3 Tumor Oxygenation and Treatment Response

Sarah Jane Lunt, PhD and Richard P. Hill, PhD

CONTENTS

SUMMARY

Solid tumor oxygenation is highly heterogeneous, often showing regions of hypoxia that demonstrate oxygen concentrations much lower than those encountered in normal tissues. Tumor hypoxia can cause treatment resistance, resulting in a poorer treatment outcome. In addition, hypoxia forms a part of the pathophysiologic microenvironment that characterizes solid tumors and is involved in disease progression, possibly through alterations in gene expression. This chapter discusses recent research focused on methods of measuring tumor hypoxia accurately and extensively, with the aim of tailoring treatment on an individual patient basis. Examples of therapeutic approaches designed to exploit tumor hypoxia directly or indirectly, are discussed.

Key Words: Tumor hypoxia; HIF-1; hypoxic markers; bioreductive drugs; gene-directed enzyme prodrug therapy.

1. INTRODUCTION

Tissue oxygenation is the result of a balance between oxygen supply and consumption, a balance that is finely regulated in normal tissues. In solid tumors this balance is disturbed, such that the supply is no longer adequate, resulting in hypoxia. The definition of hypoxia varies between studies, and the term has been used to describe severe oxygen deprivation, near 0 mmHg, or oxygen levels (~15–20 mmHg) approaching those of

From: *Cancer Drug Discovery and Development: Cancer Drug Resistance*
Edited by: B. Teicher © Humana Press Inc., Totowa, NJ

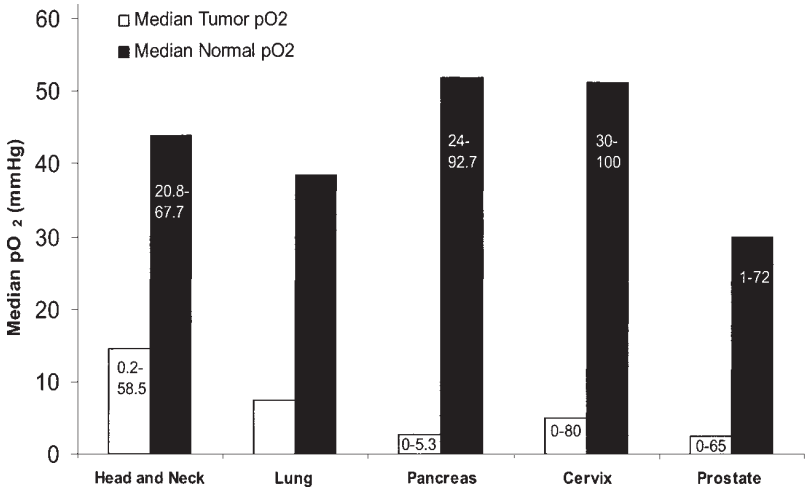

Fig. 1. Differences in median pO_2 of normal tissue (black bar) vs tumor tissue (white bar). The range of pO_2 values are shown within the bars for head and neck *(118)*, pancreas *(119)*, cervix *(120)*, and prostate cancers *(58)*. The median values for the lung samples were taken from Brown and Wilson *(6)*, and no range was available.

normal, well-oxygenated tissue. Experimentally, hypoxia is often used to describe pO_2 values below ~7.5 mmHg. Solid tumors demonstrate a low median pO_2 of ~5–20 mmHg, as compared to most normal tissues (median pO_2 of ~20–95 mmHg). Representative values for human tumors are shown in Fig. 1. Tumor oxygenation is highly heterogeneous, both within an individual tumor and between tumors *(1)*. Hypoxia in tumors has long been known to induce resistance to both radiation and chemotherapy, and clinically median oxygen partial pressures (pO_2) below ~10 mmHg are generally found to be associated with poorer treatment outcome. There is also mounting evidence of a role for tumor hypoxia in tumor progression *(2)*. As a consequence of the negative impact of tumor hypoxia on treatment outcome, recent research has focused on further elucidating the role of hypoxia in disease progression *(2,3)*, on methods of measuring tumor hypoxia accurately and extensively *(4,5)*, and on exploiting tumor hypoxia to improve treatment response *(6,7)*. This chapter provides an overview of each of these three areas.

2. HYPOXIA IN TUMORS

The tumor microenvironment is characterized by several pathophysiologic conditions including tumor hypoxia, reduced pH, and elevated interstitial fluid pressure, all of which are, to varying extents, a consequence of the disorganized structure and function of the abnormal vasculature that characterizes solid tumors *(8)*. Tumor growth and development is supported by both the pre-existing host vasculature and by neovasculature generated through the process of angiogenesis. Studies using tumors growing in window chambers have shown that this process may be initiated early in tumor growth when the tumor comprises 60–80 cells *(9)*. The host vessels do not increase in number during tumor growth (initially they may actually regress) *(10)*, and consequently, the number of preexisting vessels is reduced in comparison to the area they supply. Furthermore, the host venules

undergo morphological changes including elongation and dilation, and may become obstructed or compressed by the surrounding tumor cells *(11)*. The arterioles remain largely intact, but are restricted to the fascial surface of the tumor, resulting in longer transport distances through the arteriolar tumor supply vessels *(12)*. The neovasculature that develops through angiogenesis is highly chaotic, resulting in further spatial heterogeneity. The vessels formed are immature and demonstrate several abnormalities, being dilated, tortuous, and lacking in enervation. They often have an incomplete or missing endothelial cell layer and basement membrane that makes them more permeable *(13)*. In addition, they are prone to excessive branching, blind ends and neovascular shunts (Fig. 2). These abnormalities result in increased geometric resistance, plasma channels containing few or no red blood cells, and longitudinal pO_2 gradients, all of which contribute to aberrant flow and the development of a pathophysiologic tumor microenvironment that is extremely heterogeneous, both within an individual tumor and between different tumor types *(1,8,11)*.

The existence of hypoxic cells in tumors was highlighted in 1955 by Thomlinson and Gray, who noted that regions of necrosis were often observed in human lung cancers at distances of 160–200 μm away from the supporting vasculature. As these distances were consistent with the calculated diffusion distances of oxygen from the capillary network, it was suggested that viable chronically hypoxic cells alongside regions of necrosis were a feature of solid tumors as a consequence of oxygen diffusion limitations *(14)*. The diffusion radius of oxygen depends on the rate of oxygen consumption by the cells and the pO_2 in the adjacent vessel(s); thus, the distance from the blood vessels to the edge of the necrotic region (sometimes called the tumor cord radius) may vary from tumor to tumor and within tumors because of declining levels of oxygen and pO_2 in the vasculature as it progresses through the tumor parenchyma. In addition, the existence of a highly chaotic vascular network and plasma channels as well as rheologic effects, such as altered blood viscosity and subsequent slow flow rates, can lead to the development of regions of hypoxia in areas that would appear to have an adequate vascular network, possibly through a reduction in the intravessel blood oxyhemoglobin saturation. Oxyhemoglobin saturation levels in a tumor can be much lower than those in normal vessels, possibly because of increased oxygen consumption to facilitate rapid proliferation or through an increase in extraction of oxygen from hemoglobin at low pO_2 as a result of sluggish flow *(8)*.

Oxygen diffusion limitations may be expected to give rise to tumor cells that are hypoxic over extended periods of time, referred to as chronically hypoxic cells. However, there are also regions in tumors that fluctuate between normoxic and hypoxic states, and this is referred to as acute, transient, or perfusion-limited hypoxia. The existence of transiently hypoxic tumor cells was investigated in studies using fluorescent dyes that stain cells adjacent to functional vasculature. The administration of two dyes either simultaneously or sequentially showed colocalization only for simultaneous administration. With sequential administration, there was mismatch in the staining patterns of these dyes, consistent with variations in perfusion *(15)*. This study went on to demonstrate that differences in vascular flow were reflective of differences in viable hypoxic cells in the tumors over time. Fluctuations in tumor blood flow were also observed by direct measurements of regional blood flow using laser Doppler techniques *(16)*, and indirectly by measuring temporal changes in tumor temperature *(17)*.

However, the situation is at once more complex and more subtle. The dye mismatch studies could only identify fluctuating flow and acute hypoxia as a result of complete

