Abstract

It has been appreciated for more than 50 years that very low levels of oxygenation, or hypoxia, both protect cells from killing by X-irradiation and are present in solid tumors but not in normal tissues. Until recently, however, there has been no definitive proof that hypoxia in human tumors contributes to radiotherapy treatment failure. We now know that hypoxia in solid tumors is not only a major problem for radiation therapy but also leads to resistance to most anticancer drugs and, importantly, appears to accelerate malignant progression and increase metastasis. To date, efforts to overcome the problem of hypoxia have had only limited success. However, the recent development of new drugs that are nontoxic until they are activated in the hypoxic cell opens a new era. The first of these new drugs to be tested clinically, tirapazamine, a drug that is highly toxic to hypoxic but not aerobic cells, has already demonstrated efficacy in selective potentiation of cisplatin in randomized Phase III trials with non-small cell lung cancer. The unique presence of hypoxic cells in human tumors provides an important target for selective cancer therapy.

Introduction

Research on methods of overcoming the problem for radiotherapy of hypoxic cells in solid tumors has been ongoing for almost 50 years. During that time, interest among basic researchers has waxed and waned as promising new directions emerged, only to fail in clinical trials. However, as increasingly sophisticated concepts have replaced earlier, simpler ideas, the prospect of overcoming and eventually exploiting this fundamental difference between normal and malignant tissues appears more realistic. Recent clinical studies have for the first time unequivocally demonstrated that hypoxia in solid tumors is a major problem for radiotherapy, and that low oxygenation can accelerate malignant progression and metastasis, thereby creating a poorer prognosis irrespective of which cancer treatment is used. Development of new drugs that are selectively toxic to hypoxic cells, of which TPZ is a prototype, has a solid theoretical and preclinical base and is also showing positive clinical results. This review will trace some of the important concepts and developments that have brought us to the present in this field.

The Problem for Radiotherapy of Hypoxia in Solid Tumors

Although it had been appreciated for several years that lowering the oxygenation of tissues made them more resistant to damage by ionizing radiation (1), it was the pioneering studies of Gray and colleagues soon after World War II that established the universality of the radiation resistance conferred by hypoxia as well as providing early insight into the mechanism of action. In their landmark paper, Gray et al. (2) showed that hypoxia conferred resistance to radiation damage of a wide range of cells and tissues using various end points. They showed further that it was the presence of oxygen at the time of irradiation that caused radiation sensitivity rather than any metabolic effects, as had been previously supposed. The oxygen effect may be unique in biology in having such a broad applicability: it applies to enzymes in solution; to bacteria; to yeast; and to plant and mammalian cells irrespective of their genetic background. A typical radiation killing curve for mammalian cells under aerobic and hypoxic conditions is shown in Fig. 1. The difference in radiation sensitivity between the aerobic and hypoxic cells, which is known as the oxygen enhancement ratio and is defined as the ratio of doses to produce the same level of cell kill under hypoxic to aerobic conditions, is normally in the range 2.5–3 for mammalian cells. Although this ratio may not seem large, the difference in cell kill for a given radiation dose can be several orders of magnitude, as can be seen in Fig. 1 for a dose of 14 Gy (dashed vertical line). The reason for the universality of this effect is that oxygen reacts chemically with the fundamental biological lesion produced by ionizing radiation, a radical in DNA. Oxygen, being the most electron-affinic molecule in the cell, reacts extremely rapidly with the free electron of the free radical, thereby “fixing” (making permanent) the damage. In the absence of oxygen, much of the radical damage can be restored to its undamaged form by hydrogen donation from nonprotein sulfhydryls in the cells. This mechanism is shown in Fig. 1. Thus, ionizing radiation is severely compromised in its ability to kill hypoxic cells. This is true for all cell types, both normal and malignant. However, the degree of resistance can be changed somewhat by the nature of the radiation. Very densely ionizing radiation such as α particles shows no effect of cellular oxygenation on their killing ability, with particles of intermediate ionization densities such as fast neutrons having an intermediate effect (3). This has been the motivation for developing neutron beams for therapy, but the expense and other problems of such modalities have prevented their widespread acceptance. For the foreseeable future, the vast majority of radiotherapy will be conducted with X-rays.

