Proceedings of the Oxygen Homeostasis/Hypoxia Meeting

Bennett Kaufman,1 Ori Scharf,1 Jeffrey Arbeit,2 Margaret Ashcroft,3 J. Martin Brown,4 Richard K. Bruck,5 J. Donald Chaplin,6 Sydney M. Evans,7 Amato J. Giaccia,4 Adrian L. Harris,8 Eric Huang,9 Randall Johnson,18 William Kaelin, Jr,10 Cameron J. Koch,9 Patrick Maxwell,8 James Mitchell,9 Len Neckers,9 Garth Powis,11 Joseph Rajendran,12 Gregg L. Semenza,13 Jonathan Simons,14 Erik Storkebaum,15 Michael J. Welch,16 Murray Whitelaw,17 Giovanni Melillo,7 and S. Percy Ivy9

1TRI Inc., Rockville, Maryland; 2University of California, San Francisco, California; 3Institute for Cancer Research, London, United Kingdom; 4Stanford University, Stanford, California; 5University of Texas, Southwestern Medical Center, Dallas, Texas; 6Fox Chase Cancer Center, Philadelphia, Pennsylvania; 7University of Pennsylvania, Philadelphia, Pennsylvania; 8Oxford University, Oxford, United Kingdom; 9Inventigen Drug Branch, CTEP, DCTD, National Cancer Institute, Rockville, Maryland; 10Dana-Farber Cancer Institute, Boston, Massachusetts; 11University of Arizona, Tucson, Arizona; 12University of Washington, Seattle, Washington; 13Johns Hopkins University, Baltimore, Maryland; 14Winship Cancer Institute, Atlanta, Georgia; 15University of Leuven, Leuven, Belgium; 16Washington University, St. Louis, Missouri; 17Adelaide University, Adelaide, Australia; and 18University of California, San Diego, California

Abstract

The first Oxygen Homeostasis/Hypoxia Meeting was held on February 12, 2003, at the Sheraton National Hotel, Washington, D.C. The meeting was hosted by Drs. S. Percy Ivy and Giovanni Melillo of the National Cancer Institute, NCI. The purpose of the meeting was to stimulate collaborations among the participants who are engaged in different areas of hypoxia research and application, including basic research on hypoxia, and its induction and consequences; the development of drugs targeting hypoxia and factors involved in pathways leading to (or controlled by) hypoxia; and the development and application of hypoxia imaging technologies and reagents.

Introduction

Many human tumors contain a significant fraction of hypoxic cells, due to uncontrollable cell growth and insufficient vascularization (1–3). Oxygen tension measurements of several cancer types using the Eppendorf histograph electrode show a range of oxygen tension in the tumor tissues, which is significantly lower than the adjacent normal tissues (4–6). The reduction in oxygen tension within tumors confers resistance to both radiotherapy and chemotherapy, and in many cases correlates with poor prognosis (1, 3, 7–11).

Over the last several years, great progress has been made concerning our understanding of the molecular mechanism underlying hypoxia. Hypoxia-inducible factor 1 (HIF-1), a basic helix-loop-helix Per-Arnt-Sim transcription factor, has been identified as a master regulator of the transcriptional response to oxygen deprivation (reviewed in Ref. 12). HIF-1 is a heterodimer composed of α and β subunits, uniquely regulated by oxygen levels, and a β subunit, known as aryl hydrocarbon receptor nuclear translocator, constitutively expressed (13). Under hypoxic conditions, the α subunit is rapidly ubiquitinated and targeted for proteasomal degradation in a process involving hydroxylation of two prolyl residues, at positions 402 and 564, and recognition by the product of the von Hippel-Lindau (VHL) tumor suppressor gene (pVHL; Refs. 14, 15). Under hypoxic conditions, HIF-1α accumulates and in complex with HIF-1β targets the hypoxia-responsive element containing genes of which the products mediate angiogenesis; glucose metabolism; cell proliferation; and survival, migration, and invasion (12). Genetic abnormalities observed frequently in human cancers, including loss-of-function mutations (e.g., VHL, p53, and PTEN), are also associated with increased expression of HIF-1α and HIF-1-inducible genes (16–18).

The difference in oxygen tension in tumor compared with normal tissues may also be exploited for selectivity. Indeed, the presence of hypoxic regions in solid tumors may be exploited for the development of tumor-specific imaging agents, which may be of great benefit in visualizing tumors both diagnostically and for monitoring the results of tumor therapy. Several methodologies, such as magnetic resonance imaging (MRI), Overhauser-enhanced MRI, positron-emission tomography (PET; Ref. 19), and electron paramagnetic resonance, have been developed for imaging tumors, which may then be treated with hypoxia-selective drugs like tirapazamine (TPZ; Ref. 20).