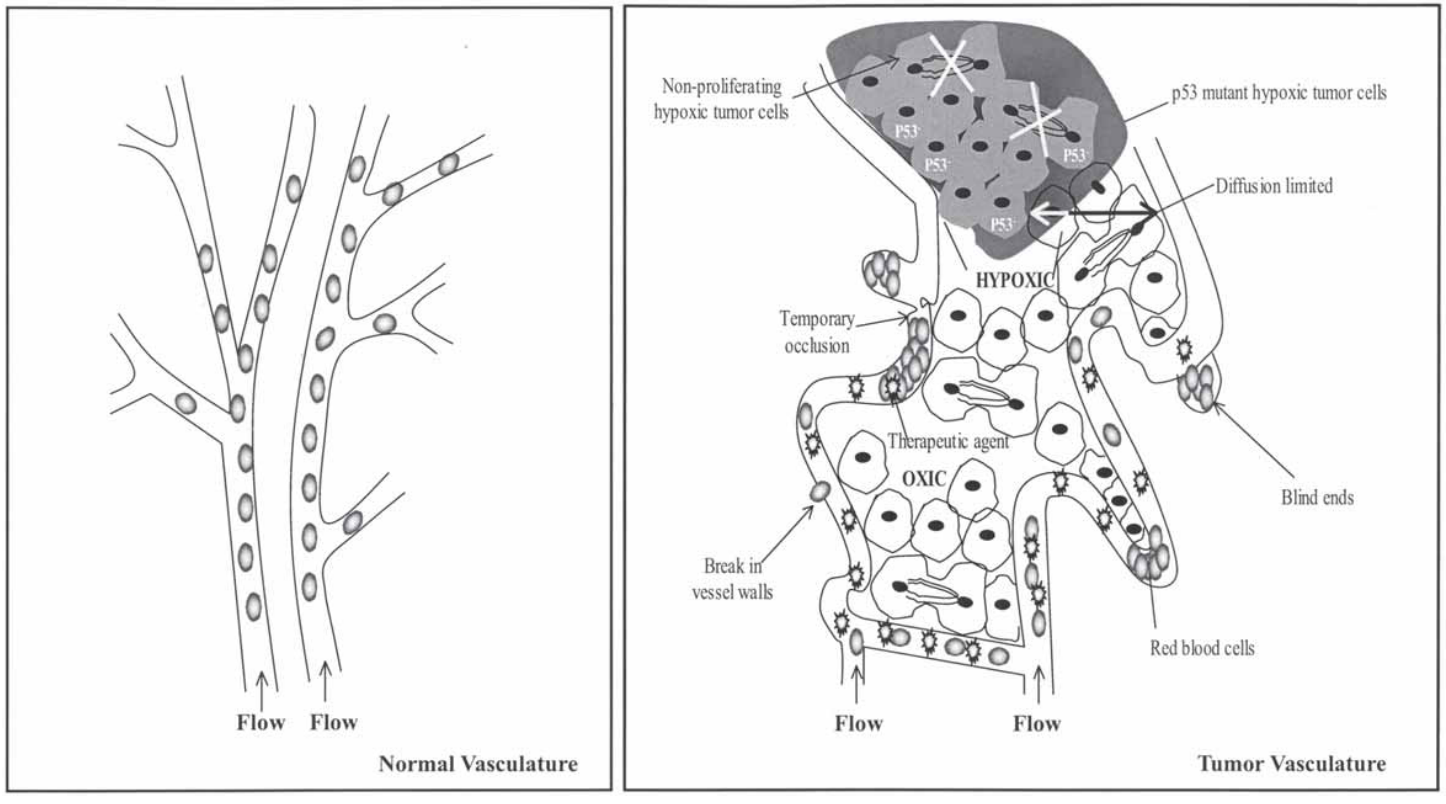

Fig. 2 Schematic representation of normal vasculature versus tumor vasculature, illustrating the development of tumor hypoxia and potential methods of drug resistance. Normal vasculature is evenly spaced and organized, ensuring a sufficient supply of oxygen and nutrients. Tumor vasculature shows several structural abnormalities as indicated on the figure. These abnormalities lead to functional deficiencies, which result in regions of hypoxia and drug resistance. Temporary occlusions in the vasculature may lead to perfusion limited hypoxia. They may also block the delivery of therapeutic agents. The tortuous nature of the vasculature and abnormalities such as blind ends combined with sluggish blood flow can lead to diffusion limited hypoxia. These diffusion limitations may apply to some chemotherapeutic drugs as well as oxygen and nutrients. Hypoxia *per se* may reduce cell proliferation, which reduces the efficacy of many conventional drugs. Hypoxia may also select for p53 mutant cell lines with increased apoptotic resistance and consequently increased resistance to some drugs. (Modified from ref. 6.)

occlusion and subsequent reopening of the vessels, whereas studies measuring red cell flux over a two hour period demonstrated that complete occlusion may be a relatively rare event *(18)*. More recent data from studies focused on measuring changes in tissue pO_2 continuously over 30- to 90-min periods demonstrated frequent fluctuations above and below 5 mmHg *(19,20)*. This is suggestive of a higher frequency of tumor cell exposure to cyclic hypoxia-reoxygenation than previously anticipated. The impact of this cyclic hypoxia on tumor progression and therapeutic response is not well established, although a major product of hypoxia-reoxygenation is the reactive oxygen species, superoxide anion, which could result in enhanced mutagenic frequency, potentially contributing to tumor progression.

Hypoxic cells are known to be approximately three times more resistant to ionizing radiation than oxygenated cells, because oxygen can chemically modify, and thus prevent, direct chemical repair of the damage caused by the initial radiation-induced radicals *(21)*. The oxygen level required for half-maximal sensitization to radiation is widely reported to be about 3 mmHg for mammalian cells, but recent work has suggested that the value may be higher in tumors (~7.5 mmHg) because of higher levels of nonprotein sulphydryls, particularly cysteine *(22)*.

The role of hypoxia in resistance to chemotherapeutic drugs is less well defined, but there are several proposed mechanisms. Hypoxic areas distant from functional vasculature will have limited diffusion of therapeutic agents, as will cells surrounding chaotic vasculature and plasma channels where perfusion may be limited. Reduced drug delivery is likely to be associated with reduced efficacy. Furthermore, many traditional chemotherapeutic agents were designed to target dividing cells. Under hypoxic conditions cellular proliferation is reduced, impacting negatively on the cytotoxic effects of these agents. Additionally, some drugs require oxygen to modify DNA damage, similar to radiotherapy *(6,23)*. As well as these physiological constraints, hypoxia contributes to genetic and epigenetic changes *(2)* including the upregulation of several genes involved in drug resistance and the selection of cells with p53 mutations, which can increase cellular resistance to apoptosis and reduce drug sensitivity (*see* Fig. 2) *(6)*.

3. MARKERS OF HYPOXIA

The link between solid tumor hypoxia and both treatment resistance and disease progression makes the ability to measure tumor hypoxia accurately and extensively extremely desirable. As such, different approaches have been, and are being, examined to achieve this aim and thus tailor treatment accordingly. The majority of clinical data relating tumor hypoxia to treatment resistance and/or disease progression is based currently on pO_2 measurements taken using the Eppendorf polarographic electrode system *(5,24)*. This system involves a fine-needle probe with a sampling volume of approximately 500 cells that automatically progresses through the tissue in a stepping motion, thereby measuring pO_2 at multiple points within a tumor *(25)*. However, the system is limited to easily accessible tumors and does not distinguish regions of necrosis, or even normal cells. Furthermore, the Eppendorf electrode gives no information as to the location or kinetics of tumor hypoxia, in relation to proximity to the vasculature or the acute versus chronic hypoxia status of the cells in the hypoxic region. To overcome these limitations, there have been numerous studies into alternative methods of measuring tumor hypoxia, with particular attention paid to the use of exogenous and endogenous markers that can be used with biopsy specimens. Alternative mechanisms of measuring

tumor hypoxia would need to fulfill certain criteria for successful application in the clinic, in particular that they are associated with prognosis. Ideally, the degree of hypoxia would also correlate to results obtained using the Eppendorf electrode, the current gold standard. In addition, some method of standardization, both in the biopsy procedure and subsequent analyses would be required to validate widespread use.

3.1. Exogenous Markers

Three alternative possibilities for evaluating tumor hypoxia are exogenous markers (2-nitroimidazoles), endogenous markers (genes upregulated by hypoxia), and noninvasive imaging. Each of these methods has potential advantages and limitations. The 2-nitroimidazole compounds used as exogenous markers were developed originally as radiosensitizers for use in conjunction with conventional radiotherapy. They are metabolized in, and bind strictly to, hypoxic cells. The most commonly used 2-nitroimidazoles are pimonidazole (1-(2-nitro-1-imidazolyl)-3-*N*-piperidino-2-propanol) and EF-5 (nitroimidazole [2-(2-nitro-1*H*-imidazol-1-yl)-*N*-(2,2,3,3,3-pentafluoropropyl)acetamide]). These markers exhibit comparable mechanisms of activation, and are reduced by viable hypoxic cells to generate reduction products that form adducts in the cells that are easily detectable through immunohistochemistry *(26)*. Both markers are reduced at oxygen concentrations below ~10 mmHg, and they tend to mark regions more distant from the vasculature than the endogenous markers CA-IX or glucose transporter (Glut)-1 (*see* Subheading 3.2.).

Tumor hypoxia as marked by pimonidazole has been shown to correlate with other methods of hypoxic detection known to indicate levels of hypoxia that affect cellular radiosensitivity (radiobiologically relevant hypoxia), in both murine and human tumors. This would imply that pimonidazole labeling is representative of radiobiologically relevant hypoxia *(5)*. However, pimonidazole labeling was found to show only a weak, nonsignificant correlation with tumor hypoxia assessed using the Eppendorf electrode method in human tumors *(27)*. A similar result was seen for EF-5 in squamous cell carcinomas *(28)*, and in brain tumors *(29)*. One possible explanation for this disparity may be the inherent differences in the techniques; the Eppendorf electrode measures discrete volumes of cells in a stepping method through the tumor before amalgamating the results to give an overall definition of the hypoxic nature of the tumor. In contrast, the use of exogenous markers specifies hypoxia on an individual cell basis and is dependent on the level of hypoxia and exposure time to the markers. Furthermore, the heterogeneity of tumor oxygenation requires the examination of multiple tissue sections to provide an overall picture of the hypoxic status of a tumor. Such analyses have rarely been performed to date.

Nevertheless, current data would suggest that exogenous markers can indicate radiobiologically relevant hypoxia in tumors accessible for biopsy, thereby overcoming a major difficulty of the Eppendorf electrode method. In addition, they can provide information on hypoxia at a cellular level and allow hypoxia to be assessed in relation to other parameters such as vascular density or regions of necrosis. Both pimonidazole and EF-5 have been successfully applied in the clinic. One study measured tumor hypoxia using pimonidazole binding in patients selected for a clinical trial of *a*ccelerated *r*adiotherapy, *c*arb*o*gen, *n*icotinamide (ARCON), a therapeutic approach aimed at improving the response of hypoxic regions to radiotherapy. Pimonidazole binding was found to correlate with poor prognosis, primarily in patients that did not receive ARCON, suggesting that ARCON was successful in reducing the impact of hypoxia on treatment response *(30)*.

3.2. Endogenous Markers

Endogenous markers share the advantages of the exogenous markers, with the further benefit that there is no need to administer any agents before obtaining a tissue biopsy. This enables retrospective analyses as well as current evaluation, provided that the biopsy material has been stored adequately. This method of marking hypoxia is focused on the hypoxia-specific expression of proteins. Hypoxic gene expression is primarily regulated by a heterodimeric transcription factor, hypoxia inducible factor (HIF)-1. HIF-1 is responsible for the hypoxia-mediated transcriptional regulation of a wide selection of genes initiated through a cognate recognition sequence, to which HIF-1 binds upstream of the coding region (*see* Subheading 6.). This is termed the hypoxia response element (HRE), and all the known HIF-1-responsive genes have been found to contain HREs of 50 bp or less, with a conserved region functionally essential for HIF-1 binding *(7,31)*. Thus, studies of endogenous markers of hypoxia have considered the use of HIF-1α, the hypoxia-regulated element of HIF-1, or genes upregulated by HIF-1, most commonly carbonic anhydrase 9 (*CA-IX*) or *Glut-1*. HIF-1α is targeted for rapid degradation by the proteasome under normoxic conditions (*see* Subheading 3.4.) *(32)*. Thus, CA-IX and Glut-1 have an advantage over HIF-1α in that they are neither rapidly degraded on exposure to oxygen, nor stabilized in response to hypoxia, potential problems in the removal and preparation of biopsy specimens for analysis. However, if the specimens are prepared correctly and rapidly, HIF-1α should be indicative of the hypoxic state of cells at the specific time of the biopsy, whereas CA-IX and Glut-1 may be more representative of long term or diffusion limited hypoxia *(4)*. The simultaneous analysis of these markers with differing expression profiles could potentially distinguish between areas of diffusion limited and transient perfusion limited hypoxia, providing HIF-1α is not constitutively expressed, as appears to be the case in some tumors *(33)*.