Insight into how hypoxia can develop in tumors was provided by Thomlinson and Gray (4), who showed with histological sections of human tumors that there was a constant distance across tumor tissue between blood vessels and necrosis. Furthermore, they showed that this distance (usually 100–150 μm) was predicted as the oxygen diffusion distance, given the oxygen partial pressure in capillaries and the rate of oxygen consumption of cells. They suggested that viable hypoxic (and hence radioresistant) cells would be adjacent to the necrotic areas in tumors. This theory has stood the test of time. There are now several different methods of visualizing hypoxic cells in sections of rodent and human tumors (5–7), and these have generally shown hypoxia at the predicted distances from blood vessels and also adjacent to necrosis if present.

Of the techniques presently available for detecting hypoxia in human tumors, the most quantitative and widely used is a commercially available, polarographic oxygen electrode (PO2 histograph; Eppendorf, Hamburg, Germany). The sensing electrode, mounted on...
the question of whether the more hypoxic tumors had an inferior radiation resistance than those in adjacent normal tissues. In Fig. 2 would be expected to have regions with cells at greater values of less than approximately 0.5 mm Hg. Thus, the tumor shown which radiation resistance starts to develop with full resistance at than 10 mm Hg, and half have a median greater than this (8 –12). This at different sites, roughly half the tumors have a median value of less in Fig. 2 is typical in the sense that in many series of human tumors show a distribution with much lower oxygen tension. The one shown values less than 10 mm Hg. Tumors, on the other hand, invariably caused by the needle. Using this system, a histogram of oxygen tensions can be obtained from multiple points along different tracks through the tissue. An example of measurements made in a neck node demonstrating cells to stop or slow their rate of progression through the cell cycle (19, 20). This effect is not the result of a generalized decrease in ATP or energy status of the cell but is likely to be caused by specific proteins induced under hypoxic conditions (21–24). Because most anticancer drugs are more effective against rapidly proliferating cells than slowly or nonproliferating cells, this slowing of cell proliferation with increasing distance from the vasculature will lead to decreased cell killing at these increased distances.

Second, the concentration of anticancer drugs will be higher closer to blood vessels than further away. This is a consequence not only of the geometry, in which the drug being provided by a central vessel has to diffuse out over a much greater volume at the periphery of the cord, but also of the fact that many anticancer drugs, because of their reactivity, will be limited in their diffusion from the blood vessel. This is particularly true for agents that physically bind to DNA, such as intercalators (25–28). Even for non-DNA intercalators, such as melphalan, there can be a problem in nonhomogeneous distribution from the blood vessel (29).

There are also other ways in which hypoxia might contribute to drug resistance. One is through amplification of genes conferring drug resist-
Tumor Hypoxia Increases Malignant Progression and Metastasis

Recent studies have shown that hypoxia in solid tumors has an important consequence in addition to conferring a direct resistance to radiation and chemotherapy. Graeber et al. (37) have demonstrated that low oxygen levels cause apoptosis in minimally transformed mouse embryo fibroblasts, and that this apoptosis depends to a large extent on wild-type p53. Using these same cells growing as solid tumors in immune-deprived mice, they further showed that apoptosis colocalizes with hypoxic regions in tumors derived from p53 wild-type mice. In tumors derived from p53−/− cells, there was much less apoptosis and no colocalization with tumor hypoxia. These findings provide evidence that hypoxia, by selecting for mutant p53, might predispose tumors to a more malignant phenotype. In addition to this selection of cells with mutant p53 (hence, cells more likely to mutate), hypoxia and the tumor microenvironment have also been shown to cause mutations directly (38, 39). Clinical data support this conclusion: studies with soft tissue sarcomas (40) and with carcinoma of the cervix (41, 42) have shown that hypoxia is an independent and highly significant prognostic factor predisposing tumors to metastatic spread.

Tumor hypoxia also stimulates tumor progression by promoting angiogenesis through the induction of proangiogenic proteins such as vascular endothelial growth factor (43). Most genes induced by hypoxia are regulated by the hypoxia-inducible transcription factor HIF-1, a protein that therefore plays a very important role in tumor development (44–46).

Fluctuating Blood Flow in Tumors

Despite the strong evidence for the Thomlinson-Gray model of development of hypoxia in tumors, a number of observations began to develop in the late 1970s that did not seem to fit the model. One was from our own experiments with the hypoxic radiosensitizer misonidazole, which, when given at very high doses to tumor-bearing mice, produced extensive tumor cell kill that was located around regions of necrosis (47). According to the classic model, this should have eliminated or markedly reduced the number of viable hypoxic cells in the tumor. However, when we measured the fraction of radiation-resistant (and therefore, hypoxic) cells in the tumor, we saw little change (48).