HIF-1 in Hypoxia and Cancer. Dr. Amato Giaccia (Stanford University, Stanford, CA) discussed HIF-1α induction and expression, and the VHL tumor suppressor gene. HIF-1α protein is unstable at normal oxygen concentrations, and is stabilized at low oxygen tension (21). The predominant form of HIF-1 is a heterodimer of HIF-1α and HIF-1β, ultimately signaling downstream events resulting in gene transcription and cell growth. HIF expression can be regulated by both genetic (p53, VHL, factor-inhibiting HIF, and others) and pharmacological (topotecan, rapamycin, geldanamycins, and others) factors. It has been shown that merely inhibiting HIF-1 alone will not eradicate tumor growth. Screening the National Cancer Institute (NCI) database of 60 human cancer cell lines (NIH60) for VHL/HIF-1α-based cytotoxicity is one approach for discovering new drugs. Several drugs screened through the NIH60 cell line library showed cytotoxicity similar to topotecan against VHL-expressing cells (21–23).

Dr. Gregg Semenza (Johns Hopkins University, Baltimore, MD) discussed tumor hypoxia, and its association with increased invasiveness and metastasis, followed by treatment failure and subsequent patient mortality. HIF-1α activates >70 target genes, most of which are induced in a cell-type-specific manner, and, as such, differ among tumors. The genes targeted by HIF-1α produce proteins needed for angiogenesis, glucose transport and metabolism, cell survival (resistance to radiation, chemotherapeutics, and hypoxia), and invasion. Additionally, HIF-1α activity may also be induced by loss of tumor suppressor gene function and gain of oncogene function, because although induction of HIF-1α is due to intratumoral hypoxia in most cases, it is also induced by oxygen-independent mechanism(s) (12). Angiogenesis is activated by increased vascular endothelial growth factor (VEGF) expression mediated by HIF-1α through hypoxia, activation of...
tyrosine kinases, phosphatidylinositol 3'-kinase-AKT-mTOR, RAF-mitogen-activated protein/extracellular signal-regulated kinase extracellular signal-regulated kinase, prostaglandin E2, cytokines, Bcl-2 overexpression, or loss of function of tumor suppressors such as VHL, p53, PTEN, and others (Fig. 1). Therefore, agents inhibiting these factors, such as inhibitors of receptor tyrosine kinase, cyclooxygenase 2, heat shock protein (Hsp)90, mTOR, and mitogen-activated protein/extracellular signal-regulated kinase, can inhibit angiogenesis by interfering with HIF-1α activity (24–27).

Drs. Patrick Maxwell (Oxford University, Oxford, United Kingdom), Eric Huang (Adelaide University, Adelaide, Australia) described several aspects of HIF-1α structure, function, and regulation. Under normoxic conditions, HIF-1α degradation by the ubiquitin-proteasome pathway is controlled by an oxygen-dependent degradation domain (ODD) that enables an oxygen-dependent signal to effect degradation of the protein. Removal of the ODD stabilizes HIF-1α and prevents its degradation. Ubiquitination (and ultimately, degradation) of HIF-1α is VHL-dependent. Mutation analysis of the ODD region (amino acids 401–603) has shown that Leu-574 and Pro-564 are required for VHL-mediated HIF-1α degradation. Prolyl hydroxylases also facilitate the hydroxylation and subsequent ubiquitination of HIF-1α, and its degradation in the presence of VHL. Pro-564 in HIF-1α is hydroxylated by a prolyl hydroxylase enzyme, and pVHL preferentially binds the hydroxylated form of the HIF-1α proline. HIF-1α contains an activation domain in its COOH-terminal region (CAD), which, under hypoxic conditions, interacts with coactivators such as p300. This hypoxic activation can be blocked by hydroxylation of the asparagine (Asn) in position 803, inhibiting p300 association required for activation of HIF transcription.

The enzyme responsible for the Asn hydroxylation is Factor Inhibiting HIF-1 (FIH-1, an asparaginyl hydroxylase), located predominantly in the cytoplasm. Using point mutation experiments, it was shown that the valine in position 802 within the CAD region of the HIF-1α is essential for FIH-1 binding and activity (28–30). Hydroxylase activity is also increased in the presence of ascorbate and/or iron. Thus, oxygen, iron, and ascorbate can work through HIF prolyl/ asparaginyl hydroxylases to signal cellular oxygen sensors. It is believed that prolyl hydroxylases control stabilization of the HIF-1α/HIF-1β complex, whereas asparaginyl hydroxylases control its activation (31–35). Under hypoxic conditions, both p53 and p21 tumor suppressors are required for cell cycle arrest via a mechanism involving HIF-1α. ODD overexpression causes elevation in p21/p53 expression levels (36, 37).