Endogenous markers have been linked with outcome. HIF-1α expression has been demonstrated as indicative of a worse prognosis *(34–36)*, as has CA-IX *(37–40)* and, to a lesser degree, Glut-1 *(41)*. However, the published results are not consistent, as HIF-1α expression was also correlated with significantly improved disease-free and overall survival in head and neck squamous cell carcinoma patients *(42)*. Likewise, CA-IX expression in renal carcinoma was indicative of an improved prognosis in one study *(43)*. It is possible that this difference could be because of differences in HIF-1 activity, as renal carcinomas have demonstrated defective regulation of HIF-1α *(33)*. In the previously mentioned ARCON study (*see* Subheading 3.1.), a greater hypoxic fraction as revealed by CA-IX expression did not correlate with outcome, despite a good correlation between CA-IX expression and pimonidazole binding *(30)*. Thus, the current data are not conclusive and, to date, potential limitations of these studies associated with the handling of the biopsies and with heterogeneity in labeling from one region of the tumor to another have not been adequately addressed. A further potential limitation of these markers is that their expression may also have alternative mechanisms of regulation distinct from that of hypoxia, and thus they may not necessarily be specific markers of tumor oxygenation, but may also reflect other changes within the tumor microenvironment *(7,44,45)*. This does not rule out the future use of these markers, but rather suggests that further study is required to elucidate the most appropriate approach.

3.3. Noninvasive Imaging

Noninvasive imaging methods of quantifying tumor hypoxia would be of great therapeutic benefit, as they are not restricted to accessible tumors, although they are not applicable to retrospective studies. A major advantage of noninvasive imaging is the potential to measure hypoxia dynamically and to monitor the activity of therapeutic agents in relation to tumor hypoxia, thus verifying whether hypoxia is influencing drug delivery or whether resistance is the result of alternative causes *(46)*. Such methods can potentially enable real-time imaging of fluctuations in flow and hypoxia, possibly allowing for measurement of both diffusion-limited and transient perfusion-limited hypoxia. Two imaging mechanisms that have been widely studied are positron emission tomography (PET) and single-photon emission computed tomography (SPECT) *(47)*. Both PET and SPECT involve the introduction of isotope-labeled hypoxia-targeted drugs and the emitted radiation is used to generate an image. However, the results obtained to date do not correlate well with those generated using the Eppendorf electrode method *(26)*.

A further imaging method that is being investigated is blood-oxygenation level-dependent (BOLD) imaging, a functional magnetic resonance technique. Unlike PET and SPECT, isotope-labeled agents are not required. Instead, BOLD imaging relies on the inherent magnetic properties of hemoglobin, which vary according to its oxygenation state *(26)*. Thus, BOLD imaging permits a direct measurement of (blood) oxygenation without the use of any other agents, similar to endogenous markers. However, this method also has limited spatial resolution, and the linkage between blood oxygenation levels and tissue oxygenation levels is likely indirect because of the heterogeneous and chaotically organized vasculature in tumors. The same concern also applies to other magnetic resonance and computed tomography techniques for measuring blood perfusion in tumors. The use of noninvasive imaging methods to measure tumor hypoxia is still at a relatively early stage of application *(48)*. Results to date are promising but further work is needed to determine their true potential.

4. TUMOR HYPOXIA AND DISEASE PROGRESSION

The advent of the Eppendorf electrode as a reliable and reproducible method of measuring tumor pO_2 in the clinic permitted widespread clinical studies focused on both the incidence of hypoxia in solid tumors and consequent treatment outcome, primarily over the past decade. These studies have revealed substantial levels of hypoxia in cervical carcinoma *(49–51)*, head and neck carcinoma *(52–54)*, soft tissue sarcoma *(55,56)*, and prostate carcinoma *(57–59)*. Furthermore, tumor hypoxia has been linked with a poorer prognosis/reduced survival outcome in cervix carcinoma, head and neck carcinoma and soft tissue sarcoma *(49–52,56)* (Table 1). Current data are suggestive of a similar link in prostate carcinoma *(59)*. There are several potential explanations for this link. As discussed above, hypoxic tumor cells are known to be refractory to radiotherapy, and common chemotherapeutic drugs and this may reduce treatment efficacy. However, the effect of hypoxia on treatment outcome was apparent in patients treated with radiotherapy, chemotherapy, or surgical resection. In addition, there was a correlation between tumor hypoxia and distant spread as well as local failure. Both of these observations imply that hypoxia is associated with more aggressive disease as well as being involved in treatment resistance *(49,50)*.

Experimental data are indicative of a role for tumor hypoxia in disease progression. Early work demonstrated that a 24-h exposure of three murine tumor cell lines to hypoxia

Table 1
Clinical Studies Showing a Correlation Between Tumor pO_2 Measured Using the Eppendorf Electrode and Treatment Outcome

Tumor type	*Treatment*	*Patient number*	*Association*	*Reference*
Cervical carcinoma	Radiation	106	DFS, DMFS	Fyles et al. 2002 *(49)*
	Radiation	40	DFS, LRC	Sundfor et al. 2000 *(121)*
	Radiation	51	DFS	Knocke et al. 1999 *(122)*
	Radiation or surgery	89	DFS	Hockel et al. 1999 *(123)*
Head and neck carcinoma	Radiation and/or chemotherapy	194	DSS	Rudat et al. 2001 *(124)*
	Radiation and chemotherapy	41	DFS	Rudat et al. 2000 *(125)*
	Radiation	35	LRC	Nordsmark et al. 2000 *(54)*
	Radiation or radiochemotherapy	59	DFS	Stadler et al. 1999 *(126)*
	Radiation and/or chemotherapy	63	DFS, LRC	Brizel et al. 1999 *(52)*
	Radiation	35	LRC	Nordsmark et al. 1996 *(53)*
Soft tissue Sarcoma	Surgery and/or radiation	28	DSS	Nordsmark et al. 2001 *(55)*
	Radiation, surgery and hyperthermia	22	DMFS	Brizel et al. 1996 *(56)*

DFS, disease-free survival; DSS, disease-specific survival: LRC, locoregional control; DMFS, distant metastases-free survival.

in vitro, before intravenous injection in vivo, resulted in enhanced metastatic ability *(60)*. Interestingly, this effect was transient, suggesting a potential role for hypoxia-mediated alterations in gene expression. Concomitant with this, a significant correlation between hypoxic fraction, as measured using the Eppendorf electrodes, and micrometastases in the lungs was demonstrated for one of the cell lines (murine fibrosarcoma) *(61)*. Similarly, a correlation between the formation of macroscopic metastases and tumor hypoxia has been demonstrated using an orthotopic pancreatic xenograft model *(62)*. Comparable results have been seen in a human melanoma cell line, in accord with its level of expression of vascular endothelial growth factor (*VEGF*), a hypoxia-regulated gene. An increase in the number of metastases following hypoxic exposure, and consequent induction of *VEGF*, was seen only in a cell line with low constitutive expression of *VEGF*. A melanoma cell line with high constitutive expression of *VEGF* did not demonstrate increased metastatic potential, despite similar hypoxic induction. Thus, hypoxia-enhanced metastases would appear to occur in a manner specific to individual tumor cells *(63)*.

Experimentally induced acute hypoxia (12 cycles 5–7% O_2 for 10 min, followed by 10 min of air each day) in murine fibrosarcoma-bearing mice significantly increased the formation of micrometastases in the lung relative to both control (air breathing) and chronic hypoxia (5–7% O_2 for 120 min each day) treatment groups *(64)*. Similarly, mice bearing orthotopically implanted cervix carcinoma xenografts exposed to the same acute hypoxia treatment demonstrated an increase in lymph node metastases. These data are indicative of a causal role for acute hypoxia in metastases formation, both in blood borne and lymphatic metastases *(65)*. The exact mechanisms through which this occurs remain to be elucidated, but a possibility is that regions of acute hypoxia may contribute to metastatic disease because of their increased cell viability and proximity to tumor vasculature.

5. TUMOR HYPOXIA AND GENOMIC INSTABILITY

There is no definitive explanation for how hypoxia might contribute to a more aggressive phenotype, but experimental studies suggest an assortment of genetic alterations endowing hypoxic cells with a survival advantage. These phenotypic changes could arise from hypoxia-mediated upregulation of gene transcription or via hypoxia-mediated genomic instability *(2,66)*. The tumor suppressor gene *p53* is involved in the apoptotic response to DNA damage and accumulates under hypoxic conditions. It is commonly mutated in many cancers, resulting in a survival advantage under conditions characteristic of the tumor microenvironment. Oncogenically transformed $p53^{+/+}$ and $p53^{-/-}$ murine embryonic fibroblasts demonstrated a clear survival advantage for p53-deficient cells following hypoxic exposure. The p53-deficient line was extremely resistant to apoptosis. In addition, if the two cell populations were mixed at a ratio of 1:1000 of $p53^{-/-}$:$p53^{+/+}$ and cultured under repeated rounds of hypoxia, the percentage of $p53^{-/-}$ cells increased following each treatment until they became the predominant cells in the culture. Consistent with this result, tumors grown from the $p53^{+/+}$ cells in vivo demonstrated a substantially higher apoptotic frequency as compared to tumors from the $p53^{-/-}$ cells despite similar hypoxic profiles *(67)*. This provides evidence for hypoxia-mediated selection of mutant variants.

Transient inactivation of p53 under hypoxic conditions may also lead to increased resistance to stress-induced apoptosis. Recent studies in a murine fibrosarcoma model have demonstrated that hypoxia can upregulate the expression of murine double minute 2 (mdm2), a negative regulator of p53 that targets p53 protein for degradation by the proteasomal

degradation pathway, consequently downregulating p53 protein levels. The tumor cells with increased mdm2 expression were found to be more efficient at forming lung metastases following intravenous injection, because they were more resistant to apoptosis induced by the stress of being arrested in the lung environment *(68)*.

Hypoxic tumors have also been reported to demonstrate a higher incidence of mutations than the same tumor cell line grown under oxic conditions in vitro, and studies involving intermittent exposure to hypoxia and reoxygenation in vitro resulted in increased mutation frequency relative to the number of exposures *(69)*. This suggests that in vivo exposure to fluctuating levels of hypoxia can result in increased mutation levels and possibly genomic instability. In addition, tumor hypoxia has been found to reduce the expression of genes involved in DNA mismatch repair *(70)*. These hypoxia-mediated effects could contribute to treatment resistance as well as disease progression *per se*, potentially through reduced drug efficacy as a consequence of decreased apoptotic ability or deregulated expression of genes involved in drug resistance.