Also, in carefully performed experiments with tumors growing between two transparent windows, Yamaura and Matsuzawa (49) found that after a large, fixed dose of radiation, tumors regrew from regions that were highly vascularized rather than (as expected) areas adjacent to necrosis. Although these authors did not invoke acute or fluctuating hypoxia as an explanation for this, it seemed to us that this was a hypothesis that could account for both our own data and their data. We therefore suggested that blood flow in tumors could fluctuate, thereby producing temporary regions of acute hypoxia (48).

Later studies using vascular casting techniques with window chamber preparations identified the structural basis for inhomogeneities in blood flow in transplanted tumors (50–52). These investigations showed that tumor blood vessels are tortuous, highly irregular, have arterial venous shunts, blind ends, and lack smooth muscles and innervation. As a result, blood flow would be expected to be sluggish and highly irregular. At about the same time as these anatomical studies, fluctuating blood flow in transplanted tumors was convincingly demonstrated using two diffusible dyes injected i.v., either together or several minutes apart (53–55). Essentially, these investigators found that in tumor sections from mice injected with the two dyes several minutes apart, a fraction of the blood vessels was labeled with one dye or the other (not both), implying vessel opening or closing in the time interval between the two injections.

One of the important implications of fluctuating blood flow is that not only would the hypoxic cells produced by temporary cessation of blood flow be resistant to ionizing radiation, but it is also likely that they would be resistant (because of inadequate drug delivery) to any anticancer drug that had a half-life in the blood stream that was less than or equal to the time for which the blood vessel remained closed. Thus, as in the case of...
chronic hypoxia, acutely hypoxic cells would be expected to be resistant to both radiation and commonly used anticancer drugs.

Are both of these types of hypoxia, acute and chronic, present in human tumors? Several investigators have clearly demonstrated the presence of chronically hypoxic cells using hypoxia-activated markers (7). Acute hypoxia cannot be visualized directly in human tumors, but studies by Hill et al. (56) of tumor blood flow using laser Doppler probes have shown that fluctuating blood flow occurs in human tumors in a manner similar to that in mouse tumors. Thus, we can be reasonably confident that both of these types of hypoxia are common in human tumors.

**Hypoxic Cell Radiosensitizers: A Good Idea That Might Have Worked**

One of the earliest attempts to overcome the problem of the resistance of hypoxic cells in tumors to radiotherapy was to increase oxygen levels in the blood stream, thereby increasing the diffusion distance of oxygen. A number of trials were performed with patients breathing 100% oxygen at a pressure of 3 atmospheres, but the results were mixed (57–59). In retrospect, we can now appreciate that this strategy would only reduce the number of chronically hypoxic cells; it would not be expected to change the proportion of acutely hypoxic cells.

As interest waned in the use of hyperbaric oxygen, an apparently superior solution seemed at hand. Following pioneering studies by Adams et al. (60, 61) on the use of electron-affinic drugs to sensitize hypoxic bacteria and mammalian cells *in vitro*, a drug was identified as showing activity with transplanted murine tumors (62, 63). This drug, a 5-nitroimidazole already used clinically as Flagel, showed excellent activity in sensitizing mouse tumors to radiation. Almost immediately a more active drug, a 2-nitroimidazole, later named misonidazole, became available (64). The results obtained for these drugs were quite remarkable: with large, single doses of radiation, large degrees of sensitization of tumors with no concomitant sensitization of normal tissues were found by a number of groups (64–66). Clinical trials were started almost immediately, but a major side effect with multiple doses emerged that had not been seen in preclinical studies: misonidazole produced a severe neuropathy, both peripheral and central, that limited the dose of drug that could be used with radiotherapy (67). Not surprisingly, almost all of the clinical trials of radiotherapy combined with misonidazole turned out to be negative (68), an outcome consistent with the small degree of radiosensitization expected with the clinically used doses (69). It should be noted, however, that a more recent meta-analysis of all of these trials has demonstrated a small but significant benefit of misonidazole and other hypoxic radiosensitizers when added to radiotherapy (70). At the time, however, the problem of neurotoxicity seemed to be an overwhelming limitation to the success of misonidazole.

