[53] In retrospect, the use of hyperbaric oxygen was stopped somewhat prematurely. This was partly the result of the introduction of hypoxic radiosensitizers and partly because of problems with patients' compliance; it has been claimed that hyperbaric treatment caused significant suffering, but the discomfort associated with such a treatment must be considered minor compared to the often life-threatening complications associated with chemotherapy, which is used with less restrictive indications.

The use of high-oxygen-content gas breathing under normobaric conditions to radiosensitize human tumors has also been tried clinically, but it failed to show any dramatic improvement.[54-56] In the most recent study this may have been the result of size limitation,[56] but in previous studies it may have been caused by the failure to achieve the optimum preirradiation gas breathing time.[54,55] Experimental studies have shown that the amount of time is critical for the enhancement of radiation damage and that it can vary from tumor to tumor.[43-45,57]

Hypoxic Cell Radiosensitizers

An alternative approach to the hypoxia problem is the use of chemical agents that mimic oxygen and preferentially sensitize the resistant population to radiation. The advantage of these drugs over oxygen is that they are not rapidly metabolized by the tumor cells through which they diffuse and thus

TABLE 3-2 Multicenter Randomized Trials with Nitroimidazoles

Site and Study	No. of Patients	Sensitizer	Endpoint*	RT and Sensitizer	RT Alone
HEAD AND NECK CARCINOMA					
DAHANCA 2 (1989)	626	MISO	Control (5 yrs)	41%	34% ($p < 0.05$)
MRC (1984)	267	MISO	Control (>2 yrs)	40%	36% (NS)
EORTC (1986)	163	MISO	Control (3 yrs)	52%	44% (NS)
RTOG (1987)	306	MISO	Control (3 yrs)	19%	24% (NS)
RTOG 79-04 (1987)	42	MISO	Control (2 yrs)	17%	10% (NS)
DAHANCA 5 (1992)	414	NIM	Control (5 yrs)	49%	34% ($p < 0.002$)
RTOG 85-27 (1995)	500	ETA	Control (2 yrs)	39%	38% (NS)
European multicenter (1991)	374	ETA	Control (2 yrs)	57%	58% (NS)
UTERINE CERVIX CARCINOMA					
Scandinavian study (1989)	331	MISO	Control (5 yrs)	50%	54% (NS)
MRC (1984)	153	MISO	Control (>2 yrs)	59%	58% (NS)
RTOG (1987)	119	MISO	Control (3 yrs)	53%	54% (NS)
MRC (1993)	183	PIM	Control (4 yrs)	64%	80% ($p < 0.01$)
GLIOBLASTOMA					
Scandinavian study (1985)	244	MISO	Survival	10	10 (NS)
MRC (1983)	384	MISO	Survival	8	9 (NS)
EORTC (1983)	163	MISO	Survival	11	12 (NS)
RTOG (1986)	318	MISO	Survival	11	13 (NS)
BRONCHOGENIC CARCINOMA					
RTOG (1987)	117	MISO	Survival	7	7 (NS)
RTOG (1989)	268	MISO	Survival	7	8 (NS)

DAHANCA, *Danish Head and Neck Cancer study;* EORTC, *European Organization for Research and Treatment of Cancer;* ETA, *etanidazole;* MISO, *misonidazole;* MRC, *Medical Research Council;* NIM, *nimorazole;* NS, *not significant;* PIM, *pimonidazole;* RT, *radiation therapy;* RTOG, *Radiation Therapy Oncology Group.*
**Endpoints were control (loco-regional control) and survival (median survival in months). See Overgaard[61] for additional information.*

the drugs can penetrate further than oxygen and so reach all the tumor cells. In the early 1960s, researchers found that the efficiency of radiosensitization was directly related to electron-affinity[58] and that ultimately led to in vitro studies demonstrating preferential radiosensitization of hypoxic cells by highly electron-affinic nitroaromatic compounds.[59,60] Several of these compounds were later shown to be effective at enhancing radiation damage in tumors in vivo,[61] and as a result, they underwent clinical testing.

The drugs reaching clinical evaluation include metronidazole, misonidazole, benznidazole, desmethylmisonidazole, etanidazole, pimonidazole, nimorazole, ornidazole, sanazole, and doranidazole. Initial clinical studies were with metronidazole in brain tumors and were followed, in the latter part of the 1970s, by a boom in clinical trials exploring the potential of misonidazole as a radiosensitizer.[53,61,62] The results from the multicenter randomized trials are summarized in Table 3-2. Most of the trials with misonidazole were unable to generate any significant improvement in radiation response, although a benefit was seen in some trials, especially the second Danish Head and Neck Cancer study (DAHANCA 2), which found a highly significant improvement in the stratification subgroup of pharynx tumors but not in the prognostically better glottic carcinomas.[63] The overall impression of the "misonidazole-era" was a prolongation of the inconclusive experience from the hyperbaric oxygen trials, namely, that the problems related to hypoxia had not been ruled out indefinately.[61] Therefore, the search for more efficient or less toxic hypoxic sensitizers continues. Furthermore, the experience from the misonidazole trials has been taken into account to select a more homogeneous tumor population in which hypoxia is more likely to be present.

Results from subsequent randomized trials with other nitroaromatic compounds have been conflicting. The European pimonidazole trial in uterine cervix was disappointing,[64] whereas the two other multicenter trials in head and neck cancer, using etanidazole, showed no benefit.[61,65] On the other hand, studies with the low toxic drug nimorazole given to patients with supraglottic and pharynx carcinomas (DAHANCA 5) showed a highly significant benefit in terms of improved locoregional tumor control and disease-free survival rates (Figure 3-4),[66] thereby confirming the result of the DAHANCA 2 study. More recent trials with the 3-nitrotriazole compound, sanazole (AK-2123), in uterine cervical cancer[67] and doranidazole in pancreatic cancer[68] demonstrated significant improvements in both local tumor control and overall survival.

The potential benefit of using hypoxic radiosensitizers to improve radiotherapy is probably best illustrated from a recent meta-analysis of randomized clinical studies in squamous cell carcinoma of the head and neck.[69] These results are summarized in Figure 3-5 and clearly showed that radiosensitizer modification of tumor hypoxia significantly improved locoregional tumor control and overall survival with odds ratios of 0.71 and 0.87, respectively. Although the overall observed gain were small (5% to 10% for local control and 0% to 6% for survival) they are actually relevant. We can conclude that the nonsignificant outcome of most clinical trials (see Table 3-2) is not the result of a biological lack of importance of hypoxia, but in most cases is considered a consequence of poor clinical trials methodology with an overly optimistic study design and an expected treatment gain that goes far beyond what is reasonable. Overall, the results with nitroimidazoles add to the general consensus that if a nontoxic hypoxic modification can be applied, then such treatments may certainly be relevant as a baseline therapy together with radiotherapy for cancers such as advanced head and neck cancer.

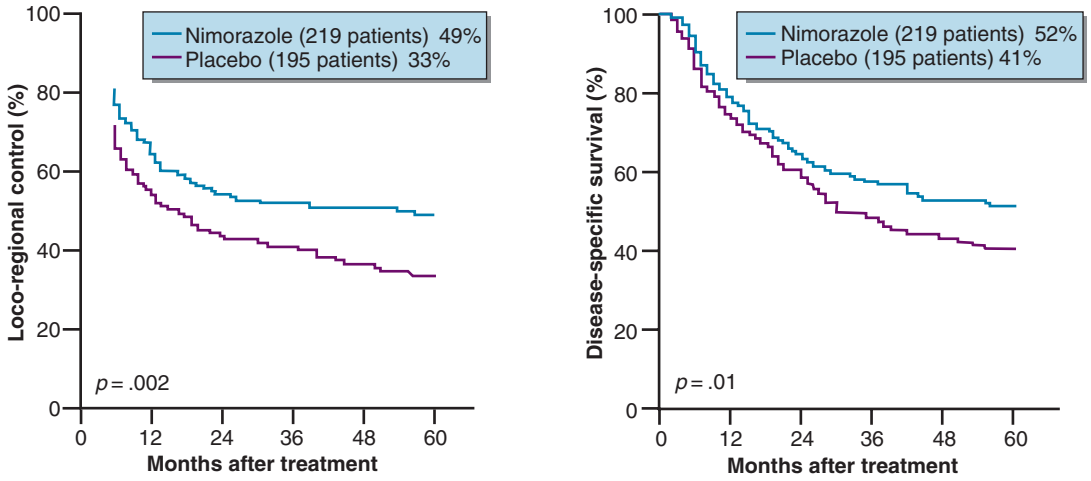
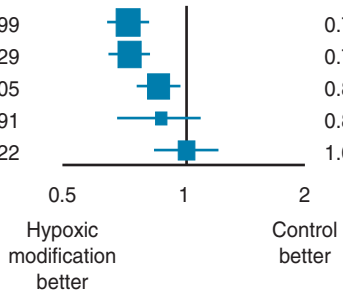


Figure 3-4 Actuarial estimated loco-regional tumor control and disease-specific survival rate in patients randomized to receive nimorazole or placebo in conjunction with conventional radiotherapy for carcinoma of the pharynx and supraglottic larynx.
Reprinted from Overgaard J, Sand Hansen H, Overgaard M, et al: A randomised double-blind phase III study of nimorazole as a hypoxic radiosensitizer of primary radiotherapy in supraglottic larynx and pharynx carcinoma. Results of the Danish head and neck cancer study [DAHANCA] protocol 5-85. Radiother Oncol 46:135–146, 1998, with permission form Elsevier Science.

Head and neck cancer–meta-analysis–summary

Endpoint	Events/Total: Hypoxic Modification	Events/Total: Control	Odds ratio and 95% CI	Odds Ratio	Risk Reduction	NNT**
Loco-regional control	1203/2406	1383/2399		0.71 (0.63–0.80)*	8% (5–10%)*	13
Disease-specific survival	1175/2335	1347/2329		0.73 (0.64–0.82)	7% (5–10%)	14
Overall survival	1450/2312	1519/2305		0.87 (0.77–0.98)	3% (0–6%)	31
Distant metastasis	159/1427	179/1391		0.87 (0.69–1.09)	2% (–1–4%)	57
Radiotherapy complications	307/1864	297/1822		1.00 (0.82–1.23)	0% (–3–2%)	>>



Meta-analysis–hypoxic modification of radiotherapy in HNSCC

*95% confidence interval.
**Numbers of patients needed to treat to achieve benefit in one patients.

Figure 3-5 Meta-analysis of hypoxic modification of radiotherapy in squamous cell carcinomas of the head and neck. Results show summary data from 32 randomized trials (including 4805 patients). Patients received radiation alone or radiation with a hypoxic modifier that included high-oxygen-content gas breathing under normobaric or hyperbaric conditions, or a hypoxic radiosensitizer.
Adapted from Overgaard J: Hypoxic modification of radiotherapy in squamous cell carcinoma of the head and neck—a systemic review and meta-analysis. Radiother Oncol 100:22–32, 2011.

Such a strategy has been adopted in Denmark where nimorazole has become part of the standard radiotherapy treatment in cancer of the head and neck.