6. HYPOXIA-MEDIATED GENE EXPRESSION

Hypoxia imposes a stress on cells, thereby inducing a response to improve survival. High throughput screens, such as microarray analysis and differential display, have enabled the discovery of a large number of genes that respond to hypoxia, including proapoptotic and antiapoptotic genes, genes involved in invasion, metabolism, growth arrest and differentiation, and synthesis of DNA, RNA, and proteins. Apart from HIF-1, hypoxia-mediated gene expression can also be regulated through several transcription factors such as the cyclic AMP-response-element-binding (CREB) protein, the activator protein 1 (AP-1), the nuclear factor- κB (NFκB), the early growth response-1 protein (EGR-1), and p53 *(2,3)*. HIF-1 has been the most extensively studied, and is responsible for the transcriptional regulation of over 60 genes involved in survival mechanisms, including angiogenesis (e.g., *VEGF,* endoglin, leptin, transforming growth factor-β3), metabolism (e.g., hexokinases 1 and 2, *Glut-1*, lactate dehydrogenase A, phosphoglycerate kinase 1, triosephosphate isomerase), and proliferation (e.g., cyclin G2, insulin-like growth factor [IGF]-2, IGF-binding protein [BP]-1, *TGF*-α, *TGF*-β3), many of which are classically associated with cancer *(7,71)*. HIF-1 is a heterodimer composed of two subunits, HIF-1α and HIF-1β. HIF-1β is constitutively expressed such that HIF-1α is the regulatory subunit of HIF-1, undergoing rapid posttranslational oxygen dependent degradation *(32)* with a half-life of about 5 min following reoxygenation *(72–75)*. The ubiquitin-proteasome pathway is known to be involved through the interaction of HIF-1α with the von Hippel Lindau protein (pVHL), the product of the VHL tumor suppressor gene *(33,76)*. pVHL interacts physically with HIF-1α via its β-domain and targets it for degradation *(77)*. This interaction is regulated through hydroxylation of conserved proline residues 402 and 564 on HIF-1α by prolyl hydroxylase proteins 1–3 *(7,78,79)*. These HIF-1α prolyl hydroxylase proteins require molecular oxygen as a substrate, providing a mechanism through which oxygen dependent degradation of HIF-1α is achieved *(7)*.

Under hypoxic conditions, there is an instantaneous and strong stabilization of HIF-1α protein, and thus, the HIF-1 dimer is formed and induces expression of its downstream genes. HIF-1 activation occurs only when there is nuclear translocation of HIF-1α protein, allowing it to dimerize with HIF-1β *(73,77)* and its coactivator CBP/p300. CBP/p300 is a general transcriptional coactivator that binds to the HIF-1α transactivation domain, an interaction that is also oxygen dependent, as factor inhibiting HIF-1(FIH) mediates hydroxylation of asparagine residue 803 and acts to inhibit this interaction under normoxia *(80)*.

There is a variety of both clinical and experimental data to suggest a role for HIF-1 in tumor progression, although not all reports support such a role *(42)*. Clinically, evidence of HIF-1α overexpression is found in many human tumors *(34,35,81–84)*, such that HIF-1α is reported to be expressed in over 90% of all colon, lung and prostate cancers, whereas there is no corresponding expression in normal tissue *(31,71)*. Clinical studies have linked this overexpression with subsequent poor prognosis in carcinoma of the head and neck, ovaries, oesophagus, brain, breast, cervix, and uterus *(7)*. Experimentally, it has been demonstrated that by disrupting the ability of HIF-1α to interact with its transcriptional coactivator CBP/p300, thereby inhibiting HIF-1 activation in a dominant negative manner, tumor growth could be restricted *(85)*. A study of naturally occurring pancreatic cell lines with constitutive expression of HIF-1α, alongside corresponding low-expressing variants, demonstrated better tumor growth of those expressing HIF-1α. Furthermore, the cell lines expressing HIF-1α demonstrated improved survival in response to hypoxia and glucose deprivation in vitro, a result that could be replicated in the low-expressing cell lines by stable transfection of HIF-1α *(86)*.

Together, the clinical and experimental data suggest that HIF-1 is involved in potentiating tumor growth, although the precise mechanisms through which this may be achieved remain unclear. One possibility is that HIF-1 may enhance the ability of the tumor cells to utilize the restricted nutrients of the microenvironment most efficiently. The introduction of an HIF-1α expression vector into a human colon carcinoma line, thereby upregulating HIF-1α expression, demonstrated a significantly enhanced ability to invade through Matrigel under hypoxic conditions. Correspondingly, inhibition of HIF-1α through targeted degradation with small-interfering RNA (siRNA) reduced the invasive-capacity of this cell line. Subsequent analyses of gene expression in both murine embryonic stem cells and human VHL-deficient tumor cells demonstrated HIF-1-dependent induction of genes such as urokinase-type plasminogen activator receptor and matrix metalloproteinase 2, both of which are involved in the degradation of the basement membrane. Specific inhibition of urokinase-type plasminogen activator receptor inhibited invasion. Taken together, these data provide evidence for a role for HIF-1 in enhanced tumor cell invasion, a vital characteristic of tumor metastasis *(87)*.

7. HYPOXIA-TARGETED THERAPY

In view of its effect on progression, coupled with its negative implications for both radiotherapy and common chemotherapy agents, tumor hypoxia has traditionally been viewed as a therapeutic obstacle. However, because it is predominantly a tumor-specific condition, recent work focused on its potential for targeted therapeutic approaches. Traditionally, attempts were made to reoxygenate the tumor cells, thereby rendering them susceptible to conventional therapies; however, current work has concentrated on the development of drugs designed to elicit a cytotoxic response selectively under hypoxic conditions, or to target genes upregulated by the hypoxic environment (Table 2; Fig. 3).

7.1. Bioreductive Drugs

Bioreductive prodrugs represent a group of drugs that are enzymatically reduced to yield a cytotoxic moiety, a process that is facilitated under hypoxic conditions. This reduction is catalyzed by a variety of reductases, most commonly cytochrome P450 reductase and the cytochrome P450 family. In general, there is an initial formation of a one-electron-reduced intermediate, which is further reduced to elicit toxicity. This inter-

Table 2
Hypoxia-Targeted Therapies: Examples of Promising Studies for Different Hypoxia-Directed Therapies Along With an Outline of Their Mechanism of Hypoxia-Selective Toxicity

Therapy	*Name*	*Mechanism of toxicity*	*Reference*
Bioreductive prodrugs	Tirapazamine (TPZ)	Reduced to form an oxidizing radical.	Brown 1993 (review) *(127)*
	AQ4N	Acts as a topoisomerase poison. Has a bystander effect.	Patterson et al. 2000 *(128)*
	NLCQ-1	DNA-targeted reactive electrophile.	Papadopoulou and Bloomer 2003 (review) *(129)*
GDEPT studies	LDH/cytosine deaminase with 5-FC	Facilitates activation of 5-FC to its toxic form 5-FU by producing the bacterial enzyme cytosine deaminase	Dachs et al. 1997 *(108)*
	LDH/P450R with RB6145	Enhances tumor response by increasing levels of required reductase enzyme, P450R	Patterson et al. 2002 *(109)*
	LDH/P450R with TPZ and radiation	Enhances tumor response by increasing levels of required reductase enzyme, P450R	Cowen et al. 2004 *(112)*
HIF-1-targeted studies	Topotecan	Inhibits HIF-1α translation in a topoisomerase I-dependent manner.	Rapisarda et al. 2002, 2004 *(115,117)*
	YC-1	Inhibits HIF-1α at posttranscriptional level.	Yeo et al. 2003 *(116)*
	Dominant-negative variant of HIF-1α	Inhibits functional HIF-1 formation in a dominant-negative manner.	Chen et al. 2003 *(130)*
	Antisense HIF-1α- and B7-1-mediated immunotherapy	Inhibits HIF-1α expression potentiating the antitumor effects of B7-1-mediated immunotherapy	Sun et al. 2001 *(131)*
	Antisense HIF-1α and VHL protein	Inhibits HIF-1α expression potentiating the antitumor effects of overexpression of the tumor suppressor pVHL	Sun et al. 2003 *(132)*

TPZ, tirapazamine; GDEPT, gene-directed enzyme prodrug therapy; LDH, lactate dehydrogenase; P450R, P450 reductase; 5-FC, 5-fluorocytosine, 5-FU, 5-fluorouracil; HIF-1, hypoxia inducible factor 1; YC-1, 3-(5¢-hydroxymethyl-2¢-furyl)-1-benzylindazole; VHL, von Hippel Lindau; pVHL, von Hippel Lindau protein.

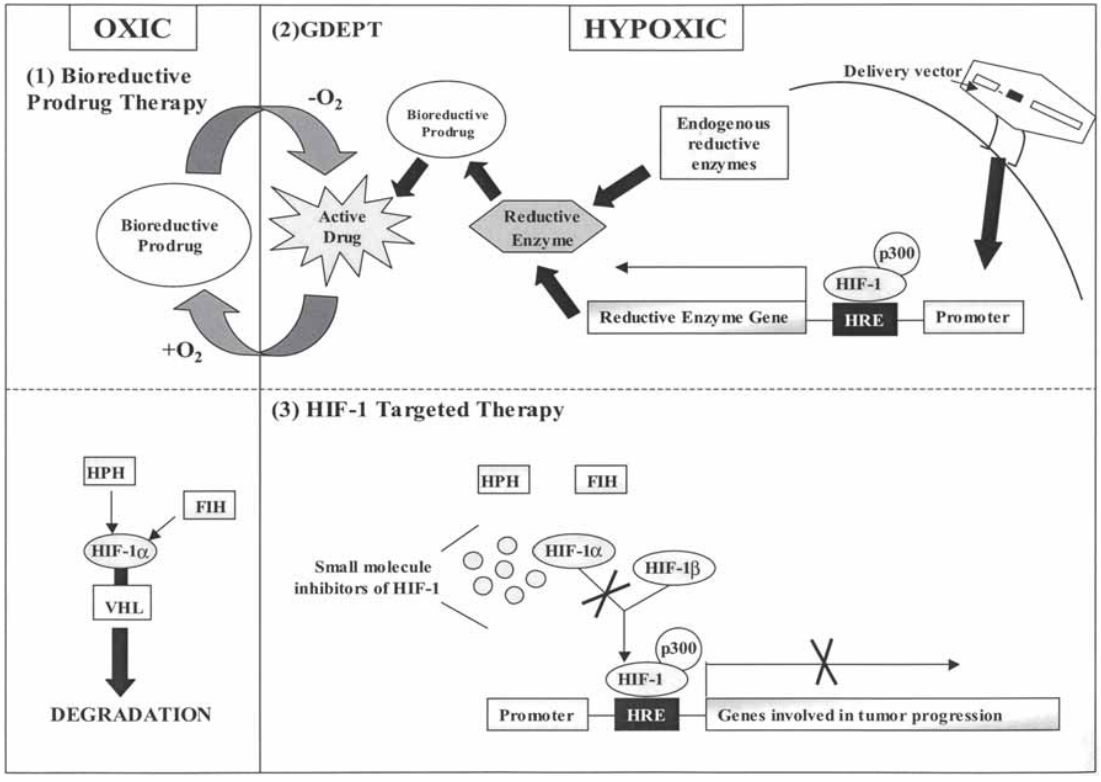

Fig. 3. Schematic outline of some examples of hypoxia-targeted therapies. Both hypoxic and oxic conditions are indicated. (1) Bioreductive prodrugs are reduced by specific reductive enzymes under hypoxic conditions to form an active drug able to elicit a cytotoxic response. Under oxic conditions these drugs undergo a process of futile cycling where they are back-oxidized into their nontoxic form. (2) Gene directed enzyme prodrug therapy (GDEPT) can be used to target expression of the reductive enzymes required for prodrug activation to hypoxic regions, thus enhancing prodrug efficacy. This is commonly achieved through use of the hypoxia inducible factor (HIF)-1/hypoxia response element (HRE) system. (3) As HIF-1, a transcription factor responsible for the regulation of many target genes, is normally only formed under hypoxic conditions it also represents a hypoxia-specific target. HIF-1-directed therapy generally targets HIF-1α. Targeting approaches have included the use of small molecule inhibitors, or dominant negative, small interfering RNA or antisense variants, thereby inhibiting the transcriptional regulation of downstream target genes involved in tumor progression.