But could the neurotoxicity of these drugs be eliminated while retaining their radiosensitizing properties? It seemed to us that this was a possibility. Because the mechanism of hypoxic radiosensitizers is similar to that of oxygen in that they fix the free radical damage to DNA, the extent of their radiosensitization depends on the concentration of drug at the time of irradiation. Drug toxicity, on the other hand, is likely to depend on area under the concentration–time curve for specific tissues. This is different from anticancer drugs whose efficacy and toxicity are both dependent on area under the concentration–time curve. This feature of radiosensitizers made it attractive to try to design drugs rationally to minimize tissue exposure, particularly to neural tissues. William Lee, who led a chemistry group at SRI International, and my laboratory at Stanford University set about to do just this. Our strategy was based on the simple concept illustrated in Fig. 5. Consistent with this concept, we

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5 M. Evans and C. J. Koch, personal communication.

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found that as we decreased the lipophilicity of the compounds while keeping the 2-nitroimidazole moiety that had been shown to determine radiosensitivity, we were able to reduce drug exposure to the brain and to reduce toxicity (71, 72). Eventually, however, at too great a hydrophilicity, the drugs failed to enter the tumor cells and hence lost antitumor efficacy. The optimum drug, SR2508, later known as etanidazole, was three to four times less toxic to mice but had the same antitumor efficacy (73). Subsequent clinical trials confirmed that three times more etanidazole than misonidazole could be given without producing neurotoxicity (74).

Why was this drug not more effective? In the first place, giving three times more drug only increased the expected radiosensitization of the hypoxic cells from an enhancement ratio of 1.1–1.2 to approximately 1.4. However, a more serious problem is that it is not the cells at maximum radiation resistance but those at intermediate oxygenation and intermediate radioresistance that dominate the response to fractionated irradiation. This has been demonstrated in a recent theoretical analysis of the influence of hypoxia on radiotherapy and is likely to be a problem with any hypoxic cell radiosensitizer (76). Unfortunately, although hypoxic cell radiosensitizers at clinically realistic doses can sensitize the maximally hypoxic cells to radiation killing, they have little effect on the radiosensitization of the cells at intermediate hypoxia and radiosensitivity. This is a consequence of the logarithmic nature of the curve of radiosensitization versus dose; as cell sensitivity increases, it takes geometrically more drug to increase their radiosensitivity still further. Thus, a hypoxic cell partially sensitized by some oxygen takes much more drug to increase its sensitivity by a given amount compared to a fully hypoxic cell. It thus seems unlikely that hypoxic cell radiosensitizers, even newer ones with greater potency, will influence the outcome of fractionated radiotherapy. However, they may and probably should play a role in those situations where large, single doses of radiation are given, such as with stereotactic radiotherapy of brain tumors.

**Hypoxic Cytotoxins: Turning Hypoxia from Problem to Advantage**

Can the low oxygen levels in tumors be turned into a therapeutic advantage? Such a possibility was proposed over 25 years ago by Lin et al. (77), who reasoned that compounds based on the quinone structure of mitomycin C might be more active in hypoxic tumors. It was known at that time that mitomycin C required metabolic reduction of the benzoquinone ring to produce the cytotoxic bifunctional alkylating agent. Lin et al. reasoned that a lower oxidation reduction (redox) potential for tumor tissue relative to most normal tissues (which had been suggested by earlier work of Cater and Philips; Ref. 78) could increase reductive activation of these quinone derivatives in tumors. Although this was not the correct mechanism for the increased

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Fig. 5. The rationale for producing a less toxic hypoxic cell radiosensitizer than misonidazole was based on changing the side chain of the drug so as to reduce lipophilicity, thereby increasing the drug clearance rate and inhibiting its access to neural tissues. This figure was adapted from Ref. 72.
cytotoxicity of mitomycin C and certain analogues toward hypoxic cells (much lower levels of hypoxia are needed to change cellular redox potential), these studies were important in suggesting the potential of hypoxia-activated drugs and led to the concept of selectively killing the hypoxic cells in solid tumors (79–83).