Dose Modification Based on Hemoglobin

One of the major factors influencing the delivery of oxygen to tumors is the concentration of hemoglobin. It is, therefore, not surprising that low hemoglobin concentration in general has a negative impact on tumor radiation response. In a review of 51 studies involving 17,272 patients the prognostic relationship between hemoglobin concentration and local tumor control were analyzed, and of these, 39 studies (14,482 patients) showed a correlation, whereas only 12 (2790 patients) did not.[70] However, the relationship between hemoglobin concentration and tumor oxygenation status is not clear because a large (397 patients) international multicenter study in head and neck cancer failed to show a correlation between these parameters.[40]

Although a well-documented causal relationship between hemoglobin concentration, tumor oxygenation, and response to radiotherapy has not been shown, it is likely that such a relationship does exist and there is thus a rationale for investigating the possibility of improving the outcome of radiotherapy in relevant tumor sites in patients with low hemoglobin concentration given curative radiotherapy. This was investigated in two randomized trials using transfusion to raise the hemoglobin levels.[66,71] Despite an initial positive report from the Canadian trial in uterine cervix carcinoma, both studies concluded that the use of such transfusions did not significantly improve treatment outcome. In the DAHANCA 5 study, transfusion was given several days before radiotherapy and

adaptation may have occurred. Based on preclinical data it was hypothesized that any increase in tumor hypoxic fraction induced by anemia will be only transient, with tumors adapting to the lowered oxygen delivery[72]; transfusing anemic animals decreased tumor hypoxia, but this effect also was only transient and the tumors were able to adapt to the increased oxygen level. This suggests that when correcting for anemia it may not necessarily be the final hemoglobin concentration by itself that is important. Rather, an increasing hemoglobin concentration occurring at the time when the tumors are regressing during radiotherapy may be more likely to result in an increased oxygen supply to tumors and a subsequent improvement in response to radiotherapy.

Increasing the hemoglobin concentration by stimulation with erythropoietin (EPO), a hormone normally secreted from the kidney in response to tissue hypoxia and low serum levels, has also been investigated. Several preclinical studies have shown that the induction of anemia in animals could be corrected by serial injection with EPO and that this EPO treatment also overcame the anemia-induced radiation resistance.[73,74] The concept of using EPO to correct for anemia has also been tested in several clinical trials. However, although low hemoglobin can be effectively and safely improved by EPO,[75,76] patients treated with radiation and EPO had a poorer outcome than the control arms not treated with EPO.[77] Although this clearly raises concerns about the use of such agents to improve radiation therapy through a manipulation of hemoglobin levels, it does not make the concept of having a high hemoglobin concentration during radiation therapy an irrelevant issue.

Other "hemoglobin-related" methods for improving tumor oxygenation have been investigated.[78] These include the use of artificial blood substances, such as perfluorocarbons,[79] which are small particles capable of carrying more oxygen than hemoglobin, or by manipulating the oxygen unloading capacity of blood by modifying the oxy-hemoglobin dissociation curve. This can be achieved either by increasing the red blood cell 2,3-DPG content,[80] 2,3-DPG being one of the most important allosteric factors controlling the hemoglobin-oxygen dissociation curve, or using antilipidemic drugs.[81] Although each of these approaches has been shown to improve the oxygenation status of experimental tumors or enhance radiation damage, none of them have yet reached controlled clinical testing; thus, their potential usefulness in the clinic is uncertain.

Changing Oxygen Consumption

A novel approach that is currently receiving attention focuses on reducing tumor hypoxia by decreasing the oxygen consumption of cells close to blood vessels and thereby increasing the oxygen diffusion distance, which makes more oxygen available to the hypoxic cells. This may be achieved using the drug metformin, which has undergone extensive clinical evaluation for the treatment of diabetes and has been linked to decreased rates of certain types of cancer.[82] One preclinical study has clearly shown that high doses of metformin can decrease cellular oxygen consumption in vitro.[83] Additional in vivo data from that study demonstrated that metformin could improve tumor radiation response, but whether or not this was the direct result of reduced oxygen consumption is not entirely known. Further studies in this area are clearly warranted.

Dealing with the Problem of Fluctuating (Acute) Hypoxia

Although several of the procedures used to combat radiation resistance caused by hypoxic cells have met with some success, the results are far from satisfactory. A possible explanation is that most of the procedures used clinically seem to operate primarily against diffusion-limited chronic hypoxia and have little or no influence on fluctuating hypoxia, which is caused by transient variations in tumor blood flow.[9]

Experimental studies have clearly demonstrated that the vitamin B3 analog, nicotinamide, can enhance radiation damage in a variety of murine tumor models using both single and fractionated treatments (Figure 3-6).[9] The enhancement of radiation damage appears to depend on the tumor type, drug dose, and time of irradiation after drug administration.[9] The drug can also enhance radiation damage in certain normal tissues, but generally the effects are less pronounced than those seen in tumors.[9] Nicotinamide seems to primarily prevent or reduce the transient fluctuations in tumor blood flow that normally lead to the development of acute hypoxia.[9] This finding led to the suggestion that the optimal approach would be to combine nicotinamide with treatments that specifically overcome chronic hypoxia. This was subsequently demonstrated with hyperthermia,[84] perfluorochemical emulsions,[85] and carbogen breathing.[57,86-88] Combining nicotinamide with carbogen has undergone testing in a number of European clinical studies, and the results in head and neck[89] and bladder cancer[90] demonstrated an improved response to radiation therapy.

Bioreductive Drugs

The early preclinical studies with electron-affinic radiosensitizers showed that these agents, which were relatively nontoxic to cells under normal oxygenated conditions, were reduced to a more toxic form under hypoxia.[91] This led to the development of various bioreductive drugs that preferentially killed the radiation-resistant hypoxic tumor cell population. Basically, these drugs can be divided into three major groups, as illustrated in Figure 3-7. They are the quinones, nitroaromatics, and N-oxides.[92]

The quinine derivative, Mitomycin-C (MMC), is probably the prototype bioreductive drug.[93] It has been used clinically for many years as a chemo-radiosensitizer, long before it was realized it had preferential effects against hypoxic cells. It is activated by bioreduction to form products that crosslink DNA and therefore produce cell killing. Several randomized clinical trials in patients with squamous cell carcinoma of the head and neck were undertaken, specifically using MMC to counteract the effects of hypoxia, but not all showed a benefit.[94-98] This may not be surprising when one considers that MMC actually has a small differential killing effect between aerobic and hypoxic cells (see Figure 3-7) and was only administered once or twice during the entire course of radiotherapy. Attempts to find more efficient quinones have been undertaken and to that end porfiromycin, RH1, and EO9 were developed.[92] Of these EO9 is currently being evaluated in a phase II trial in bladder cancer.

The finding that misonidazole was preferentially toxic toward hypoxic cells led to numerous efforts to find other nitroimidazoles that were better. The first drug developed was RSU-1069 (see Figure 3-7). This compound has the classic 2-nitroimidazole radiosensitizing properties, but also an aziridine ring at the terminal end of the chain, which gave the molecule substantial potency as a hypoxic cell cytotoxin, both in vitro and in vivo.[99] In large-animal studies it was found to cause gastrointestinal toxicity and a less toxic prodrug was therefore developed (RB-6145), which is reduced in vivo to RSU-1069. Although this drug was found to have potent antitumor activity in experimental systems, further animal studies revealed that this drug induced blindness; this is perhaps not surprising when one realizes that the retina is hypoxic, thus further development of this drug was halted. However, other

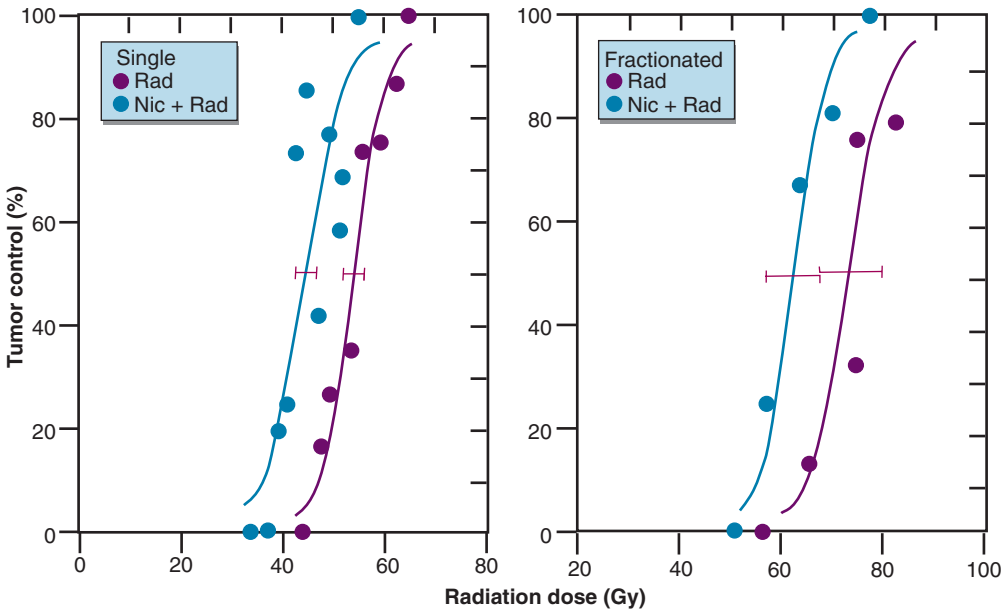


Figure 3-6 Effect of nicotinamide (500-1000 mg/kg) on local tumor control measured as a function of the total radiation dose given either as a single treatment to C3H mammary carcinomas or in fractionated schedule to the carcinoma NT. The drug was i.p. injected 30-60 minutes before irradiation. *Modified from Horsman MR, Chaplin DJ, Overgaard J: Combination of nicotinamide and hyperthermia to eliminate radioresistant chronically and acutely hypoxic tumor cells. Cancer Res 50:7430–7436, 1990, and Kjellen E, Joiner MC, Collier JM, et al: A therapeutic benefit from combining normobaric carbogen or oxygen with nicotinamide in fractionated x-ray treatments. Radiother Oncol 22:81–91, 1991. Copyright 1991, with permission from Elsevier Science.*

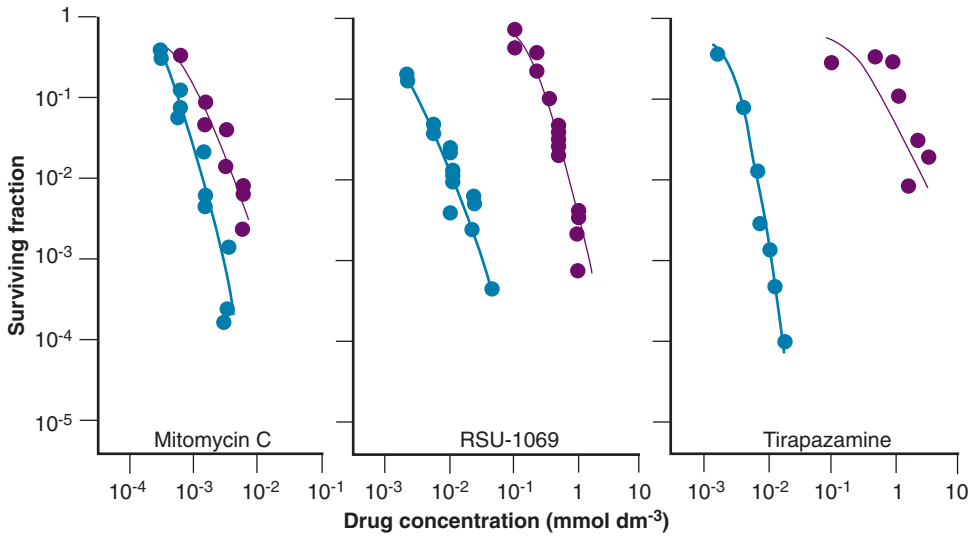


Figure 3-7 Survival of mammalian cells exposed to mitomycin C, RSU 1069, or tirapazamine under aerobic *(red symbols)* of hypoxic *(blue symbols)* conditions.
Adapted from Stratford IJ, Stephens MA: The differential hypoxic cytotoxicity of bioreductive agents determined in vitro by the MTT assay. Int J Radiat Oncol Biol Phys 16:973–976, 1989, and Hall EJ: Radiobiology for the radiobiologist. ed 4. Philadelphia, 1994, JB Lippincott.

nitro-containing compounds have been developed, including NLCQ-1, CB 1954, SN 23862, PR-104, and TH-302,[92] of which the latter is currently under Phase II/III clinical evaluation albeit in combination with chemotherapy.