Dr. Jonathan Simons (Winship Cancer Institute, Atlanta, GA) discussed clinical opportunities for using HIF as a biomarker of angiogenic switching and metastatic potential. HIF-1α was found to be expressed in 79 of 141 malignant primary tumors of different types, and 24 of 36 metastatic tumors, but in 0 of 12 benign tumor types. HIF-1α overexpression is an early event in transgenic prostate carcinogenesis, and the metastatic potential increases with increasing HIF-1α translocation into the nucleus. Efforts are being made toward new drug discovery, targeting HIF-1α in VHL+/- prostate cancer tumors. Prostate cancer cell growth can be triggered by epidermal growth factor receptor-mediated downstream signaling of HIF-1α induction through the phosphatidylinositol 3'-kinase pathway, and this may be targeted by use of nonsteroidal antiandrogens. The compound 2-methoxyestradiol has been shown to selectively inhibit nuclear HIF-1α (38, 39), and is now being used in Phase I and II clinical trials. Additional investigation is required to determine the mechanism of action of agents suppressing HIF-1α, and to discover new extra- and intracellular signaling agents.

**Targeting Hypoxia and Signaling.** Dr. William Kaelin, Jr. (Dana-Farber Cancer Institute, Boston, MA) discussed therapeutic opportunities. Inactivation of VHL results in tumor development, whereas adding the VHL protein causes inhibition of tumors (40). Suppression of HIF is necessary for tumor suppression by VHL, as HIF regulates growth factors such as VEGF, platelet-derived growth factor, and transforming growth factor α, which affect tumor growth (Fig. 1; 31–35).
Ref. 41). Sugen 5416 is a VEGF inhibitor and was shown to cause symptomatic improvement in VHL patients with hemangioblastomas without inducing tumor regression. RTK787 (a Kinase insert Domain Receptor+ platelet-derived growth factor receptor inhibitor) has been used in renal cancer, in which ~50% of the cells are VHL mutated. This treatment resulted in a drop in tumor blood flow and in tumor regression. HIF was found to accumulate in cells lacking VHL.

As noted above, the role of hypoxia in tumorigenesis can be mediated through its effects on oncogene expression. For instance, hypoxia activates transcription of the *met* proto-oncogene, and hypoxic areas of tumors overexpress Met tyrosine kinase, which sensitizes cells to the growth-stimulating effects of hepatocyte growth factor. Inhibition of Met expression prevents hypoxia-induced invasive growth (42). Dephosphorylated (but not phosphorylated) HIF-1α can bind to the tumor suppressor p53 and promote p53-dependent apoptosis. Depletion of dephosphorylated HIF-1α, such as by geldanamycin, can suppress p53 induction and subsequent apoptosis (43).

Dr. Margaret Ashcroft (Institute for Cancer Research, London, United Kingdom) expanded on the relationships between oncogene expression and hypoxia. HIF-1 activation can be facilitated by many factors in addition to hypoxia, such as growth factors, loss of tumor suppressor function, and activation by oncogenes such as Ras, Src, and Myc (44). In response to growth factors such as insulin-like growth factor-I, insulin, and heregulin, HIF-1α protein synthesis is increased (45, 46), although the precise mechanism for this remains unclear. Interestingly, the PI3K-Akt/protein kinase B signaling pathway has been shown to play a role in up-regulating HIF-1α expression in response to growth factors and oncogenic signals. Previous studies have shown that expression of a constitutively active form of Akt/protein kinase B enhances HIF-1α protein levels (17) via a VHL-independent mechanism (47, 48), and growth factor-mediated induction of HIF-1α can be blocked by the PI3K inhibitor, LY294002. The PI3K signaling pathway has a number of downstream targets (Fig. 1), including mTOR, which has been implicated in regulating HIF-1α expression (21, 49). A requirement for the PI3K signaling pathway in hypoxia-mediated induction of HIF-1α appears to be cell type specific and remains a matter for debate (50–52). However, recent studies have shown that the PI3K pathway is necessary for the induction of HIF-1α early in hypoxia (52), indicating that the kinetics of HIF-1α induction may be differentially regulated in response to different stimuli.