mediate can be back-oxidized in the presence of oxygen in a process known as futile cycling, thereby preventing the production of the toxic species (*see* Fig. 3). However, futile cycling produces a superoxide radical that can induce aerobic toxicity to varying extents *(88–90)*. Because cytotoxic bioreductive drugs selectively target hypoxic tumor cells, combination therapy in conjunction with radiation or classic chemotherapeutic drugs, which are more effective against well oxygenated cells, should result in a greatly enhanced response through the complimentary killing of the cells refractory to the different treatment modalities *(6,91)*. However, the stringent hypoxic requirement of many of these drugs represents a potential problem, in that bioreductive drugs generally elicit a cytotoxic response at oxygen concentrations only below ~3 mmHg. In contrast, cellular radioresistance becomes apparent below ~20 mmHg. Thus, combined treatment with a bioreductive drug and radiotherapy might result in a survival advantage for cells at intermediate oxygen concentrations *(92)*.

Clinically, the most widely studied bioreductive drug is tirapazamine (TPZ), a benzotriazine di-*N*-oxide that elicits cytotoxicity through a nitroxide radical intermediate that causes single- and double-stranded DNA breaks *(90,93)*. Unlike most bioreductive drugs, the toxicity range of TPZ extends to intermediate oxygen concentrations. Thus, TPZ can target those cells that are radioresistant but too well oxygenated to represent a suitable target for the majority of bioreductive drugs. Furthermore, TPZ enhances the toxicity of cisplatin *(94)*. It has achieved some success in clinical trials in combination with cisplatin *(95–98)*, and/or with radiotherapy *(99–102)* (*see* Table 3). The combination of TPZ and cisplatin was found to improve significantly median survival and response rate in a phase III trial of patients presenting with non-small cell lung cancer *(98)*. Further studies began in 2004 with this drug in combination with radiation and cisplatin in head and neck cancers *(102a)*. However, despite the success achieved to date in the clinic, there is evidence for dose-limiting toxicity and the development of analogs that may have a better therapeutic ratio is in progress *(6)*.

Aside from TPZ, we are aware of only one other bioreductive prodrug that underwent clinical trials in 2004, AQ4N. AQ4N is a di-N-oxide prodrug that is reduced under conditions of low-oxygen tension to form the active species, AQ4, an alkylaminoanthraquinone metabolite *(103)*. The process of futile cycling, in which the active drug is back-oxidized into its nontoxic form on the reintroduction of oxygen, typical of most bioreductive drugs, does not occur. Instead, oxygen completely inhibits the reducing enzyme, cytochrome P450 (isoform 3A; CYP3A), and once formed, AQ4 is highly stable. AQ4 is a DNA affinic, topoisomerase II poison and, as such, targets predominantly cycling cells, a potential drawback when targeting hypoxic tumor cells, which generally demonstrate substantially reduced proliferation rates. However, the stability of AQ4 enables cytotoxic targeting of transiently hypoxic tumor cells as they become oxic, thus facilitating an improved response when combined with fractionated radiotherapy, which can cause reoxygenation of hypoxic cells in tumors *(21)*. AQ4 is also able to diffuse into surrounding oxygenated tumor cells and elicit a cytotoxic response, thus causing a bystander effect *(6,104–107)*. Interim results have been released recently of a phase I clinical trial examining the safety of AQ4N in patients with advanced esophageal carcinoma undergoing palliative radiotherapy. The current results, in 13 of an anticipated 22 patients, are promising (KuDOS Pharmaceuticals 2004; http://www.kudospharma.co.uk/news/current_item.php?time=1092673109&page_id=44).

Table 3
Clinical Trials With Tirapazamine

	Treatment	*Tumor type*	*Patient number*	*Reference*
Phase I				
	TPZ and cyclophosphamide	Pediatric solid tumors	23	Aquino et al. 2004 *(133)*
		Advanced malignant solid tumors	28	Hoff et al. 2001 *(134)*
	TPZ, carboplatin, and paclitaxel	Advanced malignant solid tumors	42	Lara et al. 2003 *(135)*
	TPZ, cisplatin, and radiotherapy	Advanced head and neck carcinoma	16	Rischin et al. 2001 *(102)*
	TPZ and radiotherapy	Advanced malignant solid tumors	43	Shulman et al. 1999 *(136)*
	TPZ and cisplatin	Recurrent cervical carcinoma	12	Aghajanian et al. 1997 *(137)*
		Advanced malignant solid tumors	13	Johnson et al. 1997 *(95)*
	TPZ	Advanced malignant solid tumors	28	Senan et al. 1997 *(138)*
Phase I/II				
	TPZ, cisplatin, and radiotherapy	Locally advanced cervix carcinoma	15	Craighead et al. 2000 *(100)*
Phase II				
	TPZ and radiotherapy	Glioblastoma multiforme	124	Del Rowe et al. 2000 *(101)*
		Advanced head and neck carcinoma	40	Lee et al 1998 *(99)*
	TPZ and cisplatin	Malignant melanoma	48, 48	Bedikian et al. *1997*, 1999 *(97,139)*
				Richin et al. 2005 *(102a)*
		Advanced non-small cell lung carcinoma	44	Treat et al 1998 *(140)*
			20	Miller et al 1997 *(96)*
Phase III				
	TPZ and cisplatin	Non-small cell lung carcinoma	446	von Pawel et al. 2000 *(98)*

Phase I, II, and III clinical trials are indicated alongside tumor type and the patient numbers involved. TPZ, tirapazamine.

7.2. Gene-Directed Enzyme Prodrug Therapy

One limitation of bioreductive drug therapy is the requirement for specific reductase enzymes for cytotoxic activation *(88,90)*. The presence and level of reductase enzymes is highly heterogeneous in tumors, and thus, the efficiency of a drug will demonstrate a corresponding degree of tumor-specific variability. This has led to the development of gene-directed enzyme prodrug therapy approaches, which can be used to develop a further degree of selectivity through conferment of specificity in the expression of the drug metabolizing enzyme. This was originally demonstrated through the exploitation of the HIF-1/HRE system to drive the expression of the enzyme cytosine deaminase. This enzyme is required for reduction of the prodrug 5-fluorocytosine (5-FC) to its active form, 5-fluorouracil. Mammalian cells are resistant to 5-FC, because the enzyme cytosine deaminase is not produced at sufficiently high levels to elicit significant reduction of the drug. The use of HRE-driven expression of cytosine deaminase was shown to selectively sensitize hypoxic tumor cells to 5-FC in vitro *(108)*.

More recently, the generation of a tumor cell line stably expressing HRE-mediated cytochrome P450 reductase was used to demonstrate a 30-fold increase, both in vitro and in vivo, in the toxic effect of the bioreductive drug RSU1069 *(109)*, a 2-nitroimidazole that achieves toxicity under hypoxic conditions through an alkylating aziridine group as its active species *(110,111)*. A similar effect was observed in a separate study using the HIF-1/HRE system to drive expression of human P450 reductase in hypoxic tumor regions. To facilitate delivery of the gene to these regions, an adenoviral vector was generated, allowing infection of both dividing and quiescent tumor cells on intratumoral injection of the virus. Administration of this viral vector before combined TPZ and radiation treatment resulted in cure in 85% of treated mice, irrespective of tumor size on treatment, a significant improvement on TPZ and radiation treatment alone *(112)*. There are a substantial number of gene-directed enzyme prodrug therapy-based studies examining a variety of different reductive enzymes, prodrugs, and delivery methods; some promising examples are shown in Table 2. A key difficulty with regard to this type of approach is delivery of these vectors to all the tumor cells, particularly as regions of hypoxia are a requisite for activation. Thus, this strategy does not circumvent one of the problems tumor hypoxia presents for conventional therapeutic approaches, namely that hypoxic tumor cells are either distant from vasculature or proximal to faulty vasculature, limiting the diffusion of therapeutic agents.

7.3. Inhibition of HIF-1

Another therapeutic approach is to target HIF-1 specifically and thus reduce or eliminate its expression. Recent data have shown HIF-1 to be upregulated in response to radiation, potentiating endothelial cell radioresistance through upregulation of VEGF and bFGF *(113)*. There is also evidence to suggest that HIF-1 can render cells resistant to chemotherapy. Exposure of HIF-1α-positive (HIF-1α+/+$^{+/+}$) and negative (HIF-1α$^{-/-}$) murine embryonic fibroblast cell lines to two chemotherapeutic agents revealed a substantially lower IC_{50} (concentration that yields a 50% inhibition of growth) in the HIF-1α$^{-/-}$ line, an effect that was mimicked in vivo. There was an increased incidence of apoptosis in the HIF-1α$^{-/-}$ line, in accord with a decreased ability to repair double-stranded DNA breaks *(114)*.

One potential strategy to target HIF-1 is the use of small molecule inhibitors *(115,116)*. A high-throughput screen identified four potential small molecule inhibitors of HIF-1α,

and studies of one of these agents, the Camptothecin analogue, Topotecan, demonstrated a dose-dependent reduction in hypoxia-regulated expression of VEGF mRNA and protein *(115)*, and of HIF-1α protein *(117)*. Further studies showed the effect of Topotecan on inhibition of HIF-1α protein accumulation to occur at the translational level and to be dependent on the presence of topoisomerase I, the target of this drug. The exact mechanism remains unclear *(117)*.

Another small molecule inhibitor of HIF-1α, 3-(5′-hydroxymethyl-2′-furyl)-1-benzylindazole (YC-1), has been shown to delay tumor growth in vivo. YC-1 was originally developed to treat circulatory disorders and it inhibits platelet aggregation and vascular contraction through the activation of soluble guanylyl cyclase. YC-1 has also been found to completely inhibit HIF-1α at the posttranscriptional level. Similar to Topotecan, exposure to YC-1 under hypoxia in vitro was found to reduce HIF-1α protein, and HIF-1 regulated genes, in a dose-dependent manner in a selection of cell lines. Furthermore, treatment of tumors in vivo with YC-1 induced a growth delay. Immunohistochemical analysis demonstrated no HIF-1α staining in tumors from mice that had received YC-1, in contrast to the detection of HIF-1α in untreated tumors *(116)*. The investigators attributed the reduced growth of the tumors to the inhibition of angiogenesis, because there was reduced immunostaining for the endothelial marker CD31 and concordant reduction in VEGF protein expression.