Perhaps the most promising group of bioreductives is the organic nitroxides, of which the benzotriazene di-N-oxide, tirapazamine, is the lead compound (see Figure 3-7). The parent moiety shows limited toxicity toward aerobic cells, but after reduction under hypoxic conditions, a product is formed that has been shown to be highly toxic and can substantially enhance radiation damage to tumors in vivo.[100] Most clinical studies have involved combining tirapazamine with chemotherapy, although there have been a few trials with radiation with or without chemotherapy.[92] The results from the phase II trials generally showed promise, but in the few randomized trials that have been completed the results were somewhat disappointing. However, it has now been suggested that the benefit of tirapazamine might be achieved if one could select patients who had hypoxic tumors before treatment. Other N-oxides currently under development include chlorambucil N-oxide, SN30000, and AQ4N (Banoxantrone), the latter being combined with radiation in a number of clinical trials.[92]

VASCULAR TARGETING AGENTS

Angiogenesis Inhibitors

The tumor's vascular supply plays a critical role in determining the tumor's microenvironmental factors that influence radiotherapy.[101] This vasculature develops from the normal tissue vessels via the process of angiogenesis,[102] which is a highly complex process triggered by the release of specific growth factors from the tumor cells.[103] These growth factors initiate a series of physical steps, including local degradation of the basement membrane surrounding capillaries, invasion of surrounding stroma by the endothelial cells in the direction of the angiogenic stimulus, proliferation of the endothelial cells, and, finally organization of the endothelial cells into three-dimensional structures that connect with other similar structures to form the new blood vessel network.[103] The importance of this process makes it an attractive target for therapy and numerous approaches for inhibiting the various steps in the angiogenic process have been tested in preclinical models.[104,105] Many of these therapies have now moved into clinical evaluation[106] and, of these, the antivascular endothelial growth factor (anti-VEGF) antibody bevacizumab (Avastin) has been shown to improve outcome in a number of chemotherapy-based trials.[107] Preclinical studies using rodent and human tumor xenografts show that certain angiogenesis inhibitors can be effectively combined with radiation to improve tumor response (Table 3-3),[105] and as a result, a limited number of clinical studies have been initiated combining certain angiogenesis inhibitors with radiation therapy.[106]

The consensus opinion is that the improvement in radiation response found in preclinical studies is the consequence of normalization of the tumor vasculature, resulting in a decrease in tumor hypoxia.[108] Although there are certainly preclinical studies showing an improved tumor oxygenation status with such treatment, there are just as many studies showing no change and even a decrease in tumor oxygenation.[105] These findings not only make it unclear as to the role of vessel normalization in influencing the combination of angiogenesis inhibitors with radiation, but they also indicate that timing and sequencing of the two modalities may be critical for an optimal benefit.

TABLE 3-3 List of Vascular Targeting Agents That Have Been Combined with Radiation

Angiogenesis Inhibitors	Vascular Disrupting Agents
Suramin	Hyperthermia
TNP-470	Photodynamic therapy
Angiostatin	Colchicine
Endostatin	Tumor necrosis factor
Arginine deiminase	Arsenic trioxide
Thrombospondin	Flavone acetic acid
Thalidomide	5,6-dimethylxanthenone acetic acid
Anginex	Combretastatin A-4 phosphate
Anti-VEGF(R) antibodies	AVE 8062
SU 5416	ZD 6126
SU 6668	OXi4503
SU 11248	MN-029
SU 11657	NPI-2358
PTK787/ZK 222584	
ZD 6474	
Metastat	

See Horsman and Siemann[105] for additional information.

Vascular Disrupting Agents

An alternative approach for targeting tumor vasculature involves using agents that can damage the already established tumor vessels.[104,105] This is not a new concept; it was first demonstrated with the tubulin binding agent colchicine back in the 1940s.[109] Since then a number of vascular disrupting agents (VDAs) have been proved capable of preferentially damaging tumor vessels, leading to a reduction in tumor perfusion which results in an increase in tumor ischemia and necrosis, subsequently producing an inhibitory effect on tumor growth.[105] The VDAs include physical treatments (e.g., hyperthermia, photodynamic therapy, and even radiation), chemotherapeutic agents (e.g., tumor necrosis factor, vinca alkaloids, and arsenic trioxides), and small molecule agents (e.g., flavonoid derivatives such as 5,6-dimethylxanthenone-4-acetic acid; tubulin binding drugs such as Combretastatin A-4 phosphate).

As with the angiogenesis inhibitors, several of the VDAs have been combined with radiation (see Table 3-3), and significant improvements in response have been seen in preclinical models.[105] This is illustrated in Figure 3-8 using a C3H mammary carcinoma grown in CDF1 mice. The radiation dose needed to control 50% of treated animals (±95% confidence intervals) following single dose radiation treatment alone was found to be 53 Gy (51-56). This was significantly reduced (Chi-squared test; $p < 0.05$) to 46 Gy (42-49) when tumors were locally irradiated, but 30 minutes later mice were given a single intraperitoneal injection of a large but nontoxic dose of Combretastatin A-4 phosphate (CA4P), the lead VDA in clinical evaluation.[105,107] On its own, in this tumor model, such a drug dose will only slow the growth of the tumors by about 2 days. The enhancement of tumor radiation damage is known to be time and schedule dependent, with the greatest effect seen when the drug is given within a few hours after irradiating.[105] It is also tumor specific with no enhancement of radiation response seen in normal tissues. This has been shown for CA4P in acutely responding normal skin (see Figure 3-8) or late-responding bladder and lung.[105] These differences between the tumor and normal tissue results are entirely consistent with the drugs' ability to induce damage in tumor vessels but not vessels in normal tissue.[105]

RADIATION PROTECTORS

Sulfhydryl-Containing Compounds

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

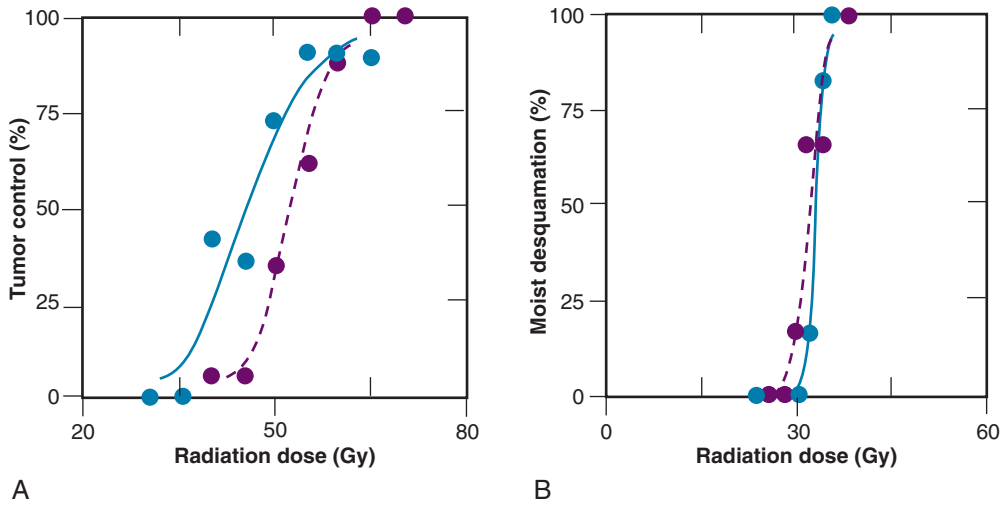


Figure 3-8 Effect of Combretastatin A-4 disodium phosphate (250 mg/kg) on either local control of a C3H mammary carcinoma **(A)** or the development of moist desquamation in the foot skin of CDF1 mice **(B)** following radiation treatment. Radiation was either given alone *(red symbols)* or 30 minutes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

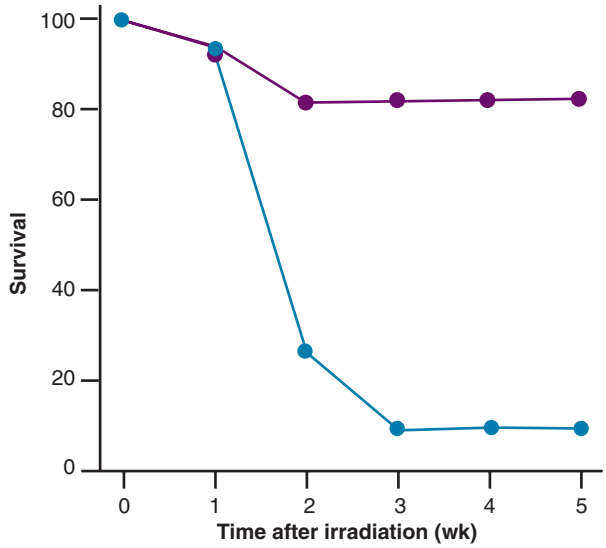


Figure 3-9 Percentage of rodents surviving after whole-body irradiation with 8 Gy. Animals were either control irradiated (blue symbols) or given radiation after injection with 575 mg of cysteine *(red symbols)*.
Adapted from Patt HM, Tyree EB, Straub RL, et al: Cysteine protection against X irradiation. Science 110:213–214, 1949. Copyright 1949 American Association for the Advancement of Science.

establishment of authoritative guidelines.[114] The lack of interest was probably related to the fact that there is no fail-safe modification of traditional radiotherapy.[115] Many normal tissues are dose limiting; therefore, evaluating the therapeutic benefit of a radioprotector requires either that the protector have absolute normal tissue selectivity (and thus fewer complications with an unchanged rate of tumor control for a given radiation dose) or, if selectivity is uncertain, that an increase in radiation dose may be needed to maintain the same rate of tumor control. However, this scenario requires that the protective effect on tumor tissues is predictable and exceeded by the protection offered to all relevant normal tissues. In addition, the "perfect" radioprotector must have an acceptable toxicity profile and must be easy to handle if generalized clinical use is to be expected with routine fractionated radiotherapy.[116-118]

It is not entirely clear how amifostine induces radioprotection. The drug must first undergo dephosphorylation to its active metabolite WR-1065, which is further metabolized to the disulfide WR-33278; the latter metabolite may also afford some protection, although to a lesser extent.[119] Several mechanisms are involved in radioprotection, depending on the quality of the radiation. Protection against sparsely ionizing radiation, such as x-rays, is mainly obtained by scavenging of free radicals[120,121]; such scavenging has also been observed with glutathione, superoxide dismutase and its mimetics, and isoflavones like genistein. Because WR-1065 and WR-33278 react with free radicals in competition with oxygen, the protection obtained by scavenging is highly influenced by oxygen tension (Figure 3-10). Here the protection is maximal at intermediate levels of oxygen (20% to 50% oxygen in the inspired air). At higher oxygen tensions, WR-1065 is counterbalanced by excess oxygen and the protection is gradually lost. The degree of protection is also diminished at low-oxygen tensions where scavenging of free radicals is no longer important because the lack of oxygen by itself provides radioprotection. Additional and complex mechanisms are undoubtedly involved. Some of these may involve hypoxia created locally by direct interaction of thiols with oxygen, chemical repair by thiol-donation of hydrogen, or decreased accessibility of radiolytic attack sites by induction of DNA packaging.[116]