It is becoming clear that the pathways regulating hypoxia-mediated induction of HIF-1α are separable from those regulating growth factor-mediated induction of HIF-1α. A better understanding of how these pathways converge to affect HIF-1α activity overall in an abnormally growing tumor mass may help us determine how and when we could best inhibit HIF-1α activity therapeutically. Left unanswered are the questions regarding whether the mechanisms that regulate HIF-1α induction are separable from those that regulate HIF-1α activity. More specifically, are there additional biochemical modifications yet to be identified that correlate with HIF-1α activation? If so, it may be interesting to determine whether antibodies to such modifications could be exploited as a diagnostic tool for drug discovery.

Dr. Len Neckers (NCI) described the relationship between Hsp90 and HIF-1α (Fig. 1), and the connections to tumorigenesis (53). Hsp90 comprises 1–2% of total cell protein, and this increases under stress. Hsp90 is a chaperone protein and has a nuclear binding site, where it is associated with either ADP or ATP (54, 55). When bound to ADP, it recruits cochaperones, whereas association with ATP causes the release of these cochaperones and the binding of others. HIF-1α is a “client” protein of Hsp90, and binds to it in the presence of ADP and a set of cochaperones. The ADP configuration leads to ubiquitination and degradation of the HIF-1α. Hsp90 inhibitors (geldanamycins and radicicol) bind to the nucleotide pocket and allow Hsp90 to attain the conformation of ADP-bound. This binding has very high affinity that cannot be displaced by nucleotides. Thus, the cycle is blocked, and the Hsp90 is ubiquitinated and degraded. These inhibitors of Hsp90 promote VHL-independent degradation of HIF-1α. The binding of Hsp90 to HIF-1α is through the NH2 terminus of the HIF protein. Geldanamycin promotes the down-regulation of HIF-1α and inhibits its transcriptional activity (56, 57).

Dr. Garth Pows (University of Arizona, Tucson, AZ) discussed the potential of reducing the effects of HIF-1α expression by inhibiting one of its activators, thioredoxin (58). Thioredoxin reductase uses NADPH to oxidize protein cysteines. Thioredoxin is required for increased activation and protein expression of HIF-1α, as well as increased vascular density and VEGF expression (59). Compounds such as PX836 (which inhibits thioredoxin reductase) and PX12 (which inhibits the phosphorylated reductase) inhibit HIF and VEGF expression. These compounds do not affect the expression of HIF-1β. Another inhibitor, PX478, has shown 2 logs of cell kill of both PC3 prostate cancer cells and OVCAR-3 ovarian cancer cells *in vivo* by inhibiting HIF. In prostate cancer cells, response continued after cessation of treatment, whereas in the ovarian cell line the tumor grew back when drug treatment was discontinued.

Dr. Giovanni Melillo (NCI) discussed targeting the hypoxic tumor by the use of both selective and nonselective inhibitors. Selective inhibitors target protein-protein interaction, protein-DNA binding, and transcriptional activity, whereas nonselective ones affect downstream signaling and other indirect pathways. Nonselective agents include topoisomerase inhibitors, geldanamycins, rapamycin, mitogen-activated protein kinase inhibitors, radicicol, histone deacetylase inhibitors, thioredoxin inhibitors (such as described by Dr. Garth Pows), and, possibly, epidermal growth factor receptor inhibitors. Topotecan, for instance, inhibits HIF-1-dependent expression of a luciferase reporter gene and the accumulation of HIF-1α protein under hypoxic conditions, probably by a mechanism involving transcription-mediated DNA damage. It remains to be determined what the best target is for small-molecule selective (60) or nonselective agents, and how to develop and test those agents (61).

**Preclinical Models and the Role of HIF in the Inflammatory Process.** Dr. Erik Storkebaum (University of Leuven, Leuven, Belgium) presented the role of HIF-2α and VEGF in tumor vascularization and growth. This group created HIF-2α knockout (KO) mice, which show reduced vascular volume vessel diameter. However, tumors in HIF-2α KO mice grow faster, due to reduced apoptosis. This group also investigated the role of hypoxia and VEGF in motor neuron degeneration. When the HIF binding site is deleted from the VEGF gene (VEGFΔV), the resulting mice suffer from abnormal motor performance, similar to amytrophic lateral sclerosis. They hypothesize that in addition to its endothelial proliferative activity, VEGF also has a neuronal protective activity (62, 63). When crossing VEGFΔV with amytrophic lateral sclerosis mice, the survival decreased compared with amytrophic lateral sclerosis mice crossed with mice having normal VEGF. The VEGFΔV mice are also unusually sensitive to transient ischemic attacks, and mice that are HIF-1α−/− are more sensitive to spinal cord ischemia. These facts led the investigators to hypothesize that VEGF protects mice against spinal cord ischemic injury. The role of HIF-2α and VEGF in lung maturation was also investigated. There is 60% lethality in HIF-2α KO mice, and the neonatal mice that are carried to term succumb to respiratory distress. These mice have immature type 2 pneumocytes, causing a lack of surfactant production, resulting in a failure to lower surface tension, promoting alveolar collapse. HIF-2α KO mice have reduced
pulmonary VEGF expression as a result of the lack of HIF-2α, and this is believed to be the cause of the respiratory distress syndrome. In utero injection of VEGF was able to restore lung function and to promote lung ventilation.