The use of HIF-1 inhibitors is still relatively unexplored. Current data are promising and imply a potential role for the use of these drugs in cancer therapy *(7)*. It is of note that the small molecule inhibitors so far identified act to inhibit HIF-1 through indirect mechanisms, and as such may have effects distinct from those of HIF-1α inhibition. Also, HIF-1α protein levels, although principally regulated through hypoxia-inhibited proteasomal degradation, are also affected by growth factors and cytokines *(7)*, and by tumor suppressor mutations and oncogene activation *(71)*, both of which are common occurrences in solid tumors. Thus, therapeutics targeted at HIF-1α have the potential for a broader spectrum of efficacy than more specific hypoxia-targeted therapeutics. Direct inhibition of HIF-1α has been achieved experimentally through siRNA approaches or the use of dominant negative variants and antisense DNA (*see* Table 2), although the practicalities of such applications in the clinic require further study. A greater degree of specificity than that achieved with the small molecule inhibitors would allow for analysis of the specific role of HIF-1α *per se* in tumor progression, and thus the potential for subsequent development of more directed therapeutics.

8. SUMMARY

Tumor hypoxia results in resistance to common therapeutic regimens, and has also been shown to be associated with disease progression. Tumor hypoxia is extremely heterogeneous both spatially and temporally, and thus, the ability to assess the hypoxic status of tumors in individual patients and tailor treatment accordingly is desirable. Methods of measuring tumor hypoxia have been, and are being, developed with the eventual aim of measuring the overall hypoxic fraction, and preferably the nature of hypoxia (chronic vs transient), in all tumors, irrespective of location. Noninvasive methods are most desirable but are currently at an early stage of testing. Endogenous markers have the potential to indicate the nature of hypoxia, with the added benefit of allowing retrospective analysis of stored patient samples, a useful research tool. In line with this, the impact of tumor hypoxia on treatment outcome and disease progression is being

evaluated to improve prognosis, and in addition, drug development through improved understanding of the causal relationships. Finally, therapeutic methods designed to target hypoxic tumor cells are being explored for use in combination with conventional therapies to improve treatment response and reduce local failure and metastatic dissemination. At present, few of these approaches are in clinical trials. However, an increasing level of information on all aspects of tumor hypoxia is developing from recent research that should enable the development of improved therapeutic strategies that are more effective in their methods of delivery and targeting of hypoxia. Targeting tumor hypoxia is a dynamic and promising area of therapeutic study.

REFERENCES

1. Vaupel P, Kelleher DK, Höckel M. Oxygen status of malignant tumors: pathogenesis of hypoxia and significance for tumor therapy. Semin Oncol 2001; 28:29–35.
2. Subarsky P, Hill RP. The hypoxic tumour microenvironment and metastatic progression. Clin Exp Metastasis 2003; 20:237–250.
3. Le QT, Denko NC, Giaccia AJ. Hypoxic gene expression and metastasis. Cancer Metastasis Rev 2004; 23:293–310.
4. Bussink J, Kaanders JH, van der Kogel AJ. Tumor hypoxia at the micro-regional level: clinical relevance and predictive value of exogenous and endogenous hypoxic cell markers. Radiother Oncol 2003; 67:3–15.
5. Olive PL, Banath JP, Aquino-Parsons C. Measuring hypoxia in solid tumours—is there a gold standard? Acta Oncol 2001; 40:917–923.
6. Brown JM, Wilson WR. Exploiting tumour hypoxia in cancer treatment. Nat Rev Cancer 2004; 4:437–447.
7. Semenza GL. Targeting HIF-1 for cancer therapy. Nat Rev Cancer 2003; 3:721–732.
8. Dewhirst MW. Concepts of oxygen transport at the microcirculatory level. Semin Radiat Oncol 1998; 8:143–150.
9. Li CY, Shan S, Huang Q, et al. Initial stages of tumor cell-induced angiogenesis: evaluation via skin window chambers in rodent models. J Natl Cancer Inst 2000; 92:143–147.
10. Yancopoulos GD, Davis S, Gale NW, Rudge JS, Wiegand SJ, Holash J. Vascular-specific growth factors and blood vessel formation. Nature 2000; 407:242–248.
11. Vaupel P, Kallinowski F, Okunieff P. Blood flow, oxygen and nutrient supply, and metabolic microenvironment of human tumors: a review. Cancer Res 1989; 49:6449–6465.
12. Dewhirst MW, Ong ET, Braun RD, et al. Quantification of longitudinal tissue pO_2 gradients in window chamber tumours: impact on tumour hypoxia. Br J Cancer 1999; 79:1717–1722.
13. Baluk P, Morikawa S, Haskell A, Mancuso M, McDonald DM. Abnormalities of basement membrane on blood vessels and endothelial sprouts in tumors. Am J Pathol 2003; 163:1801–1815.
14. Thomlinson RH, Gray LH. The histological structure of some human lung cancers and the possible implications for radiotherapy. Br J Cancer 1955; 9:539–549.
15. Chaplin DJ, Olive PL, Durand RE. Intermittent blood flow in a murine tumor: radiobiological effects. Cancer Res 1987; 47:597–601.
16. Pigott KH, Hill SA, Chaplin DJ, Saunders MI. Microregional fluctuations in perfusion within human tumours detected using laser Doppler flowmetry. Radiother Oncol 1996; 40:45–50.
17. Li X, Brown SL, Hill RP. Factors influencing the thermosensitivity of two rodent tumors. Radiat Res 1992; 130:211–219.
18. 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 1996; 56:5522–5528.
19. Dewhirst MW, Braun RD, Lanzen JL. Temporal changes in pO_2 of R3230AC tumors in Fischer-344 rats. Int J Radiat Oncol Biol Phys 1998; 42:723–726.
20. Brurberg KG, Graff BA, Rofstad EK. Temporal heterogeneity in oxygen tension in human melanoma xenografts. Br J Cancer 2003; 89:350–356.
21. Hall EJ. Radiobiology for the Radiologist. Philadelphia: Lippincott Williams & Wilkins, 2000.
22. Horan AD, Koch CJ. The K_m for radiosensitization of human tumor cells by oxygen is much greater than 3 mmHg and is further increased by elevated levels of cysteine. Radiat Res 2001; 156:388–398.
23. Teicher BA. Hypoxia and drug resistance. Cancer Metastasis Rev 1994; 13:139–168.

24. Kallinowski F, Zander R, Hoeckel M, Vaupel P. Tumor tissue oxygenation as evaluated by computerized-pO_2-histography. Int J Radiat Oncol Biol Phys 1990; 19:953–961.
25. Milosevic M, Fyles A, Hedley D, Hill R. The human tumor microenvironment: Invasive (needle) measurement of oxygen and interstitial fluid pressure. Semin Radiat Oncol 2004; 14:249–258.
26. Evans SM, Koch CJ. Prognostic significance of tumor oxygenation in humans. Cancer Lett 2003; 195:1–16.
27. Nordsmark M, Loncaster J, Chou SC, et al. Invasive oxygen measurements and pimonidazole labeling in human cervix carcinoma. Int J Radiat Oncol Biol Phys 2001; 49:581–586.
28. Evans SM, Hahn S, Pook DR, et al. Detection of hypoxia in human squamous cell carcinoma by EF5 binding. Cancer Res 2000; 60:2018–2024.
29. Evans SM, Judy KD, Dunphy I, et al. Comparative measurements of hypoxia in human brain tumors using needle electrodes and EF5 binding. Cancer Res 2004; 64:1886–1892.
30. Kaanders JH, Wijffels KI, Marres HA, et al. Pimonidazole binding and tumor vascularity predict for treatment outcome in head and neck cancer. Cancer Res 2002; 62:7066–7074.
31. Semenza GL. Expression of hypoxia-inducible factor 1: mechanisms and consequences. Biochem Pharmacol 2000; 59:47–53.
32. Huang LE, Arany Z, Livingston DM, Bunn HF. Activation of hypoxia-inducible transcription factor depends primarily upon redox-sensitive stabilization of its alpha subunit. J Biol Chem 1996; 271:32,253–32,259.
33. Maxwell PH, Wiesener MS, Chang GW, et al. The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. Nature 1999; 399:271–275.
34. Zhong H, De Marzo AM, Laughner E, et al. Overexpression of hypoxia-inducible factor 1alpha in common human cancers and their metastases. Cancer Res 1999; 59:5830–5835.
35. Birner P, Schindl M, Obermair A, et al. Overexpression of hypoxia-inducible factor 1alpha is a marker for an unfavorable prognosis in early-stage invasive cervical cancer. Cancer Res 2000; 60:4693–4596.
36. Aebersold DM, Burri P, Beer KT, et al. Expression of hypoxia-inducible factor-1alpha: a novel predictive and prognostic parameter in the radiotherapy of oropharyngeal cancer. Cancer Res 2001; 61:2911–2916.
37. Chia SK, Wykoff CC, Watson PH, et al. Prognostic significance of a novel hypoxia-regulated marker, carbonic anhydrase IX, in invasive breast carcinoma. J Clin Oncol 2001; 19:3660–3688.
38. Koukourakis MI, Giatromanolaki A, Sivridis E, et al. Hypoxia-regulated carbonic anhydrase-9 (CA9) relates to poor vascularization and resistance of squamous cell head and neck cancer to chemoradiotherapy. Clin Cancer Res 2001; 7:3399–3403.
39. Loncaster JA, Harris AL, Davidson SE, et al. Carbonic anhydrase (CA IX) expression, a potential new intrinsic marker of hypoxia: correlations with tumor oxygen measurements and prognosis in locally advanced carcinoma of the cervix. Cancer Res 2001; 61:6394–6399.
40. Maseide K, Kandel RA, Bell RS, et al. Carbonic anhydrase IX as a marker for poor prognosis in soft tissue sarcoma. Clin Cancer Res 2004; 10:4464–4471.
41. Airley R, Loncaster J, Davidson S, et al. Glucose transporter glut-1 expression correlates with tumor hypoxia and predicts metastasis-free survival in advanced carcinoma of the cervix. Clin Cancer Res 2001; 7:928–934.
42. Beasley NJ, Leek R, Alam M, et al. Hypoxia-inducible factors HIF-1alpha and HIF-2alpha in head and neck cancer: relationship to tumor biology and treatment outcome in surgically resected patients. Cancer Res 2002; 62:2493–2497.
43. Bui MH, Seligson D, Han KR, et al. Carbonic anhydrase IX is an independent predictor of survival in advanced renal clear cell carcinoma: implications for prognosis and therapy. Clin Cancer Res 2003; 9:802–811.
44. Kaluz S, Kaluzova M, Chrastina A, et al. Lowered oxygen tension induces expression of the hypoxia marker MN/carbonic anhydrase IX in the absence of hypoxia-inducible factor 1 alpha stabilization: a role for phosphatidylinositol 3'-kinase. Cancer Res 2002; 62:4469–4477.
45. Maxwell PH, Dachs GU, Gleadle JM, et al. Hypoxia-inducible factor-1 modulates gene expression in solid tumors and influences both angiogenesis and tumor growth. Proc Natl Acad Sci U S A 1997; 94:8104–8109.
46. West CM, Jones T, Price P. The potential of positron-emission tomography to study anticancer-drug resistance. Nat Rev Cancer 2004; 4:457–469.
47. Koch CJ, Evans SM. Non-invasive PET and SPECT imaging of tissue hypoxia using isotopically labeled 2-nitroimidazoles. Adv Exp Med Biol 2003; 510:285–292.