Preclinical studies have shown that many tissues can be protected from radiation damage by amifostine (Table 3-4). However, the protection observed in different normal tissues is unfortunately heterogeneous. Some normal tissues—such as central nervous system, which often is dose limiting in radiotherapy—are not protected because amifostine probably does not cross the blood-brain barrier.[122] In other normal tissues such as salivary glands and hematopoietic system, amifostine affords significant radioprotection. These variations are probably explained by tissue variations in oxygen concentration, dephosphorylation activity, and distribution of amifostine and its metabolites.[119,121,123] To make things even more complicated, tumor protection has been impossible to rule out by preclinical experiments.[115] In addition, large single doses of irradiation have often been used, and relevant

comparison with tumor effects have been absent or difficult to translate into a clinical meaningful context.[116]

On the clinical side there have been far too many publications of phase I-II studies with limited number of patients and a few underpowered randomized studies. In addition, chemotherapy has often been applied together with radiotherapy, making it difficult to evaluate the results (Table 3-5). Despite the long list of preclinical normal tissues studies with a proven effect of amifostine, disappointingly few have been confirmed in the clinical setting. Amelioration of acute radiation toxicity has been observed in studies which often employed various types of treatment intensification like concomitant chemotherapy or accelerated radiotherapy. However, definite confirmation regarding late morbidity such as fibrosis has not been obtained. A pivotal trial by Brizel et al[128] showed that amifostine significantly protected against radiation-induced xerostomia with no apparent loss of tumor control in head and neck cancers. That was followed by amifostine being approved by the US Federal Drug Administration for use in patients undergoing postoperative fractionated radiotherapy in the head and neck region to decrease the incidence of acute and chronic xerostomia. In light of the paucity of relevant clinical data this recommendation seems premature. A later large meta-analysis of published data concluded that amifostine could significantly reduce acute and late side effects associated with radiation therapy.[129] However, that review has since been criticized

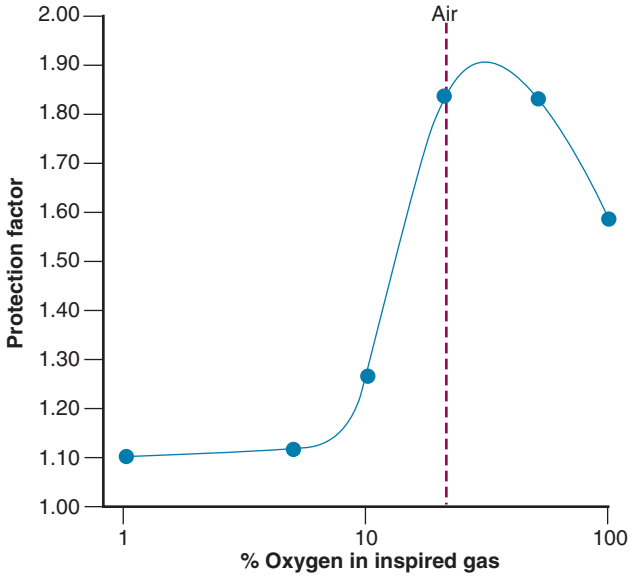


Figure 3-10 The variation in normal skin protection in mice given 400 mg/kg WR-2721 30 to 45 minutes before irradiation in mice that breathed various oxygen concentrations.
Modified from Denekamp J, Michael BD, Rojas A, et al: Radioprotection of mouse skin by WR-2721: the critical influence of oxygen tension. Int J Radiat Oncol Biol Phys 8:531–534, 1982, with permission from Elsevier Science.

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

Tissue	Protection Factor
Salivary gland	2.3-3.3
Bone marrow	1.8-3.0
Jejunum	1.5-2.1
Skin	1.4-2.1
Testis	1.5-1.6
Kidney	1.3-1.5
Bladder	1.3-1.5
Lung	1.2-1.4
Heart	>1.0
Tumor	1.0-2.8

Data from references 115 and 121,123-127.

TABLE 3-5 Randomized Clinical Trials Investigating the Effect of Amifostine on Outcome in Radiotherapy (RT) Administered Alone or with Chemotherapy (CT)

Author	Year	Reference	Site	Treatment	No. Patients
Buntzel	1998	131	Head and neck	RT and Chemo	39
Bourhis	1999	132	Head and neck	RT	26
Brizel	2000	128	Head and neck	RT	315
Momm	2001	133	Head and neck	RT	73
Wasserman	2005	134	Head and neck	RT	303
Buentzel	2006	135	Head and neck	RT and Chemo	132
Anné	2007	136	Head and neck	RT and Chemo	54
Haddad	2009	137	Head and neck	RT and Chemo	58
Antonadou	2001	138	Lung	RT	146
Antonadou	2003	139	Lung	RT and Chemo	73
Leong	2003	140	Lung	RT and Chemo	60
Komaki	2004	141	Lung	RT and Chemo	62
Movsas	2005	142	Lung	RT and Chemo	242
Lawrence	2013	143	Lung	RT and Chemo	243
Bohouslavizki	1998	144	Thyroid	RT (I-131)	50
Liu	1992	145	Rectum	RT	100
Athanassiou	2003	146	Pelvis	RT	205
Kouloulius	2004	147	Prostate	RT	67
Koukourkis	2000	148	Various	RT	140

for publication and classification bias and the quality of the trials included.[130] A more comprehensive meta-analysis found that amifostine did not change radiation-induced overall or progression free survival,[130] thus further studies are needed to explore the potential benefits of amifostine.

Modifiers of the Oxygen Supply

Because hypoxia reduces radiation sensitivity, decreasing the availability of oxygen to tissues may be way to achieve radiation protection. One way to accomplish this might be to modify hemoglobin-oxygen affinity. The most widely studied agents in this context are the substituted benzaldehyde, BW12C, and derivatives.[149,150] These agents preferentially bind to oxy-hemoglobin and so increase the affinity of the hemoglobin for oxygen.[151,152] Consequently this decreases the amount of oxygen available to the tissues. Although this radioprotects some normal tissues,[149,153] there is evidence that in certain normal tissues BW12C can actually increase perfusion,[154] which should increase oxygen delivery. Additionally, experimental studies have also shown that BW12C will significantly radioprotect tumors.[149,150]

Carbon monoxide reduces oxygen transport to tissues by binding to hemoglobin, thereby decreasing the amount available for oxygen transport, as well as causing a left shift of the hemoglobin-oxygen dissociation curve. Therefore, any oxygen that binds to the hemoglobin does so more strongly.[155] This approach to increase hypoxia and reduce radiation response has been well documented in experimental systems.[156,157] Unfortunately, this effect was demonstrated in tumors and not normal tissues. The effect of carbon monoxide in patients has also been observed, as shown in Figure 3-11, which illustrates that patients with head and neck carcinomas who were smokers (and therefore had higher carboxyhemoglobin levels) had a poorer response to radiation therapy than nonsmokers.

Because most modifiers of the oxygen supply already discussed also provide radioprotection for tumors as well as normal tissues, their use in this context must be considered limited. However, this may not be true for pentoxifylline. This is a drug that alters red blood cell deformability and inhibits platelet aggregation and fibrinolytic activity.[158] As a result, red blood cells are better able to transverse narrowed arterioles and capillaries. When given before irradiation, pentoxifylline enhances radiation damage in tumors,[159] presumably because of an improvement in oxygen delivery,[160,161] but it has no effect on the response of normal tissues.[159] However, when administered on a daily basis after irradiation, pentoxifylline had no effect on early skin reactions in mice but did significantly reduce the incidence of late reactions.[162]

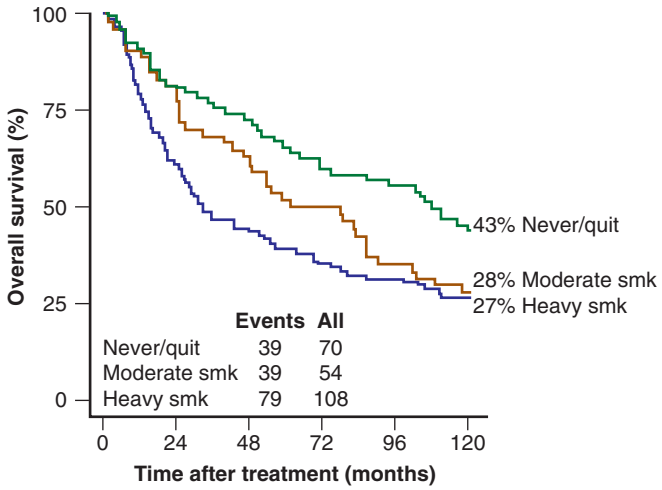


Figure 3-11 Influence of smoking (smk) during treatment on the outcome of radiotherapy in 232 patients with advanced head and neck carcinoma. Results show 10-year survival when comparing nonsmokers and quitters to moderate or heavy smokers (>20 cigarettes or one pack per day).
Redrawn from Hoff CM: Importance of hemoglobin concentration and its modification for the outcome of head and neck cancer patients treated with radiotherapy. Acta Oncol 51:419–432, 2012.

Other Radioprotectors

Various other agents have been reported to be capable of radioprotecting certain normal tissues.[163-165] These include cytokines, such as granulocyte-macrophage colony-stimulating factor, interleukin-1, tumor necrosis factor-α (TNF-α), transforming growth factor-β (TGF-β), and basic fibroblast growth factor.[166-170] Another interesting group of agents inhibit the process of apoptosis. Many tumors have acquired mutations that prevent them from undergoing radiation-induced apoptosis. Proliferating normal cells, however, are often killed by radiation-induced apoptosis, and several apoptosis inhibitors have shown radioprotective potentials in animal models. These include p53 inhibitors (pifithrin-α and pifithrin-μ), growth factors [KGF-1 (palifermin), KGF-2 (repifermin), FGF-20 (velafermin)], activators of NRF2 (triterpenoids), and inhibitors of NF-κB (CBLB502).[163-165] Another recent approach, designed to take advantage of a typically acquired defect in tumor cells, is to induce a G1 cell cycle arrest in normal cells, thereby temporarily arresting them in the relatively radioresistant G1 phase allowing for improved DNA repair (e.g., PD0332991 and 2BrIC).[163-165] Agents are also being developed that target molecular pathways involved in radiation-induced effects, including the TGF-β1/Smad, CTGF/RHO/ROCK, TNF-α, and PDGF/PDGFR pathways.[163-165] Finally, there is a group of miscellaneous inhibitors that include the angiotensin-converting enzyme inhibitor captopril, corticosteroids, prostaglandins, and essential fatty acids.[171-175] However, the mechanisms of action are not entirely clear, nor is there evidence that these agents do not also radioprotect tumors.

SUMMARY

The use of radiation to treat certain types of cancer with curative intent is a well-established and effective therapy, but there is still room for improvement. The additional use of treatments that can either increase radiation damage in tumors without affecting normal tissues, or protect normal tissues without having a similar protective effect in tumors, is clearly warranted. Because the vasculature and microenvironment of tumors differs from those of normal tissues, targeting these parameters should lead to tumor specificity. Many preclinical studies have demonstrated this to be a viable approach. But despite numerous clinical studies confirming the potential of such methods to significantly improve outcome to radiation therapy only one agent has become established in standard radiation therapy protocols. That agent is the radiosensitizer nimorazole, which is only used in Denmark and only in head and neck cancers. The use of radioprotectors is more controversial. Experimentally, several agents have been shown to radioprotect certain normal tissues, but data also demonstrate that some of these agents induce radiation protection in tumors. Results from clinical studies investigating the potential of radioprotectors have also been inconclusive, and until good human data demonstrating radioprotection of normal tissues, but not tumors, become available the use of radioprotectors must be considered experimental.