Dr. Jeffrey Arbeit (University of California, San Francisco, CA) discussed the effects of HIF-1α gain-of-function in epithelium. Human wild-type HIF-1α or mutant HIF-1αΔODD (deletion of the oxygen-dependent degradation domain) constructs were targeted to basal keratinocytes (K14). K-14 HIF-1αΔODD transgenic mice showed redness and prominent vasculature of ear skin and roughness of coat, whereas K-14 HIF-1α transgenic mice were indistinguishable from nontransgenic controls (64). Histopathologic examination of the ears revealed an increase in the number of blood vessels in the dermis of K-14 HIF-1αΔODD transgenic mice, with no change in thickness, and no inflammation or edema. In addition, there was an increase in VEGF expression in these mice. Using 12-O-tetradecanoylphorbol-13-acetate in a two-stage skin carcinogenesis model it was shown that the K-14 HIF-1αΔODD transgenic mice were relatively unaffected, and developed papilloma much later than the nontransgenic mice. The tumors that developed were also different, with denser stroma in nontransgenic mice, compared with looser stroma, dilated blood vessels, and increased vessel size in the K-14 HIF-1αΔODD transgenic mice.

In common with hypoxic tumors, infected tissue, wounds, and rheumatic joints have lower oxygen concentration than healthy tissues, and are also infiltrated by leukocytes (65). Thus, HIF-1α plays a central role in the process of cell-mediated inflammation (66). Dr. Randall Johnson (University of California, San Diego, CA) described the use of HIF-1α KO mouse models. Hypoxic responses affect normal function of inflammation pathways. During tumorigenesis, hypoxic responses affect inflammation responses to the tumor. The glycolytic function is a central aspect of inflammation. Myeloid KO of HIF-1α shows no signs of inflammation, and in an arthritis model shows no swelling. VEGF myeloid deficiency has no effect on infiltration, but there is reduction in edema. VHL deficiency, however, shows hyper-inflammatory phenotype, massive edema, and infiltration. Hypoxia up-regulates glycolytic pathways, whereas loss of HIF-1α inhibits those pathways.

Therapeutics/Radiation Therapy (RT). Dr. Adrian Harris (Oxford University, Oxford, United Kingdom) described the use of carbonic anhydrase IX (CA9) as a hypoxia marker and target for therapy. CA9 is expressed in perinecrotic areas in breast cancer, and its expression increases with increasing distance from blood vessels and decreasing O2 concentration, and is associated with the hypoxic fraction as measured by Eppendorf electrode. CA9 expression correlates with decrease in survival in breast cancer patients, as well as poor prognosis as seen in other randomized trials. Tumors that were negative for HIF and CA9 expression (45 patients) had the best prognosis relative to tumors that were positive for HIF and negative for CA9 (38 patients; P = 0.04), tumors that were HIF negative and CA9 positive (37 patients; P = 0.22), and tumors that were positive for both HIF and CA9 (78 patients; P < 0.0001). CA9 could be useful as a predictor of prognosis and survival, and could be used as a basis for stratification in trials involving radiation, anthracyclines, or antiangiogenic and hypoxia-activated drugs, such as HIF antagonists. CA9 could also be a target of chemotherapy by treatment with CA9 inhibitors, or by using it as a target of immunotherapy. Additional electrophysiology studies are required to determine the mechanism of the regulatory processes controlling CA9 expression (67).