It is important to note that specifically killing the hypoxic cells in tumors has greater therapeutic potential than oxygenating the cells or chemically sensitizing them to radiation or chemotherapy for two reasons: (a) hypoxic cytotoxins specifically kill those cells resistant to radiation and chemotherapy, thereby producing complementary cytotoxicity as shown in Fig. 4; and (b) the random fluctuations in acute hypoxia are thought to occur in tumors create a situation in which hypoxia could be a therapeutic advantage. Modeling studies, the results of which are shown in Fig. 6, illustrate that if a hypoxic cytotoxin can be given with every radiation dose in a radiotherapy regime, then the overall cell kill in a hypoxic tumor can be greater than if the tumor were fully oxygenated. This occurs when the hypoxic cytotoxin kills 50% or more of the hypoxic cells each time it is given. It is important to note that this requirement of giving the hypoxic cytotoxin with many, if not all, of the radiation doses would be difficult to achieve with a conventional chemotherapy drug that kills aerobic cells. Thus, although randomized clinical trials have demonstrated that the addition of the bioreductive drug mitomycin C to radiotherapy has shown a clear benefit (84, 85), the fact that it could only be given two times during the course of radiotherapy makes it unlikely that the drug was exploiting fluctuating hypoxia. Nonetheless, these results with mitomycin C are clearly promising for the use of hypoxia-activated agents with radiotherapy.

**TPZ: A Novel Hypoxic Cell Toxin**

Serendipity played a large role in the discovery of the benzotriazine di-N-oxide class of hypoxic cytotoxins of which TPZ is the lead compound. In the mid-1980s, William Lee and I were looking for new classes of hypoxic radiosensitizers that did not include a nitro group, which we surmised might be responsible for the neurotoxicity of the 2-nitromidazole class of radiation sensitizers. In testing a particular benzotriazine di-N-oxide, SR 4233, (later known as TPZ), we found not only that it killed hypoxic cells at much lower concentrations than those needed to radiosensitize the cells, but also that its differential toxicity to hypoxic cells was much larger than that of any known drug. The HCR, or the concentration of drug required under aerobic relative to hypoxic conditions to produce equal cytotoxicity, was in the 50–300 range for various cell lines (79). This is to be compared with a HCR of 1–5 for various cell lines for mitomycin C (83, 86–88) and a HCR of 10–20 for nitromidazoles (89, 90). Fig. 7 shows data for killing of aerobic and hypoxic mouse SCCVII tumor cells by a 1-h exposure to TPZ.

The mechanism of this selective hypoxic cytotoxicity is shown in Fig. 8. TPZ is an excellent substrate for a variety of intracellular reductases that add an electron to the drug, which then becomes protonated to form a neutral radical. This radical is highly reactive and will abstract hydrogen from macromolecules including DNA (if it is close enough), producing both single- and double-strand breaks. We have shown that the hypoxic cytotoxicity of TPZ can be fully accounted for by the double-strand breaks and resulting chromosome breaks that are produced by the drug (91). Under aerobic conditions, oxygen can remove the additional electron from the TPZ radical, thereby back-oxidizing the radical to the inactive parent drug.

However, there is a problem with this proposed mechanism. One TPZ radical could only be expected to produce one single-strand break, and yet we find some $10^4$ times more double-strand breaks than could be accounted for by the chance overlap of the single-strand breaks. Our hypothesis to account for this is that the reductase leading to DNA damage is located very close to DNA, thus creating high concentrations.
of TPZ radical close to the DNA. We have in fact shown that the activating enzyme(s) leading to DNA damage is (are) located in the cell nucleus (93) and have found that there is a high concentration of TPZ-metabolizing enzymes associated with the nuclear matrix (94). This hypothesis is illustrated in Fig. 9. We propose that the double-strand breaks leading to cell kill under hypoxic conditions are the result of high local concentrations of radicals close to the DNA that is associated with the nuclear matrix. If this is so, a number of phenomena might be predicted. First, because the replication and transcription machinery is associated with the nuclear matrix (95), we might expect major effects of TPZ on replication and transcription. In support of this, we have recently observed very dramatic decreases (of up to 80%) in DNA replication at TPZ on replication and transcription. In support of this, we have recently observed very dramatic decreases (of up to 80%) in DNA replication at only moderately toxic TPZ doses under hypoxic conditions.

This raises a number of interesting possibilities, including the fact that the double-strand breaks induced by TPZ may be the result of poisoning of topoisomerase II, producing cleavable complexes similar to those produced by other topoisomerase II poisons, such as etoposide. This is presently an active area of investigation.