CRITICAL REFERENCES

A full list of cited references is published online at www.expertconsult.com.

1. Schwarz G: über Desensibiliserung gegen Röntgen- und Radiumstrahlen. Munch Med Wochenschr 24:1–2, 1909.
2. Müller C: Eine neue Behandlungsmethode bösartiger Geschwülste. Munch Med Wochenschr 28:1490–1493, 1910.
3. Gray LH, Conger AD, Ebert M, et al: The concentration of oxygen dissolved in tissues at the time of irradiation as a factor in radiotherapy. Br J Radiol 26:638–648, 1953.
4. Thomlinson RH, Gray LH: The histological structure of some human lung cancers and the possible implications for radiotherapy. Br J Cancer 9:539–549, 1955.
5. Tannock IF: The relationship between cell proliferation and the vascular system in a transplanted mouse mammary tumour. Br J Cancer 22:258–273, 1968.
8. Chaplin DJ, Olive PL, Durand RE: Intermittent blood flow in a murine tumor: Radiobiological effects. Cancer Res 47:597–601, 1987.
9. Horsman MR: Nicotinamide and other benzamide analogs as agents for overcoming hypoxic cell radiation resistance in tumours. Acta Oncol (Madr) 34:571–587, 1995.
12. Moulder JE, Rockwell S: Hypoxic fractions of solid tumour. Int J Radiat Oncol Biol Phys 10:695–712, 1984.
13. Horsman MR, Mortensen LS, Petersen JB, et al: Imaging hypoxia to improve radiotherapy outcome. Nat Rev Clin Oncol 9:674–687, 2012.
40. Nordsmark M, Bentzen SM, Rudat V, et al: Prognostic value of tumor oxygenation in 397 head and neck tumors after primary radiation therapy. An international multi-center study. Radiother Oncol 77:18–24, 2005.
41. Overgaard J, Horsman MR: Modification of hypoxia-induced radioresistance in tumors by the use of oxygen and sensitizers. Semin Radiat Oncol 6:10–21, 1996.
43. Suit HD, Marshall N, Woerner D: Oxygen, oxygen plus carbon dioxide, and radiation therapy of a mouse mammary carcinoma. Cancer 30:1154–1158, 1972.
45. Rojas A: Radiosensitization with normobaric oxygen and carbogen. Radiother Oncol 20(Suppl 1):65–70, 1991.
53. Overgaard J: Sensitization of hypoxic tumour cells—clinical experience. Int J Radiat Biol 56:801–811, 1989.
56. Mendenhall WM, Morris CG, Amdur RJ, et al: Radiotherapy alone or combined with carbogen breathing for squamous cell carcinoma of the head and neck. Cancer 104:332–337, 2005.
57. Chaplin DJ, Horsman MR, Siemann DW: Further evaluation of nicotinamide and carbogen as a strategy to reoxygenate hypoxic cells in vivo: Importance of nicotinamide dose and pre-irradiation breathing time. Br J Cancer 68:269–273, 1993.
58. Adams GE, Cooke MS: Electron-affinic sensitization. I. A structural basis for chemical radiosensitizers in bacteria. Int J Radiat Biol 15:457–471, 1969.
59. Asquith JC, Watts ME, Patel K, et al: Electron-affinic sensitization V. Radiosensitization of hypoxic bacteria and mammalian cells in vitro by some nitroimidazoles and nitropyrazoles. Radiat Res 60:108–118, 1974.
61. Overgaard J: Clinical evaluation of nitroimidazoles as modifiers of hypoxia in solid tumors. Oncol Res 6:509–518, 1994.
66. Overgaard J, Sand Hansen H, Overgaard M, et al: A randomised double-blind phase III study of nimorazole as a hypoxic radiosensitizer of primary radiotherapy in supraglottic larynx and pharynx carcinoma. Results of the Danish head and neck cancer study (DAHANCA) protocol 5–85. Radiother Oncol 46:135–146, 1998.
67. Dobrowsky W, Huigol NG, Jayatilake RS, et al: Ak-2123 (Sanazol) as a radiation sensitizer in the treatment of stage III cervical cancer: Results of an IAEA multicentre randomized trial. Radiother Oncol 82:24–29, 2007.
68. Karasawa K, Sunamura M, Okamoto A, et al: Efficacy of novel hypoxic cell sensitizer doranidazole in the treatment of locally advanced pancreatic cancer—long-term results of a placebo-controlled randomized study. Radiother Oncol 87:326–330, 2008.
69. Overgaard J: Hypoxic modification of radiotherapy in squamous cell carcinomas of the head and neck—a systematic review and meta-analysis. Radiother Oncol 100:22–32, 2011.
70. Grau C, Overgaard J: Significance of haemoglobin concentration for treatment outcome. In Molls M, Vaupel P, editors: Medical radiology: Blood perfusion and microenvironment of human tumours, Heidelberg, Germany, 1998, Springer-Verlag, pp 101–112.
71. Grogan M, Thomas GM, Melamed I, et al: The importance of hemoglobin levels during radiotherapy for carcinoma of the cervix. Cancer 86:1528–1536, 1999.
72. Hirst DG: Anemia: A problem or an opportunity in radiotherapy? Int J Radiat Oncol Biol Phys 12:2009–2017, 1986.
77. Hoff CM: Importance of hemoglobin concentration and its modification for the outcome of head and neck cancer patients treated with radiotherapy. Acta Oncol 51:419–432, 2012.
83. Zannella VE, Dal Pra A, Muaddi H, et al: Reprogramming metabolism with metformin improves tumor oxygenation and radiotherapy response. Clin Cancer Res 19:6741–6750, 2013.
89. Janssens GO, Rademakers SE, Terhaard CH, et al: Accelerated radiotherapy with carbogen and nicotinamide for laryngeal cancer: Results of a phase III randomized trial. J Clin Oncol 30:1777–1783, 2012.
90. Hoskin PJ, Rojas AM, Bentzen SM, et al: Radiotherapy with concurrent carbogen and nicotinamide in bladder cancer. J Clin Oncol 28:4912–4918, 2010.
92. McKeown SR, Cowen RL, Williams KJ: Bioreductive drugs: From concept to clinic. Clin Oncol 19:427–442, 2007.
103. Fidler IJ, Langley RR, Kerbel RS, et al: Angiogenesis. In DeVita VT, Hellman S, Rosenberg SA, editors: Cancer principles and practice of oncology, ed 7, Philadelphia, 2005, Lippincott Williams & Wilkins, pp 129–137.
105. Horsman MR, Siemann DW: Pathophysiological effects of vascular-targeting agents and the implications for combination with conventional therapies. Cancer Res 66:11520–11539, 2006.
110. Patt HM, Tyree EB, Straub RL, et al: Cysteine protection against X irradiation. Science 110:213–214, 1949.
113. Yuhas JM: Protective drugs in cancer therapy: Optimal clinical testing and future directions. Int J Radiat Oncol Biol Phys 8:513–517, 1982.
116. Lindegaard JC, Grau C: Has the outlook improved for amifostine as a clinical radioprotector? Radiother Oncol 57:113–118, 2000.
119. Savoye C, Swenberg C, Hugot S, et al: Thiol WR-1065 and disulphide WR-33278, two metabolites of the drug ethyol (WR-2721), protect DNA against fast neutron-induced strand breakage. Int J Radiat Biol 71:193–202, 1997.
120. Denekamp J, Michael BD, Rojas A, et al: Radioprotection of mouse skin by WR-2721: The critical influence of oxygen tension. Int J Radiat Oncol Biol Phys 8:531–534, 1982.
121. Travis EL: The oxygen dependence of protection by aminothiols: Implications for normal tissues and solid tumors. Int J Radiat Oncol Biol Phys 10:1495–1501, 1984.
130. Bourhis J, Blanchard P, Maillard E, et al: Effect of amifostine on survival among patients treated with radiotherapy: A meta-analysis of individual patient data. J Clin Oncol 29:2590–2597, 2011.
149. Adams GE, Barnes DW, du-Boulay C, et al: Induction of hypoxia in normal and malignant tissues by changing the oxygen affinity of hemoglobin—implications for therapy. Int J Radiat Oncol Biol Phys 12:1299–1302, 1986.
152. Keidan AJ, Franklin IM, White RD: Effect of BW12C on oxygen affinity of haemoglobin in sickle cell disease. Lancet 1:831–834, 1986.
154. Honess DJ, Hu DE, Bleehen NM: BW12C: Effects on tumour hypoxia, tumour radiosensitivity and relative tumour and normal tissue perfusion in C3H mice. Br J Cancer 64:715–722, 1991.
155. Roughton FJW, Darling RC: The effect of carbon monoxide on the oxyhemoglobin dissociation curve. Am J Phys 141:17–31, 1944.
156. Siemann DW, Hill RP, Bush RA: The importance of the pre-irradiation breathing times of oxygen and carbogen (5% CO2; 95% O2) on the in vivo radiation response of a murine sarcoma. Int J Radiat Oncol Biol Phys 2:903–911, 1977.
158. Ward A, Clissold SP: Pentoxifylline: A review of its pharmacodynamic and pharmacokinetic properties, and its therapeutic efficacy. Drugs 34:50–97, 1987.
159. Lee I, Kim JH, Levitt SH, et al: Increases in tumor response by pentoxifylline alone or in combination with nicotinamide. Int J Radiat Oncol Biol Phys 22:425–429, 1992.
162. Dion MW, Hussey DH, Osborne JW: The effect of pentoxifylline on early and late radiation injury following fractionated irradiation in C3H mice. Int J Radiat Oncol Biol Phys 17:101–107, 1989.
163. Stewart FA, Akleyev AV, Hauer-Jensen M, et al: ICRP publication 118: ICRP statement on tissue reactions and early and late effects of radiation in normal tissues and organs–threshold doses for tissue reactions in a radiation protection context. Ann ICRP 41:211–286, 2012.
166. Hendry JH: Biological response modifiers and normal tissue injury after irradiation. Semin Radiat Oncol 4:123–132, 1994.