Dr. Sydney Evans (University of Pennsylvania, Philadelphia, PA) discussed the issue of methodological end points in clinical trials that evaluate hypoxia. The measurement of end points is complicated by multiple factors, such as the target and range of values to be measured, the area and level of hypoxic tissue, and the assay used. In a clinical trial in patients with glial tumors, 13 patients were administered 21 mg/kg fluorinated derivative of etanidazole (EF5) i.v. for analysis of tumor hypoxia, and 10 patients also had Eppendorf electrode studies. Measured parameters included EF5 binding, electrode measurements, recursive partitioning analysis classification, and time to recurrence. EF5 undergoes chemical reduction in cells, forming covalent adds with intracellular protein thiols. Drug reduction and binding is inhibited as oxygen concentration increases. The drug adds can then be detected by flow cytometry and/or fluorescence immunohistochemistry of frozen tissue sections. In all of the cases, EF5 provides quantitative assessment of tissue oxygenation in each specimen. Measurements were done using the relationship of in situ binding to identify patterns and/or levels of hypoxia in tumors and “cube reference” binding to measure maximal binding that the tissue is capable of, which is a reflection of reductive enzyme activity. The ratio of in situ binding to cube reference binding was used to report percentage of cube reference binding, which relates to mm Hg or percentage of oxygen. It was found that the majority of cells in human brain tumors are characterized by modest (2.5% O2) to physiological (>10% O2) oxia, and that time to recurrence can be predicted by EF5 binding: patients with EF5 binding less than the median had a longer time to recurrence than patients with EF5 binding greater than the median. The clinical course of this group of patients was found to be consistent with other studies in that recursive partitioning analysis classification was also predictive of time to recurrence. In summary, human glial tumors are characterized by considerable inter- and intratumoral heterogeneity; all of the tumors contain regions that are oxic, whereas some tumors contain severely hypoxic regions. The majority of cells in human glial tumors exhibit oxic to moderate levels of hypoxia. In the small group of patients studied, hypoxia was predictive of time to de novo glial tumor recurrence.

Dr. J. Martin Brown (Stanford University) discussed TPZ, which is the only purely hypoxic cytotoxin currently in the clinic. TPZ potentiates the efficacy of both fractionated irradiation and platinum-based chemotherapies given to mouse tumors and has shown activity in Phase III clinical trials with both radiotherapy and chemotherapy. However, its use in clinical trials is limited by toxicity. TPZ was shown to be preferentially toxic to hypoxic mouse squamous cell carcinoma VII cells in vitro by 100-fold under hypoxic conditions compared with normoxic conditions. In addition, TPZ was shown to potentiate multiafraction irradiation by growth delay assay, compared with TPZ or irradiation alone. Addition of a hypoxic cytotoxin to standard treatment (radiation or chemotherapeutic drug) exploits tumor hypoxia to achieve better survival. In a Phase II randomized trial in patients with stage III or IV head and neck squamous cell carcinoma, 123 patients were randomized into one of two arms. Patients in arm 1 received cisplatin (50 mg/m2) and 5-FU (360 mg/m2/day) on weeks 6 and 7, combined with radiation (70 Gy/35 Fx), whereas patients in arm 2 received a combination of TPZ/cisplatin and radiation (70 Gy/35 Fx). An interim analysis of the first 63 patients showed a nearly significant trend of superior results for the TPZ arm (P = 0.08). TPZ was also shown to potentiate cell killing by cisplatin in RIF-1 tumor in a schedule-dependent fashion, working better if given before cisplatin. No effect of TPZ on cisplatin toxicity was observed. In a Phase III randomized multicenter trial of TPZ with cisplatin in patients with advanced non-small-cell lung cancer (NSCLC), the investigators concluded that TPZ potentiated the anti-tumor efficacy of cisplatin in NSCLC without increasing its side effect. The mechanism of the selective hypoxic cytotoxicity of TPZ involves reduction of TPZ, yielding a radical that under hypoxic conditions is converted to an oxidizing radical, which causes double-strand DNA breaks by poisoning of topoisomerase II. Knowledge of
the radical mechanism is highly important, and facilitates the design of analogs with superior tumor penetration and activity (68–70).

**Imaging of Hypoxia.** Dr. J. Donald Chapman (Fox Chase Cancer Center, Philadelphia, PA) discussed the optimization of markers for tumor imaging. The classical technique for measurement of tissue oxygenation is the Eppendorf oxygen electrode. This technique has been used to show that prostate cancer patients with low tumor tissue oxygen levels have the worst prognosis (71). A number of noninvasive chemical markers of hypoxia are currently under development. Dr. Chapman has classified these into “flavors” by their mode of marking hypoxic tissues. “Vanilla” markers (e.g., MISO, 18F-labeled fluoromisonidazole, EF5, and IAZA) are those that selectively bind to viable hypoxic cells of low oxygen tension after the enzymatic activation of a bioreducible moiety (azomycin). “Chocolate” markers (e.g., [99mTc]HL-91 and [Cu]ATSM) comprise ligands, which chelate radiolabeled metals and selectively deposit in hypoxic tissues, and “chocolate swirl” markers (e.g., BMS-181321 and azomycin-cyclams [FC-334]) are those that contain both a bioreducible moiety and a ligand that chelates a radiolabeled metal. Several markers of the various classes were evaluated by kinetic studies of uptake in tumor cells in vitro. A hypoxia-specific factor was defined as the ratio of marker binding rate to, or uptake into, severely hypoxic cells compared with aerobic cells (larger is better). When tested in mouse EMT-6 and human DU-145 tumor cells, β-t-IAZGP was found to be the optimal “vanilla” marker and can be used to visualize hypoxic tumor blood volume by single-photon emission computed tomography and positron-emission tomography (PET) when labeled with 123I and 124I, respectively, whereas diazoxymycin-cyclam (FC-334) was the optimal “chocolate swirl” marker and should be evaluated for its ability to visualize hypoxic tumor volume by single-photon emission computed tomography and PET when labeled with 60Cu and 64Cu, respectively. Marker sensitivity for the noninvasive visualization of hypoxic microregions in solid tumors is governed by the pharmacokinetics of the radiolabeled agent.