48. Mazurchuk R, Zhou R, Straubinger RM, Chau RI, Grossman Z. Functional magnetic resonance (fMR) imaging of a rat brain tumor model: implications for evaluation of tumor microvasculature and therapeutic response. Magn Reson Imaging 1999; 17:537–548.
49. Fyles A, Milosevic M, Hedley D, et al. Tumor hypoxia has independent predictor impact only in patients with node-negative cervix cancer. J Clin Oncol 2002; 20:680–687.
50. Hockel M, Schlenger K, Mitze M, Schaffer U, Vaupel P. Hypoxia and radiation response in human tumors. Semin Radiat Oncol 1996; 6:3–9.
51. Rofstad EK, Sundfor K, Lyng H, Trope CG. Hypoxia-induced treatment failure in advanced squamous cell carcinoma of the uterine cervix is primarily due to hypoxia-induced radiation resistance rather than hypoxia-induced metastasis. Br J Cancer 2000; 83:354–359.
52. Brizel DM, Dodge RK, Clough RW, Dewhirst MW. Oxygenation of head and neck cancer: changes during radiotherapy and impact on treatment outcome. Radiother Oncol 1999; 53:113–117.
53. Nordsmark M, Overgaard M, Overgaard J. Pretreatment oxygenation predicts radiation response in advanced squamous cell carcinoma of the head and neck. Radiother Oncol 1996; 41:31–39.
54. Nordsmark M, Overgaard J. A confirmatory prognostic study on oxygenation status and loco-regional control in advanced head and neck squamous cell carcinoma treated by radiation therapy. Radiother Oncol 2000; 57:39–43.
55. Nordsmark M, Alsner J, Keller J, et al. Hypoxia in human soft tissue sarcomas: adverse impact on survival and no association with p53 mutations. Br J Cancer 2001; 84:1070–1075.
56. Brizel DM, Scully SP, Harrelson JM, et al. Tumor oxygenation predicts for the likelihood of distant metastases in human soft tissue sarcoma. Cancer Res 1996; 56:941–943.
57. Parker C, Milosevic M, Toi A, et al. Polarographic electrode study of tumor oxygenation in clinically localized prostate cancer. Int J Radiat Oncol Biol Phys 2004; 58:750–757.
58. Movsas B, Chapman JD, Hanlon AL, et al. Hypoxia in human prostate carcinoma: an Eppendorf pO_2 study. Am J Clin Oncol 2001; 24:458–461.
59. Movsas B, Chapman JD, Hanlon AL, et al. Hypoxic prostate/muscle pO_2 ratio predicts for biochemical failure in patients with prostate cancer: preliminary findings. Urology 2002; 60:634–639.
60. Young SD, Marshall RS, Hill RP. Hypoxia induces DNA overreplication and enhances metastatic potential of murine tumor cells. Proc Natl Acad Sci U S A 1988; 85:9533–9537.
61. De Jaeger K, Kavanagh MC, Hill RP. Relationship of hypoxia to metastatic ability in rodent tumours. Br J Cancer 2001; 84:1280–1285.
62. Buchler P, Reber HA, Lavey RS, et al. Tumor hypoxia correlates with metastatic tumor growth of pancreatic cancer in an orthotopic murine model. J Surg Res 2004; 120:295–303.
63. Rofstad EK, Danielsen T. Hypoxia-induced metastasis of human melanoma cells: involvement of vascular endothelial growth factor-mediated angiogenesis. Br J Cancer 1999; 80:1697–1707.
64. Cairns RA, Kalliomaki T, Hill RP. Acute (cyclic) hypoxia enhances spontaneous metastasis of KHT murine tumors. Cancer Res 2001; 61:8903–8908.
65. Cairns RA, Hill RP. Acute hypoxia enhances spontaneous lymph node metastasis in an orthotopic murine model of human cervical carcinoma. Cancer Res 2004; 64:2054–2061.
66. Rofstad EK. Microenvironment-induced cancer metastasis. Int J Radiat Biol 2000; 76:589–605.
67. Graeber TG, Osmanian C, Jacks T, et al. Hypoxia-mediated selection of cells with diminished apoptotic potential in solid tumours. Nature 1996; 379:88–91.
68. Zhang L, Hill RP. Hypoxia enhances metastatic efficiency by up regulating mdm2 in KHT cells and increasing resistance to apoptosis. Cancer Res 2004; 64:4180–4190.
69. Reynolds TY, Rockwell S, Glazer PM. Genetic instability induced by the tumor microenvironment. Cancer Res 1996; 56:5754–5757.
70. Mihaylova VT, Bindra RS, Yuan J, et al. Decreased expression of the DNA mismatch repair gene *Mlh1* under hypoxic stress in mammalian cells. Mol Cell Biol 2003; 23:3265–3573.
71. Maxwell PH, Pugh CW, Ratcliffe PJ. Activation of the HIF pathway in cancer. Curr Opin Genet Dev 2001; 11:293–299.
72. Huang LE, Gu J, Schau M, Bunn HF. Regulation of hypoxia-inducible factor 1alpha is mediated by an O_2-dependent degradation domain via the ubiquitin-proteasome pathway. Proc Natl Acad Sci USA 1998; 95:7987–7992.
73. Kallio PJ, Wilson WJ, O'Brien S, Makino Y, Poellinger L. Regulation of the hypoxia-inducible transcription factor 1alpha by the ubiquitin-proteasome pathway. J Biol Chem 1999; 274:6519–6525.
74. Salceda S, Caro J. Hypoxia-inducible factor 1alpha (HIF-1alpha) protein is rapidly degraded by the ubiquitin-proteasome system under normoxic conditions. Its stabilization by hypoxia depends on redox-induced changes. J Biol Chem 1997; 272:22,642–22,647.

75. Wang GL, Jiang BH, Rue EA, Semenza GL. Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O_2 tension. Proc Natl Acad Sci U S A 1995; 92:5510–5514.
76. Ohh M, Park CW, Ivan M, et al. Ubiquitination of hypoxia-inducible factor requires direct binding to the beta-domain of the von Hippel-Lindau protein. Nat Cell Biol 2000; 2:423–427.
77. Tanimoto K, Makino Y, Pereira T, Poellinger L. Mechanism of regulation of the hypoxia-inducible factor-1 alpha by the von Hippel-Lindau tumor suppressor protein. EMBO J 2000; 19:4298–4309.
78. Ivan M, Kondo K, Yang H, et al. HIFalpha targeted for VHL-mediated destruction by proline hydroxylation: implications for O_2 sensing. Science 2001; 292:464–468.
79. Jaakkola P, Mole DR, Tian YM, et al. Targeting of HIF-alpha to the von Hippel-Lindau ubiquitylation complex by O_2-regulated prolyl hydroxylation. Science 2001; 292:468–472.
80. Lando D, Gorman JJ, Whitelaw ML, Peet DJ. Oxygen-dependent regulation of hypoxia-inducible factors by prolyl and asparaginyl hydroxylation. Eur J Biochem 2003; 270:781–790.
81. Blancher C, Moore JW, Talks KL, Houlbrook S, Harris AL. Relationship of hypoxia-inducible factor (HIF)-1alpha and HIF-2alpha expression to vascular endothelial growth factor induction and hypoxia survival in human breast cancer cell lines. Cancer Res 2000; 60:7106–7113.
82. Bos R, van der Groep P, Greijer AE, et al. Levels of hypoxia-inducible factor-1alpha independently predict prognosis in patients with lymph node negative breast carcinoma. Cancer 2003; 97:1573–1581.
83. Koukourakis MI, Giatromanolaki A, Sivridis E, et al. Lactate dehydrogenase-5 (LDH-5) overexpression in non-small-cell lung cancer tissues is linked to tumour hypoxia, angiogenic factor production and poor prognosis. Br J Cancer 2003; 89:877–885.
84. Talks KL, Turley H, Gatter KC, et al. The expression and distribution of the hypoxia-inducible factors HIF-1alpha and HIF-2alpha in normal human tissues, cancers, and tumor-associated macrophages. Am J Pathol 2000; 157:411–421.
85. Kung AL, Wang S, Klco JM, Kaelin WG, Livingston DM. Suppression of tumor growth through disruption of hypoxia-inducible transcription. Nat Med 2000; 6:1335–1340.
86. Akakura N, Kobayashi M, Horiuchi I, et al. Constitutive expression of hypoxia-inducible factor-1alpha renders pancreatic cancer cells resistant to apoptosis induced by hypoxia and nutrient deprivation. Cancer Res 2001; 61:6548–6554.
87. Krishnamachary B, Berg-Dixon S, Kelly B, et al. Regulation of colon carcinoma cell invasion by hypoxia-inducible factor 1. Cancer Res 2003; 63:1138–1143.
88. Jaffar M, Williams KJ, Stratford IJ. Bioreductive and gene therapy approaches to hypoxic diseases. Adv Drug Deliv Rev 2001; 53:217–228.
89. Stratford IJ, Adams GE, Bremner JC, et al. Manipulation and exploitation of the tumour environment for therapeutic benefit. Int J Radiat Biol 1994; 65:85–94.
90. Stratford IJ, Williams KJ, Cowen RL, Jaffar M. Combining bioreductive drugs and radiation for the treatment of solid tumors. Semin Radiat Oncol 2003; 13:42–52.
91. Brown JM, Giaccia AJ. The unique physiology of solid tumors: opportunities (and problems) for cancer therapy. Cancer Res 1998; 58:1408–1416.
92. Koch CJ. Unusual oxygen concentration dependence of toxicity of SR-4233, a hypoxic cell toxin. Cancer Res 1993; 53:3992–3997.
93. Biedermann KA, Wang J, Graham RP, Brown JM. SR 4233 cytotoxicity and metabolism in DNA repair-competent and repair-deficient cell cultures. Br J Cancer 1991; 63:358–362.
94. Dorie MJ, Brown JM. Modification of the antitumor activity of chemotherapeutic drugs by the hypoxic cytotoxic agent tirapazamine. Cancer Chemother Pharmacol 1997; 39:361–366.
95. Johnson CA, Kilpatrick D, von Roemeling R, et al. Phase I trial of tirapazamine in combination with cisplatin in a single dose every 3 weeks in patients with solid tumors. J Clin Oncol 1997; 15:773–780.
96. Miller VA, Ng KK, Grant SC, et al. Phase II study of the combination of the novel bioreductive agent, tirapazamine, with cisplatin in patients with advanced non-small-cell lung cancer. Ann Oncol 1997; 8:1269–1271.
97. Bedikian AY, Legha SS, Eton O, et al. Phase II trial of tirapazamine combined with cisplatin in chemotherapy of advanced malignant melanoma. Ann Oncol 1997; 8:363–367.
98. von Pawel J, von Roemeling R, Gatzemeier U, et al. Tirapazamine plus cisplatin versus cisplatin in advanced non-small-cell lung cancer: A report of the international CATAPULT I study group. Cisplatin and Tirapazamine in Subjects with Advanced Previously Untreated Non-Small-Cell Lung Tumors. J Clin Oncol 2000; 18:1351–1359.
99. Lee DJ, Trotti A, Spencer S, et al. Concurrent tirapazamine and radiotherapy for advanced head and neck carcinomas: a Phase II study. Int J Radiat Oncol Biol Phys 1998; 42:811–815.