REFERENCES

1. Schwarz G: über Desensibiliserung gegen Röntgen- und Radiumstrahlen. Munch Med Wochenschr 24:1–2, 1909.
2. Müller C: Eine neue Behandlungsmethode bösartiger Geschwülste. Munch Med Wochenschr 28:1490–1493, 1910.
3. Gray LH, Conger AD, Ebert M, et al: The concentration of oxygen dissolved in tissues at the time of irradiation as a factor in radiotherapy. Br J Radiol 26:638–648, 1953.
4. Thomlinson RH, Gray LH: The histological structure of some human lung cancers and the possible implications for radiotherapy. Br J Cancer 9:539–549, 1955.
5. Tannock IF: The relationship between cell proliferation and the vascular system in a transplanted mouse mammary tumour. Br J Cancer 22:258–273, 1968.
6. Horsman MR, Overgaard J: Overcoming tumour radiation resistance resulting from acute hypoxia. Eur J Cancer 28A:717–718, 1992.
7. Brown JM: Evidence for acutely hypoxic cells in mouse tumours, and a possible mechanism of reoxygenation. Br J Radiol 52:650–656, 1979.
8. Chaplin DJ, Olive PL, Durand RE: Intermittent blood flow in a murine tumor: Radiobiological effects. Cancer Res 47:597–601, 1987.
9. Horsman MR: Nicotinamide and other benzamide analogs as agents for overcoming hypoxic cell radiation resistance in tumours. Acta Oncol (Madr) 34:571–587, 1995.
10. Helmlinger G, Yuan F, Dellian M, et al: Interstitial pH and pO2 gradients in solid tumors in vivo: High-resolution measurements reveal a lack of correlation. Nat Med 3:177–182, 1997.
11. Kimura H, Braun RD, Ong ET, et al: Fluctuations in red cell flux in tumor microvessels can lead to transient hypoxia and reoxygenation in tumor parenchyma. Cancer Res 56:5522–5528, 1996.
12. Moulder JE, Rockwell S: Hypoxic fractions of solid tumour. Int J Radiat Oncol Biol Phys 10:695–712, 1984.
13. Horsman MR, Mortensen LS, Petersen JB, et al: Imaging hypoxia to improve radiotherapy outcome. Nat Rev Clin Oncol 9:674–687, 2012.
14. Kolstad P: Intercapillary distance, oxygen tension and local recurrence in cervix cancer. Scand J Clin Lab Invest 106:145–157, 1968.
15. Révész L, Siracka E, Siracky J, et al: Variation of vascular density within and between tumors of the uterine cervix and its predictive value for radiotherapy. Int J Radiat Oncol Biol Phys 11:97–103, 1989.
16. Lauk S, Skates S, Goodman M, et al: Morphometric study of the vascularity of oral squamous cell carcinomas and its relation to outcome of radiation therapy. Eur J Cancer Clin Oncol 25:1431–1440, 1989.
17. Mueller-Kleiser W, Vaupel P, Manz R, et al: Intracapillary oxyhemoglobin saturation of malignant tumors in humans. Int J Radiat Oncol Biol Phys 7:1397–1404, 1981.
18. Vikram DS, Zweier JL, Kuppusamy P: Methods for noninvasive imaging of tissue hypoxia. Antioxid Redox Signal 9:1745–1756, 2007.
19. Padhani AR, Krohn KA, Lewis JS, et al: Imaging oxygenation of human tumours. Eur Radiol 17:861–872, 2007.
20. Mayr NA, Wang JZ, Zhang D, et al: Longitudinal changes in tumor perfusion pattern during the radiation therapy course and its clinical impact in cervical cancer. Int J Radiat Oncol Biol Phys 77:502–508, 2010.
21. Bisdas S, Ngyuyen SA, Anand SK, et al: Outcome prediction after surgery and chemoradiation of squamous cell carcinoma in te oral cavity, oropharynx, and hypopharynx: Use of baseline perfusion CT microcirculatory parameters vs. tumor volume. Int J Radiat Oncol Biol Phys 73:1313–1318, 2009.
22. Lehtio K, Eskola O, Vijanen T, et al: Imaging perfusion and hypoxia with PET to predict radiotherapy response in head-and-neck cancer. Int J Radiat Oncol Biol Phys 59:971–982, 2004.
23. Harris AL: Hypoxia—a key regulatory factor in tumour growth. Nat Rev Cancer 2:38–47, 2002.
24. Haugland HK, Vukovic V, Pintilie M, et al: Expression of hypoxia-inducible factor-1alpha in cervical carcinomas: Correlation with tumor oxygenation. Int J Radiat Oncol Biol Phys 53:854–861, 2002.
25. Hui EP, Chan ATC, Pezzella F, et al: Coexpression of hypoxia-inducible factors 1α and 2α, carbonic anhydrase IX, and vascular endothelial growth factor in nasopharyngeal carcinoma and relationship to survival. Clin Cancer Res 8:2595–2604, 2002.
26. Airley RE, Loncaster J, Raleigh J, et al: GLUT-1 and CAIX as intrinsic markers of hypoxia in carcinoma of the cervix: Relationship to pimonidazole binding. Int J Cancer 104:85–91, 2003.
27. Le QT, Sutpin PD, Raychaudhuri S, et al: Identification of osteopontin as a prognostic plasma marker for head and neck squamous cell carcinomas. Clin Cancer Res 9:59–67, 2003.
28. Raleigh JA, Chou SC, Arteel GE, et al: Comparisons among pimonidazole binding, oxygen electrode measurements and radiation response in C3H mouse tumors. Radiat Res 151:580–589, 1999.
29. Evans SM, Joiner B, Jenkins WT, et al: Identification of hypoxia in cells and tissues of epigastric 9L rat glioma using EF5 [2-(2-nitro-1H-imidazol-1-yl)-N-(2,2,3,3,3-pentafluoropropyl) acetamide]. Br J Cancer 72:875–882, 1995.
30. Rasey JS, Koh WJ, Evans ML, et al: Quantifying regional hypoxia in human tumors with positron emission tomography of 18F-fluoromisonidazole: A pretherapy study of 37 patients. Int J Radiat Oncol Biol Phys 36:417–428, 1996.
31. Piert M, Machulla HJ, Picchio M, et al: Hypoxia-specific tumor imaging with 18F-Fluoroazomycin arabinoside. J Nucl Med 46:106–113, 2005.
32. Urtasun RC, Parliament MB, McEwan AJ, et al: Measurement of hypoxia in human tumours by non-invasive spect imaging of iodoazomycin arabinoside. Br J Cancer 74(Suppl):S209–S212, 1996.
33. Seddon BM, Payne GS, Simmons L, et al: A phase I study of SR-4554 via intravenous administration for noninvasive investigation of tumor hypoxia by magnetic resonance spectroscopy in patients with malignancy. Clin Cancer Res 9:5101–5112, 2003.
34. Gatenby RA, Kessler HB, Rosenblum JS, et al: Oxygen distribution in squamous cell carcinoma metastases and its relationship to outcome of radiation therapy. Int J Radiat Oncol Biol Phys 14:831–838, 1988.
35. Hoeckel M, Schlenger K, Aral B, et al: Association between tumor hypoxia and malignant progression in advanced cancer of the uterine cervix. Cancer Res 56:4509–4515, 1996.
36. Brizel DM, Scully SP, Harrelson JM, et al: Tumor oxygenation predicts for the likelihood of distant metastasis in human soft tissue sarcoma. Cancer Res 56:941–943, 1996.
37. Brizel DM, Sibley GS, Prosnitz LR, et al: Tumor hypoxia adversely affects the prognosis of carcinoma of the head and neck. Int J Radiat Oncol Biol Phys 38:285–289, 1997.
38. Nordsmark M, Overgaard M, Overgaard J: Pretreatment oxygenation predicts radiation response in advanced squamous cell carcinoma of the head and neck. Radiother Oncol 41:31–39, 1996.
39. Nordsmark M, Alsner J, Keller J, et al: Hypoxia in soft tissue sarcomas: Adverse impact on survival and no association with p53 mutations. Br J Cancer 84:1070–1075, 2001.
40. Nordsmark M, Bentzen SM, Rudat V, et al: Prognostic value of tumor oxygenation in 397 head and neck tumors after primary radiation therapy. An international multi-center study. Radiother Oncol 77:18–24, 2005.
41. Overgaard J, Horsman MR: Modification of hypoxia-induced radioresistance in tumors by the use of oxygen and sensitizers. Semin Radiat Oncol 6:10–21, 1996.
42. Du Sault LA: The effect of oxygen on the response of spontaneous tumours in mice to radiotherapy. Br J Radiol 36:749–754, 1963.
43. Suit HD, Marshall N, Woerner D: Oxygen, oxygen plus carbon dioxide, and radiation therapy of a mouse mammary carcinoma. Cancer 30:1154–1158, 1972.
44. Siemann DW, Hill RP, Bush RS: Smoking: The influence of carboxyhemoglobin (HbCO) on tumor oxygenation and response to radiation. Int J Radiat Oncol Biol Phys 40:657–662, 1978.
45. Rojas A: Radiosensitization with normobaric oxygen and carbogen. Radiother Oncol 20(Suppl 1):65–70, 1991.
46. Grau C, Horsman MR, Overgaard J: Improving the radiation response in a C3H mouse mammary carcinoma by normobaric oxygen and carbogen breathing. Int J Radiat Oncol Biol Phys 22:415–419, 1992.
47. Churchill-Davidson I: The oxygen effect in radiotherapy—historical review. Front Radiat Ther Oncol 1:1–15, 1968.
48. Dische S: Hyperbaric oxygen: The Medical Research Council trials and their clinical significance. Br J Radiol 51:888–894, 1979.
49. Dische S, Anderson PJ, Sealy R, et al: Carcinoma of the cervix—anaemia, radiotherapy and hyperbaric oxygen. Br J Radiol 56:251–255, 1983.
50. Henk JM, Kunkler PB, Smith CW: Radiotherapy and hyperbaric oxygen in head and neck cancer. Final report of first controlled clinical trial. Lancet 2:101–103, 1977.
51. Henk JM, Smith CW: Radiotherapy and hyperbaric oxygen in head and neck cancer. Interim report of second clinical trial. Lancet 2:104–105, 1977.
52. Watson ER, Halnan KE, Dische S, et al: Hyperbaric oxygen and radiotherapy: A medical research council trial in carcinoma of the cervix. Br J Radiol 51:879–887, 1978.
53. Overgaard J: Sensitization of hypoxic tumour cells—clinical experience. Int J Radiat Biol 56:801–811, 1989.
54. Bergsjø P, Kolstad P: Clinical trial with atmospheric oxygen breathing during radiotherapy of cancer of the cervix. Scand J Clin Lab Invest 106(Suppl):167–171, 1968.
55. Rubin P, Hanley J, Keys HM, et al: Carbogen breathing during radiation therapy. The RTOG study. Int J Radiat Oncol Biol Phys 5:1963–1970, 1979.
56. Mendenhall WM, Morris CG, Amdur RJ, et al: Radiotherapy alone or combined with carbogen breathing for squamous cell carcinoma of the head and neck. Cancer 104:332–337, 2005.
57. Chaplin DJ, Horsman MR, Siemann DW: Further evaluation of nicotinamide and carbogen as a strategy to reoxygenate hypoxic cells in vivo: Importance of nicotinamide dose and pre-irradiation breathing time. Br J Cancer 68:269–273, 1993.
58. Adams GE, Cooke MS: Electron-affinic sensitization. I. A structural basis for chemical radiosensitizers in bacteria. Int J Radiat Biol 15:457–471, 1969.
59. Asquith JC, Watts ME, Patel K, et al: Electron-affinic sensitization V. Radiosensitization of hypoxic bacteria and mammalian cells in vitro by some nitroimidazoles and nitropyrazoles. Radiat Res 60:108–118, 1974.
60. Adams GE, Flockhart IR, Smithen CE, et al: Electron-affinic sensitization VII: A correlation between structures, one-electron reduction potentials and efficiencies of some nitroimidazoles as hypoxic cell radiosensitizers. Radiat Res 67:9–20, 1976.
61. Overgaard J: Clinical evaluation of nitroimidazoles as modifiers of hypoxia in solid tumors. Oncol Res 6:509–518, 1994.