Dr. Joseph Rajendran (University of Washington, Seattle, WA) described the application of nitroimidazoles in imaging of hypoxia. Hypoxic tumors are imaged through the use of 18F-labeled fluoromisonidazole followed by PET. Normal ratio of label uptake in tissue versus blood is 1, whereas a ratio ≥ 1.2 is indicative of hypoxia. Studies in head and neck and brain cancers clearly demonstrate the differences between pretreatment images, in which the tissue versus blood ratio is 1.4–1.5, and post-treatment images, where the ratios are ~1.1. 18F-labeled fluoromisonidazole-PET has several beneficial characteristics: the entire tumor is imaged (primary tumor and affected nodes), so geographic localization of hypoxia can be determined, quantification is possible, and serial studies can be done to gather both spatial and temporal information. Also, computed tomography images can be fused with 18F-labeled fluoromisonidazole images to precisely locate hypoxic sub-volume. Hypoxic volume determinations can serve as a prognosticator: patients with larger hypoxic volumes and greater ratios of hypoxic volume to total tumor volume are at higher risk, and display a significantly lower probability of survival. The advantages of hypoxia-directed radiotherapy are the feasibility of dose escalation and the improved cost-benefit ratio.

Dr. James Mitchell (NCI) discussed the application of MRI techniques to the determination of tissue oxygen and redox status. A hypoxia-imaging technique derived from electron paramagnetic resonance imaging, which images free radicals, is under development (72). This technique, Overhauser-enhanced MRI, uses a low-strength magnetic field, is a hybrid between electron paramagnetic resonance imaging and MRI, and uses trityl-free radical paramagnetic contrast agents. Tissue oxygen tension can be quantitated with electron paramagnetic resonance imaging, as the spin probe line width increases with increasing oxygen pressure. Likewise, tissue oxygenation can be visualized with Overhauser-enhanced MRI, with color intensity being proportional to oxygenation. This imaging correlates well with oxygen electrode measurements.

Dr. Cameron J. Koch (University of Pennsylvania) discussed the development of hypoxia marker drugs, which includes the determination of the drug binding characteristics, its pharmacokinetics, its ability to mark oxygen distribution in tissues, and validation of the assay used for its detection/binding (73). The most useful drugs will have uniform tissue accessibility (i.e., are lipophilic), have a simple pharmacology, show renal clearance, are stable in vivo, have a wide range of oxygen sensitivity, and are able to be detected in a time-independent fashion by multiple modalities. One drug meeting these criteria is the fluorinated derivative of etanidazole, EF5. EF5 is administered i.v., and adducts are detected, after clearance, by monoclonal antibodies in tissue biopsies or by flow cytometry in cell suspensions (74–76). EF5 shows uniform biodistribution in animals and humans (19, 77). In human cancer cells, EF5 displays 1% binding at a pO2 of 10%, increasing to 30% binding at a pO2 of 0.1%. The most commonly used isotopes for noninvasive imaging are 18F, 60/64Cu, 124I, and 123I, and 99mTc. Imaging of 18F-EF5 is readily obtained through PET. One unique advantage of EF5 is that by replacing one fluoride with [F-18], the same agent can be imaged using PET. Tumor tissue may be imaged in situ by PET after 2–3 h (19). 18F-EF5 allows good visualization of even small hypoxic tumors. Gastrointestinal uptake is minor, and urinary excretion can be handled readily by patients (78).