Turning back to the question of activity of TPZ when combined with standard anticancer therapies such as radiation and chemotherapeutic drugs, we have been able to show that TPZ potentiates cell kill by fractionated irradiation at clinically realistic drug and radiation doses (96). Based on these studies, a Phase II multicenter trial of TPZ with irradiation of head and neck tumors was recently reported by the Radiotherapy Oncology Group (97). The results, a 60% local control rate at 18 months for advanced head and neck cancer, are encouraging when compared to the most recent randomized study by the same cooperative group (75).

But the most clinical success has been realized by the combination of TPZ and cisplatin. In testing the concept that TPZ should enhance the antitumor efficacy of a number of anticancer drugs by the complementary cytotoxicity mechanism shown in Fig. 4, we found a remarkable potentiation of cisplatin. Fig. 10 shows the effect of combining a single dose of TPZ with cisplatin on the number of clonogenic cells in 6–8-mm-diameter RIF-1 mouse tumors. In this tumor, TPZ alone produced approximately 0.5 logs of cell kill, cisplatin alone produced approximately 2 logs of cell kills, and the combination of the two drugs given at the same time produced approximately 2.5–3 logs of cell kill. However, when TPZ was given 2–3 h before cisplatin, a large potentiation of cell kill was observed with some 6–7 logs of cell kill produced. Despite this remarkable potentiation of cisplatin toxicity to the tumor cells, we saw no evidence of any increase of cisplatin toxicity to any normal tissues or in its systemic toxicity (98). Although our original hypothesis was that the interaction of the two drugs would be the result of complementary cytotoxicity [because hypoxic cells in tumors have been shown to be resistant to cisplatin (18, 99)], we have found that this is not the most important mechanism. In fact, there is a specific cellular interaction of TPZ (when given under hypoxia) with cisplatin that mimics the time course and extent of the interaction seen with tumors in vivo. We have further shown that this interaction can be accounted for by a delay in the repair of cisplatin-induced interstrand cross-links in cells exposed to TPZ under hypoxia before cisplatin exposure (100). Exactly how TPZ might inhibit or delay the repair of cisplatin-induced interstrand cross-links is presently under investigation.

The promising preclinical data on combining TPZ with cisplatin has led to clinical trials of this combination (101–104). Table I shows the results of a Phase III randomized clinical trial in advanced non-small cell lung cancer comparing cisplatin only with the combination of TPZ plus cisplatin (103). In this trial, the overall response rate was doubled by the addition of TPZ to cisplatin, and the median life span was extended significantly. The clinical data also confirmed the preclinical studies showing no potentiation of cisplatin side effects or other toxicities in the combination group. Of note is the fact that there was no increase in hematological toxicity in the combination group, although specific TPZ-associated toxicities of increased nausea and vomiting and muscle cramps were present. These data therefore confirmed the preclinical data that TPZ can produce a tumor-selective potentiation of cisplatin cell kill in human tumors. Because the addition of TPZ to cisplatin is considerably less toxic than the addition to cisplatin of other anticancer drugs such as gemcitabine, navelbine, or Taxol, it is expected that this new bioreductive drug can be added to the best of the current two-drug combinations. To this end, a randomized Phase III trial of cisplatin + navelbine with or without TPZ will soon be started.

5 K. Peters et al., unpublished observations.
Summary

The discovery more than 50 years ago that hypoxic cells were resistant to killing by X-rays led to the hypothesis that tumors might be resistant to radiotherapy because their poor blood supply would lead to hypoxia. These early ideas have not only been confirmed in the past 5 years, but tumor hypoxia is now seen as a mechanism for resistance to many anticancer drugs as well as a predisposing factor toward increased malignancy and metastasis. However, tumor hypoxia represents a unique target for cancer therapy that could be exploited for therapeutic benefits. This review focuses on one way in which hypoxia might be exploited: the use of hypoxic cytotoxins, in general, and TPZ, the first purely hypoxic cytotoxin to enter the clinic, in particular. However, this is only one possibility. Other approaches being investigated by different groups are targeting some of the specific hypoxia-induced proteins such as HIF-1 or using hypoxia to obtain tumor-specific gene expression for gene therapy.

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References

The Hypoxic Cell: A Target for Selective Cancer Therapy—
Eighteenth Bruce F. Cain Memorial Award Lecture

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