62. Dische S: Chemical sensitizers for hypoxic cells: A decade of experience in clinical radiotherapy. Radiother Oncol 3:97–115, 1985.
63. Overgaard J, Hansen HS, Andersen AP, et al: Misonidazole combined with split-course radiotherapy in the treatment of invasive carcinoma of the larynx and pharynx: Report from the DAHANCA 2 study. Int J Radiat Oncol Biol Phys 16:1065–1068, 1989.
64. Dische S, Machin D, Chassagne D: A trial of Ro 03–8799 (pimonidazole) in carcinoma of the uterine cervix: An interim report from the Medical Research Council Working Party on advanced carcinoma of the cervix. Radiother Oncol 26:93–103, 1993.
65. Lee D-J, Cosmatos D, Marcial VA, et al: Results of an RTOG phase III trial (RTOG 85–27) comparing radiotherapy plus etanidazole (SR-2508) with radiotherapy alone for locally advanced head and neck carcinomas. Int J Radiat Oncol Biol Phys 32:567–576, 1995.
66. Overgaard J, Sand Hansen H, Overgaard M, et al: A randomised double-blind phase III study of nimorazole as a hypoxic radiosensitizer of primary radiotherapy in supraglottic larynx and pharynx carcinoma. Results of the Danish head and neck cancer study (DAHANCA) protocol 5–85. Radiother Oncol 46:135–146, 1998.
67. Dobrowsky W, Huigol NG, Jayatilake RS, et al: Ak-2123 (Sanazol) as a radiation sensitizer in the treatment of stage III cervical cancer: Results of an IAEA multicentre randomized trial. Radiother Oncol 82:24–29, 2007.
68. Karasawa K, Sunamura M, Okamoto A, et al: Efficacy of novel hypoxic cell sensitizer doranidazole in the treatment of locally advanced pancreatic cancer—long-term results of a placebo-controlled randomized study. Radiother Oncol 87:326–330, 2008.
69. Overgaard J: Hypoxic modification of radiotherapy in squamous cell carcinomas of the head and neck—a systematic review and meta-analysis. Radiother Oncol 100:22–32, 2011.
70. Grau C, Overgaard J: Significance of haemoglobin concentration for treatment outcome. In Molls M, Vaupel P, editors: Medical radiology: Blood perfusion and microenvironment of human tumours, Heidelberg, Germany, 1998, Springer-Verlag, pp 101–112.
71. Grogan M, Thomas GM, Melamed I, et al: The importance of hemoglobin levels during radiotherapy for carcinoma of the cervix. Cancer 86:1528–1536, 1999.
72. Hirst DG: Anemia: A problem or an opportunity in radiotherapy? Int J Radiat Oncol Biol Phys 12:2009–2017, 1986.
73. Stuben G, Pottgen C, Knuhmann K, et al: Erythropoietin restores the anemia-induced reduction in radiosensitivity of experimental human tumors in nude mice. Int J Radiat Oncol Biol Phys 55:1358–1362, 2003.
74. Thews O, Koenig R, Kelleher DK, et al: Enhanced radiosensitivity in experimental tumours following erythropoietin treatment of chemotherapy-induced anaemia. Br J Cancer 78:752–756, 1998.
75. Lavey RS, Dempsey WH: Erythropoietin increases hemoglobin in cancer patients during radiotherapy. Int J Radiat Oncol Biol Phys 27:1147–1152, 1993.
76. Hoskin PJ, Robinson M, Slevin N, et al: Effect of epoetin alfa on survival and cancer treatment-related anemia and fatigue in patients receiving radical radiotherapy with curative intent for head and neck cancer. J Clin Oncol 27:5751–5756, 2009.
77. Hoff CM: Importance of hemoglobin concentration and its modification for the outcome of head and neck cancer patients treated with radiotherapy. Acta Oncol 51:419–432, 2012.
78. Horsman MR, Overgaard J, Siemann D: Impact on radiotherapy. In Sieman D, editor: Tumor microenvironment, Chichester, UK, 2011, John Wiley & Sons, Ltd., pp 353–376.
79. Rockwell S: Use of a perfluorochemical emulsion to improve oxygenation in a solid tumor. Int J Radiat Oncol Biol Phys 11:97–103, 1985.
80. Siemann DW, Macler LM: Tumor radiosensitization through reductions in hemoglobin affinity. Int J Radiat Oncol Biol Phys 12:1295–1297, 1986.
81. Hirst DG, Wood PJ: Could manipulation of the binding affinity of haemoglobin for oxygen be used clinically to sensitize tumours to radiation? Radiother Oncol 20:53–57, 1991.
82. Zhang P, Li H, Tan X, et al: Association of metformin use with cancer incidence and mortality: A meta analysis. Cancer Epidemiol 37:201–218, 2013.
83. Zannella VE, Dal Pra A, Muaddi H, et al: Reprogramming metabolism with metformin improves tumor oxygenation and radiotherapy response. Clin Cancer Res 19:6741–6750, 2013.
84. Horsman MR, Chaplin DJ, Overgaard J: Combination of nicotinamide and hyperthermia to eliminate radioresistant chronically and acutely hypoxic tumor cells. Cancer Res 50:7430–7436, 1990.
85. Chaplin DJ, Horsman MR, Aoki DS: Nicotinamide, fluosol DA and carbogen: A strategy to reoxygenate acutely and chronically hypoxic cells in vivo. Br J Cancer 63:109–113, 1991.
86. Kjellen E, Joiner MC, Collier JM, et al: A therapeutic benefit from combining normobaric carbogen or oxygen with nicotinamide in fractionated x-ray treatments. Radiother Oncol 22:81–91, 1991.
87. Horsman MR, Nordsmark M, Khalil AA, et al: Reducing chronic and acute hypoxia in tumours by combining nicotinamide with carbogen breathing. Acta Oncol 33:371–376, 1994.
88. Horsman MR, Overgaard J: Preclinical studies on how to deal with patient intolerance to nicotinamide and carbogen. Radiother Oncol 70:301–309, 2004.
89. Janssens GO, Rademakers SE, Terhaard CH, et al: Accelerated radiotherapy with carbogen and nicotinamide for laryngeal cancer: Results of a phase III randomized trial. J Clin Oncol 30:1777–1783, 2012.
90. Hoskin PJ, Rojas AM, Bentzen SM, et al: Radiotherapy with concurrent carbogen and nicotinamide in bladder cancer. J Clin Oncol 28:4912–4918, 2010.
91. Hall EJ, Roizin-Towle L: Hypoxic sensitizers: Radiobiological studies at the cellular level. Radiology 117:453–457, 1975.
92. McKeown SR, Cowen RL, Williams KJ: Bioreductive drugs: From concept to clinic. Clin Oncol 19:427–442, 2007.
93. Kennedy KA, Rockwell S, Sartorelli AC: Preferential activation of mitomycin C to cytotoxic metabolites by hypoxic tumor cells. Cancer Res 40:2356–2360, 1980.
94. Weissberg JB, Son YH, Papac RJ, et al: Randomized clinical trial of mitomycin C as an adjunct to radiotherapy in head and neck cancer. Int J Radiat Oncol Biol Phys 17:3–9, 1989.
95. Haffty BG, Son YH, Sasaki CT, et al: Mitomycin C as an adjunct to postoperative radiation therapy in squamous cell carcinoma of the head and neck: Results from two randomized clinical trials. Int J Radiat Oncol Biol Phys 27:241–250, 1993.
96. Dobrowsky W, Naude J, Dobrowsky E, et al: Mitomycin C (MMC) and unconventional fractionation (V-CHART) in advanced head and neck cancer. Acta Oncol 34:270–272, 1995.
97. Grau C, Agarwal JP, Jabeen K, et al: Radiotherapy with or without mitomycin c in the treatment of locally advanced head and neck cancer: Results of the IAEA multicentre randomized trial. Radiother Oncol 67:17–27, 2003.
98. Haffty BG, Wilson LD, Son YH, et al: Concurrent chemo-radiotherapy with mitomycin C compared with porfiromycin in squamous cell cancer of the head and neck: Final results of a randomized clinical trial. Int J Radiat Oncol Biol Phys 61:5–6, 2005.
99. Stratford IJ, O'Neill P, Sheldon PW, et al: RSU 1069, a nitroimidazole containing an aziridine group: Bioreduction greatly increases cytotoxicity under hypoxic conditions. Biochem Pharmacol 35:105–109, 1986.
100. Zeman EM, Hirst VK, Lemmon MJ, et al: Enhancement of radiation-induced tumor cell killing by the hypoxic cell toxin SR 4233. Radiother Oncol 12:209–218, 1988.
101. Vaupel P, Kallinowski F, Okunieff P: Blood flow, oxygen and nutrient supply, and metabolic microenvironment of human tumors: A review. Cancer Res 49:6449–6465, 1989.
102. Folkman J: What is the evidence that tumors are angiogenesis dependent? J Natl Cancer Inst 82:4–6, 1990.
103. Fidler IJ, Langley RR, Kerbel RS, et al: Angiogenesis. In DeVita VT, Hellman S, Rosenberg SA, editors: Cancer principles and practice of oncology, ed 7, Philadelphia, 2005, Lippincott Williams & Wilkins, pp 129–137.
104. Denekamp J: Inadequate vasculature in solid tumours: Consequences for cancer research strategies. Br J Radiol 24(Suppl):111–117, 1992.
105. Horsman MR, Siemann DW: Pathophysiological effects of vascular-targeting agents and the implications for combination with conventional therapies. Cancer Res 66:11520–11539, 2006.
106. National Cancer Institute Website: <www.cancer.gov/clinicaltrials>.
107. Patterson DM, Rustin GJS: Vascular damaging agents. Clin Oncol 19:443–456, 2007.
108. Jain RK: Normalizing tumor vasculature with anti-angiogenic drug therapy: Anew paradigm for combination therapy. Nat Med 7:987–989, 2001.
109. Ludford RJ: Colchicine in the experimental chemotherapy of cancer. J Natl Cancer Inst 6:89–101, 1945.
110. Patt HM, Tyree EB, Straub RL, et al: Cysteine protection against X irradiation. Science 110:213–214, 1949.
111. Weiss JF, Landauer MR: History and development of radiation-protective agents. Int J Radiat Biol 85:539–573, 2009.
112. Yuhas JM: Active versus passive absorption kinetics as the basis for selective protection of normal tissues by S-2(3-aminopropylamino)-ethylphosphorothioic acid. Cancer Res 40:1519–1524, 1980.
113. Yuhas JM: Protective drugs in cancer therapy: Optimal clinical testing and future directions. Int J Radiat Oncol Biol Phys 8:513–517, 1982.
114. Schuchter LM, Hensley ML, Meropol NJ, et al: 2002 update of recommendations for the use of chemotherapy and radiotherapy protectants: Clinical practice guidelines of the American Society of Clinical Oncology. J Clin Oncol 20:2895–2903, 2002.
115. Denekamp J, Stewart FA, Rojas A: Is the outlook grey for WR-2721 as a clinical radioprotector. Int J Radiat Oncol Biol Phys 9:595–598, 1983.
116. Lindegaard JC, Grau C: Has the outlook improved for amifostine as a clinical radioprotector? Radiother Oncol 57:113–118, 2000.
117. Rades D, Fehlauer F, Bajrovic A, et al: Serious adverse effects of amifostine during radiotherapy in head and neck cancer patients. Radiother Oncol 70:261–264, 2004.
118. Brizel DM, Overgaard J: Does amifostine have a role in chemoradiation treatment? Lancet Oncol 4:378–381, 2003.
119. Savoye C, Swenberg C, Hugot S, et al: Thiol WR-1065 and disulphide WR-33278, two metabolites of the drug ethyol (WR-2721), protect DNA against fast neutron-induced strand breakage. Int J Radiat Biol 71:193–202, 1997.
120. Denekamp J, Michael BD, Rojas A, et al: Radioprotection of mouse skin by WR-2721: The critical influence of oxygen tension. Int J Radiat Oncol Biol Phys 8:531–534, 1982.