Dr. Michael J. Welch (Washington University, St. Louis, MO) discussed the application of copper-based compounds in imaging, specifically, using 60Cu-labeled diacetyl-bis[N4-methylthiiosemicarbazone; Ref. 79]. This copper adduct is thought to be trapped within a hypoxic cell, but is not trapped in a normoxic environment, and can be visualized by PET. Clinical studies using 60Cu-labeled diacetyl-bis[N4-methylthiiosemicarbazone] have been conducted in lung, cervical, head and neck, brain, and rectal cancers, NSCLC, and in ischemic myocardium. Dr. Welch described the results of two studies conducted in NSCLC and in cervical cancer. The NSCLC study enrolled 19 patients, 11 received RT alone, 5 received RT and chemotherapy, and 3 received chemotherapy alone. One patient showed no 60Cu-labeled diacetyl-bis[N4-methylthiiosemicarbazone] uptake. Among 14 evaluable patients, there were 8 responders (5 CR and 3 PR), and 6 nonresponders. Responders showed a pretherapy tumor: muscle ratio (T:M) of 1.5 ± 0.4, whereas nonresponders showed a T:M of 3.4 ± 0.8 (P < 0.0001). Overall survival was ~450 days for patients with a T:M ≥ 3 and >1300 days for those with T:M < 3. In the study including 14 patients with cervical cancer, 1 patient received RT alone and 13 received both RT and chemotherapy. No uptake was seen in 1 patient. The T:M was determined in these patients pre- and post-therapy, and correlated with outcome. Eight of 9 patients with a pretherapy T:M < 3.5 are currently disease-free (current T:M 1.9 ± 0.6), whereas 4 of 5 patients with T:M > 3.5 have developed a tumor recurrence (T:M 5.9 ± 3.6). Disease-free survival in patients with a T:M > 3.5 was ~175 days versus >350 days for those with a T:M < 3.5.

**Summary and Perspective.** Dr. Richard K. Bruick (University of Texas, Southwestern Medical Center, Dallas, TX) summarized many of the questions in the area of hypoxia research. Of great importance is the question of whether HIF-1 is indeed a superior target, and whether or not it is a good hypoxia marker. HIF-1 targeting can be achieved either by direct HIF-1 suppression or cytotoxic killing of HIF-1-expressing cells. The potential side effects for either strategy and the therapeutic window for a HIF-1-based approach were dis-
cussed. The structure/function differences among HIF-1α, HIF-2α, and HIF-3α need to be delineated. The consequences of hypoxia that are not mediated by HIF, the best model systems and assay techniques that will aid development of therapeutic drugs, and the best ways to move these ideas into a clinical setting, are other important questions.

To facilitate the translation of these concepts to the clinic, the Developmental Therapeutics Program staff of the NCI and the imaging group within CTEP discussed the Rapid Access to Intervention Development program, as well as other development programs within the Institute. Dr. Ivey expressed the hope that investigators in the different areas of hypoxia research, laboratory and clinical, would work together to put this area of common interest on a well-established footing, especially in terms of clinical development.

Novel targets (e.g., HIF-1) and novel agents, emerging from a very active discovery effort, need to be evaluated in appropriate preclinical models, including evaluation of relevant molecular end points. The role of novel molecular noninvasive imaging technologies should be emphasized for their potential translation to the clinical setting. Many questions remain to be answered in the clinical development of HIF-1/hypoxia-targeting agents. Are these agents going to have cytostatic or cytotoxic activity? Are they going to be active as single agents or will combination with conventional therapies be required? What is the potential interaction with antiangiogenic therapies? A rational preclinical development, patient selection, and well-designed early clinical trials will ultimately determine the utility of these potentially useful novel approaches.

References

34. Huang LE, Gu J, Schau M, Bunn HF. Regulation of hypoxia-inducible factor 1α is mediated by an O2-dependent degradation domain via the ubiquitin-proteasome pathway. Proc Natl Acad Sci USA 1998;95:7987–92.
35. Huang LE, Bunn HF. Hypoxia-inducible factor and its biologic relevance. J Biol Chem 2003;278:19575–60.
PROCEEDINGS OF THE OXYGEN HOMEOSTASIS/HYPOXIA MEETING


Downloaded from cancerres.aacrjournals.org on April 15, 2017. © 2004 American Association for Cancer Research.
Proceedings of the Oxygen Homeostasis/Hypoxia Meeting

Bennett Kaufman, Orit Scharf, Jeffrey Arbeit, et al.

Cancer Res 2004;64:3350-3356.

Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/64/9/3350

Cited articles
This article cites 77 articles, 35 of which you can access for free at:
http://cancerres.aacrjournals.org/content/64/9/3350.full.html#ref-list-1

Citing articles
This article has been cited by 5 HighWire-hosted articles. Access the articles at:
/content/64/9/3350.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.