100. Craighead PS, Pearcey R, Stuart G. A phase I/II evaluation of tirapazamine administered intravenously concurrent with cisplatin and radiotherapy in women with locally advanced cervical cancer. Int J Radiat Oncol Biol Phys 2000; 48:791–795.
101. Del Rowe J, Scott C, Werner-Wasik M, et al. Single-arm, open-label phase II study of intravenously administered tirapazamine and radiation therapy for glioblastoma multiforme. J Clin Oncol 2000; 18:1254–1259.
102. Rischin D, Peters L, Hicks R, et al. Phase I trial of concurrent tirapazamine, cisplatin, and radiotherapy in patients with advanced head and neck cancer. J Clin Oncol 2001; 19:535–542.
102a. Rischin D, Peters L, Fisher R, et al. Tirapazamine, cisplatin, and radiation versus fluorouracil, cisplatin and radiation in patients with locally advanced head and neck cancer: a randomised phase II trial of the Trans-Tasman Radiation Oncology Group (TROG 98.02). J Clin Oncol 2005; 23(1):79–87.
103. Patterson LH. Bioreductively activated antitumor N-oxides: the case of AQ4N, a unique approach to hypoxia-activated cancer chemotherapy. Drug Metab Rev 2002; 34:581–592.
104. Hejmadi MV, McKeown SR, Friery OP, McIntyre IA, Patterson LH, Hirst DG. DNA damage following combination of radiation with the bioreductive drug AQ4N: possible selective toxicity to oxic and hypoxic tumour cells. Br J Cancer 1996; 73:499–505.
105. Loadman PM, Swaine DJ, Bibby MC, Welham KJ, Patterson LH. A preclinical pharmacokinetic study of the bioreductive drug AQ4N. Drug Metab Dispos 2001; 29:422–426.
106. Patterson LH, McKeown SR. AQ4N: a new approach to hypoxia-activated cancer chemotherapy. Br J Cancer 2000; 83:1589–1593.
107. McCarthy HO, Yakkundi A, McErlane V, et al. Bioreductive GDEPT using cytochrome P450 3A4 in combination with AQ4N. Cancer Gene Ther 2003; 10:40–48.
108. Dachs GU, Patterson AV, Firth JD, et al. Targeting gene expression to hypoxic tumor cells. Nat Med 1997; 3:515–520.
109. Patterson AV, Williams KJ, Cowen RL, et al. Oxygen-sensitive enzyme-prodrug gene therapy for the eradication of radiation-resistant solid tumours. Gene Ther 2002; 9:946–954.
110. O'Neill P, McNeil SS, Jenkins TC. Induction of DNA crosslinks in vitro upon reduction of the nitroimidazole-aziridines RSU-1069 and RSU-1131. Biochem Pharmacol 1987; 36:1787–1792.
111. Stratford IJ, Walling JM, Silver AR. The differential cytotoxicity of RSU 1069: cell survival studies indicating interaction with DNA as a possible mode of action. Br J Cancer 1986; 53:339–344.
112. Cowen RL, Williams KJ, Chinje EC, et al. Hypoxia targeted gene therapy to increase the efficacy of tirapazamine as an adjuvant to radiotherapy: reversing tumor radioresistance and effecting cure. Cancer Res 2004; 64:1396–1402.
113. Moeller BJ, Cao Y, Li CY, Dewhirst MW. Radiation activates HIF-1 to regulate vascular radiosensitivity in tumors: role of reoxygenation, free radicals, and stress granules. Cancer Cell 2004; 5:429–441.
114. Unruh A, Ressel A, Mohamed HG, et al. The hypoxia-inducible factor-1 alpha is a negative factor for tumor therapy. Oncogene 2003; 22:3213–3220.
115. Rapisarda A, Uranchimeg B, Scudiero DA, et al. Identification of small molecule inhibitors of hypoxia-inducible factor 1 transcriptional activation pathway. Cancer Res 2002; 62:4316–4324.
116. Yeo EJ, Chun YS, Cho YS, et al. YC-1: a potential anticancer drug targeting hypoxia-inducible factor 1. J Natl Cancer Inst 2003; 95:516–525.
117. Rapisarda A, Uranchimeg B, Sordet O, et al. Topoisomerase I-mediated inhibition of hypoxia-inducible factor 1: mechanism and therapeutic implications. Cancer Res 2004; 64:1475–1482.
118. Becker A, Hansgen G, Bloching M, et al. Oxygenation of squamous cell carcinoma of the head and neck: comparison of primary tumors, neck node metastases, and normal tissue. Int J Radiat Oncol Biol Phys 1998; 42:35–41.
119. Koong AC, Mehta VK, Le QT, et al. Pancreatic tumors show high levels of hypoxia. Int J Radiat Oncol Biol Phys 2000; 48:919–922.
120. Lyng H, Sundfor K, Rofstad EK. Oxygen tension in human tumours measured with polarographic needle electrodes and its relationship to vascular density, necrosis and hypoxia. Radiother Oncol 1997; 44:163–169.
121. Sundfor K, Lyng H, Trope CG, Rofstad EK. Treatment outcome in advanced squamous cell carcinoma of the uterine cervix: relationships to pretreatment tumor oxygenation and vascularization. Radiother Oncol 2000; 54:101–107.

122. Knocke TH, Weitmann HD, Feldmann HJ, Selzer E, Potter R. Intratumoral pO_2-measurements as predictive assay in the treatment of carcinoma of the uterine cervix. Radiother Oncol 1999; 53:99–104.
123. Hockel M, Schlenger K, Hockel S, Vaupel P. Hypoxic cervical cancers with low apoptotic index are highly aggressive. Cancer Res 1999; 59:4525–4528.
124. Rudat V, Stadler P, Becker A, et al. Predictive value of the tumor oxygenation by means of pO_2 histography in patients with advanced head and neck cancer. Strahlenther Onkol 2001; 177:462–468.
125. Rudat V, Vanselow B, Wollensack P, et al. Repeatability and prognostic impact of the pretreatment pO_2 histography in patients with advanced head and neck cancer. Radiother Oncol 2000; 57:31–37.
126. Stadler P, Becker A, Feldmann HJ, et al. Influence of the hypoxic subvolume on the survival of patients with head and neck cancer. Int J Radiat Oncol Biol Phys 1999; 44:749–754.
127. Brown JM. SR 4233 (tirapazamine): a new anticancer drug exploiting hypoxia in solid tumours. Br J Cancer 1993; 67:1163–1170.
128. Patterson LH, McKeown SR, Ruparelia K, et al. Enhancement of chemotherapy and radiotherapy of murine tumours by AQ4N, a bioreductively activated anti-tumour agent. Br J Cancer 2000; 82:1984–1990.
129. Papadopoulou MV, Bloomer WD. NLCQ-1 (NSC 709257): exploiting hypoxia with a weak DNA-intercalating bioreductive drug. Clin Cancer Res 2003; 9:5714–5720.
130. Chen J, Zhao S, Nakada K, et al. Dominant-negative hypoxia-inducible factor-1 alpha reduces tumorigenicity of pancreatic cancer cells through the suppression of glucose metabolism. Am J Pathol 2003; 162:1283–1291.
131. Sun X, Kanwar JR, Leung E, et al. Gene transfer of antisense hypoxia inducible factor-1 alpha enhances the therapeutic efficacy of cancer immunotherapy. Gene Ther 2001; 8:638–645.
132. Sun X, Kanwar JR, Leung E, Vale M, Krissansen GW. Regression of solid tumors by engineered overexpression of von Hippel-Lindau tumor suppressor protein and antisense hypoxia-inducible factor-1alpha. Gene Ther 2003; 10:2081–2089.
133. Aquino VM, Weitman SD, Winick NJ, et al. Phase I trial of tirapazamine and cyclophosphamide in children with refractory solid tumors: a pediatric oncology group study. J Clin Oncol 2004; 22:1413–1419.
134. Hoff PM, Saad ED, Ravandi-Kashani F, Czerny E, Pazdur R. Phase I trial of i.v. administered tirapazamine plus cyclophosphamide. Anticancer Drugs 2001; 12:499–503.
135. Lara PN, Jr., Frankel P, Mack PC, et al. Tirapazamine plus carboplatin and paclitaxel in advanced malignant solid tumors: a California cancer consortium phase I and molecular correlative study. Clin Cancer Res 2003; 9:4356–4362.
136. Shulman LN, Buswell L, Riese N, et al. Phase I trial of the hypoxic cell cytotoxin tirapazamine with concurrent radiation therapy in the treatment of refractory solid tumors. Int J Radiat Oncol Biol Phys 1999; 44:349–353.
137. Aghajanian C, Brown C, O'Flaherty C, et al. Phase I study of tirapazamine and cisplatin in patients with recurrent cervical cancer. Gynecol Oncol 1997; 67:127–130.
138. Senan S, Rampling R, Graham MA, et al. Phase I and pharmacokinetic study of tirapazamine (SR 4233) administered every three weeks. Clin Cancer Res 1997; 3:31–38.
139. Bedikian AY, Legha SS, Eton O, et al. Phase II trial of escalated dose of tirapazamine combined with cisplatin in advanced malignant melanoma. Anticancer Drugs 1999; 10:735–739.
140. Treat J, Johnson E, Langer C, et al. Tirapazamine with cisplatin in patients with advanced non-small-cell lung cancer: a phase II study. J Clin Oncol 1998; 16:3524–3527.