121. Travis EL: The oxygen dependence of protection by aminothiols: Implications for normal tissues and solid tumors. Int J Radiat Oncol Biol Phys 10:1495–1501, 1984.
122. Washburn LC, Rafter JJ, Hayes RL: Prediction of the effective radioprotective dose of WR-2721 in humans through an interspecies tissue distribution study. Radiat Res 66:100–105, 1976.
123. Yuhas JM, Afzal SMF, Afzal V: Variation in normal tissue responsiveness to WR-2721. Int J Radiat Oncol Biol Phys 10:1537–1539, 1984.
124. Kruse JJ, Strootman EG, Wondergem J: Effects of amifostine on radiation-induced cardiac damage. Acta Oncol 42:4–9, 2003.
125. Bohuslavizki KH, Klutmann S, Jenicke L, et al: Radioprotection of salivary glands by S-2-(3-aminopropylamin)-ethylphosphorothioic (amifostine) obtained in a rabbit animal model. Int J Radiat Oncol Biol Phys 45:181–186, 1999.
126. Rojas A, Denekamp J: The influence of X ray dose level on normal tissue radioprotection by WR-2721. Int J Radiat Oncol Biol Phys 10:2351–2356, 1984.
127. Rojas A, Stewart FA, Soranson JA, et al: Fractionated studies with WR-2721: Normal tissues and tumour. Radiother Oncol 6:51–60, 1986.
128. Brizel DM, Wasserman TH, Henke M, et al: Phase III randomized trial of amifostine as a radioprotector in head and neck cancer. J Clin Oncol 18:3339–3345, 2000.
129. Sasse AD, Clark LG, Sasse EC, et al: Amifostine reduces side effects and improves complete response rate during radiotherapy: Results of a meta-analysis. Int J Radiat Oncol Biol Phys 64:784–791, 2006.
130. Bourhis J, Blanchard P, Maillard E, et al: Effect of amifostine on survival among patients treated with radiotherapy: A meta-analysis of individual patient data. J Clin Oncol 29:2590–2597, 2011.
131. Buntzel J, Kuttner K, Frohlich D, et al: Selective cytoprotection with amifostine in concurrent radiochemotherapy for head and neck cancer. Ann Oncol 9:505–509, 1998.
132. Bourhis J, De Crevoisier R, Abdulkarim B, et al: A randomized of very accelerated radiotherapy with and without amifostine in advanced head and neck squamous cell carcinoma. Int J Radiat Oncol Biol Phys 46:1105–1108, 1999.
133. Momm F, Bechtold C, Rudat V, et al: Alteration of radiation-induced hematotoxicity by amifostine. Int J Radiat Oncol Biol Phys 51:947–951, 2001.
134. Wasserman TH, Brizel DM, Henke M, et al: Influence of intravenous amifostine on xerostomia, tumor control, and survival after radiotherapy for head-and-neck cancer: 2-year follow-up of a prospective, randomized, phase III trial. Int J Radiat Oncol Biol Phys 63:985–990, 2005.
135. Buentzel J, Micke O, Adamietz IA, et al: Intravenous amifostine during chemoradiotherapy for head-and-neck cancer: A randomized placebo-controlled phase III study. Int J Radiat Oncol Biol Phys 64:684–691, 2006.
136. Anné PR, Machtay M, Rosenthal DI, et al: A phase II trial of subcutaneous amifostine and radiation therapy in patients with head-and-neck cancer. Int J Radiat Oncol Biol Phys 67:445–452, 2007.
137. Haddad R, Sonis S, Posner M, et al: Randomized phase 2 study of concomitant chemoradiotherapy using weekly carboplatin/paclitaxel with or without daily subcutaneous amifostine in patients with locally advanced head and neck cancer. Cancer 115:4514–4523, 2009.
138. Antonadou D, Coliarakis N, Synodinou M, et al: Randomized phase III trial of radiation treatment ± amifostine in patients with advanced-stage lung cancer. Int J Radiat Oncol Biol Phys 51:915–922, 2001.
139. Antonadou D, Throuvalas N, Petridis A, et al: Effect of amifostine on toxicities associated with radiochemotherapy in patients with locally advanced non–small-cell lung cancer. Int J Radiat Oncol Biol Phys 57:402–408, 2003.
140. Leong SS, Tan EH, Fong KW, et al: Randomized double-blind trial of combined modality treatment with or without amifostine in unresectable stage III non–small-cell lung cancer. J Clin Oncol 2003:1767–1774, 2003.
141. Komaki R, Lee JS, Milas L, et al: Effects of amifostine on acute toxicity from concurrent chemotherapy and radiotherapy for inoperable non–small-cell lung cancer: Report of a randomized comparative trial. Int J Radiat Oncol Biol Phys 58:1369–1377, 2004.
142. Movsas B, Scott C, Langer C, et al: Randomized trial of amifostine in locally advanced non–small-cell lung cancer patients receiving chemotherapy and hyper fractionated radiation: Radiation therapy oncology group trial 98–01. J Clin Oncol 23:2145–2154, 2005.
143. Lawrence YR, Paulus R, Langer C, et al: The addition of amifostine to carboplatin and paclitaxel based chemoradiation in locally advanced non–small cell lung cancer: Long-term follow-up of Radiation Therapy Oncology Group (RTOG) randomized trial 9801. Lung Cancer 80:298–305, 2013.
144. Bohuslavizki KH, Klutmann S, Bleckmann C, et al: Salivary gland protection by amifostine in high-dose radioiodine therapy of differentiated thyroid cancer. Strahlenther Onkol 175:57–61, 1999.
145. Liu T, Liu Y, He S, et al: Use of radiation with or without WR-2721 in advanced rectal cancer. Cancer 69:2820–2825, 1992.
146. Athanassiou H, Antonadou D, Coliarakis N, et al: Protective effect of amifostine during fractionated radiotherapy in patients with pelvic carcinomas: Results of a randomized trial. Int J Radiat Oncol Biol Phys 56:1154–1160, 2003.
147. Kouloulias VE, Kouvaris JR, Pissakas G, et al: A phase II randomized study of topical intrarectal administration of amifostine for the prevention of acute radiation-induced rectal toxicity. Strahlenther Onkol 180:557–562, 2004.
148. Koukourakis MI, Kyrias G, Kakolyris S, et al: Subcutaneous administration of amifostine during fractionated radiotherapy: A randomized phase II study. J Clin Oncol 18:2226–2233, 2000.
149. Adams GE, Barnes DW, du Boulay C, et al: Induction of hypoxia in normal and malignant tissues by changing the oxygen affinity of hemoglobin—implications for therapy. Int J Radiat Oncol Biol Phys 12:1299–1302, 1986.
150. Adams GE, Stratford IJ, Nethersell AB, et al: Induction of severe tumor hypoxia by modifiers of the oxygen affinity of hemoglobin. Int J Radiat Oncol Biol Phys 16:1179–1182, 1989.
151. Beddell CR, Goodford PJ, Kneen G, et al: Substituted benzaldehydes designed to increase the oxygen affinity of human haemoglobin and inhibit the sickling of sickle erythrocytes. Br J Pharmacol 82:397–407, 1984.
152. Keidan AJ, Franklin IM, White RD: Effect of BW12C on oxygen affinity of haemoglobin in sickle cell disease. Lancet 1:831–834, 1986.
153. Van den Aardweg GJMJ, Hopewell JW, Barnes DWH, et al: Modification of the radiation response of pig skin by manipulation of tissue oxygen tension using anaesthetics and administration of BW12C. Int J Radiat Oncol Biol Phys 16:1191–1194, 1989.
154. Honess DJ, Hu DE, Bleehen NM: BW12C: Effects on tumour hypoxia, tumour radiosensitivity and relative tumour and normal tissue perfusion in C3H mice. Br J Cancer 64:715–722, 1991.
155. Roughton FJW, Darling RC: The effect of carbon monoxide on the oxyhemoglobin dissociation curve. Am J Phys 141:17–31, 1944.
156. Siemann DW, Hill RP, Bush RA: The importance of the pre-irradiation breathing times of oxygen and carbogen (5% CO2; 95% O2) on the in vivo radiation response of a murine sarcoma. Int J Radiat Oncol Biol Phys 2:903–911, 1977.
157. Grau C, Khalil AA, Nordsmark M, et al: The relationship between carbon monoxide breathing, tumour oxygenation and local tumour control in the C3H mammary carcinoma in vivo. Br J Cancer 69:50–57, 1994.
158. Ward A, Clissold SP: Pentoxifylline: A review of its pharmacodynamic and pharmacokinetic properties, and its therapeutic efficacy. Drugs 34:50–97, 1987.
159. Lee I, Kim JH, Levitt SH, et al: Increases in tumor response by pentoxifylline alone or in combination with nicotinamide. Int J Radiat Oncol Biol Phys 22:425–429, 1992.
160. Honess DJ, Andrews MS, Ward R, et al: Pentoxifylline increases RIF-1 tumour pO_2 in a manner compatible with its ability to increase relative tumour perfusion. Acta Oncol 34:385–389, 1995.
161. Price MJ, Li LT, Tward JD, et al: Effect of nicotinamide and pentoxifylline on normal tissue and FSA tumor oxygenation. Acta Oncol 34:391–395, 1995.
162. Dion MW, Hussey DH, Osborne JW: The effect of pentoxifylline on early and late radiation injury following fractionated irradiation in C3H mice. Int J Radiat Oncol Biol Phys 17:101–107, 1989.
163. Stewart FA, Akleyev AV, Hauer-Jensen M, et al: ICRP publication 118: ICRP statement on tissue reactions and early and late effects of radiation in normal tissues and organs–threshold doses for tissue reactions in a radiation protection context. Ann ICRP 41:211–286, 2012.
164. Bourgier C, Levy A, Vozenin M-C, et al: Pharmacological strategies to spare normal tissues from radiation damage: Useless or overlooked therapeutics? Cancer Metastasis Rev 31:699–712, 2012.
165. Maier P, Wenz F, Herskind C: Radioprotection of normal tissue cells. Strahlenther Onkol 2014. [Epub ahead of print].
166. Hendry JH: Biological response modifiers and normal tissue injury after irradiation. Semin Radiat Oncol 4:123–132, 1994.
167. Bernstein EF, Harisiadis L, Salomon G, et al: Transforming growth factor-β improves healing of radiation-impaired wounds. J Invest Dermatol 97:430–434, 1991.
168. Hancock SL: Effects of tumor necrosis factor α on the radiation response of murine intestinal stem cells and lung. Radiation Research Society Meeting, Salt Lake City, Utah, March 1992 (abstract P-06–5).
169. Hancock SL, Chung RT, Cox RS, et al: Interleukin 1β initially sensitizes and subsequently protects murine intestinal stem cells exposed to photon radiation. Cancer Res 51:2280–2285, 1991.
170. Okunieff P, Abraham EH, Moini M, et al: Basic fibroblast growth factor radioprotects bone marrow and not RIF-1 tumor. Acta Oncol 34:435–438, 1995.
171. Robbins MEC, Hopewell JW: Physiological factors affecting renal radiation tolerance: A guide to the treatment of late effects. Br J Cancer 53:265–267, 1986.
172. Halnan KE: The effect of corticosteroids on the radiation skin reaction. Br J Radiol 35:403–408, 1962.
173. Walden TL, Patchen M, Snyder SL: 16,16-dimethyl prostaglandin-E2 increases survival in mice following irradiation. Radiat Res 109:540–549, 1987.
174. Hansen WR, Pelka AE, Nelson AK, et al: Subcutaneous or topical administration of 16,16 dimethyl prostaglandin-E2 protects from radiation-induced alopecia in mice. Int J Radiat Oncol Biol Phys 23:333–337, 1992.
175. Hopewell JW, Robbins MEC, van den Aardweg GJMJ: The modulation of radiation-induced damage to pig skin by essential fatty acids. Br J Cancer 68:1–7, 1993.