Published in final edited form as:

Semin Oncol. 2008 June ; 35(3): 262–273. doi:10.1053/j.seminoncol.2008.03.001.

Novel Imaging Approaches to Head and Neck Cancer Seminars in Oncology

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Abstract

An inadequate supply of oxygen, hypoxia, is an important factor contributing to resistance to treatment in a number of tumor types, including head and neck cancer. Novel imaging methods have been applied to studies of this important prognostic factor. Mammalian cells need oxygen to live but O₂ also participates in the cytotoxic effects of ionizing radiation. Hypoxia is often the result of abnormal blood vessels supplying the tumor, increased diffusion distances to tumor cells, and reduced O₂ transport capacity of the blood. Its consequences are mediated by a series of hypoxia-initiated genomic changes activating angiogenesis, glycolysis and other processes that enable tumor cells to survive or escape the O₂-deficient environment. Hypoxia has been shown to be important in overall diminished therapeutic response, malignant progression, increased probability of recurrence, loco-regional spread and distant metastases. Strategies are being developed to surmount the cure-limiting consequences of hypoxia, but methods are needed to select patients most likely to benefit from these new treatments. Even though hypoxia is a common tumor phenotype, it is by no means universal and is often heterogeneous within an individual patient. This review considers the biology of hypoxia, its consequences with respect to treatment, methods for measuring oxygenation in tissues, modern techniques for imaging of regional hypoxia and how information about the oxygenation status of tumors might impact treatment.

Biology of Hypoxia

Cancers are not just clonal populations of cells gone awry. They are complex multicellular tissues in which individual cells respond to intercellular signals, their microenvironment, and the therapies directed against them. Cellular heterogeneity of solid tumors involves a mixture of malignant cells, stromal elements, infiltrating inflammatory (defensive) cells and necrotic areas. Heterogeneity in solid tumors includes a loss in the hierarchical organization of the vasculature, including a loss of capillary endothelium, increased vessel permeability, stasis, shunting and even reversal of flow (1). The heterogeneous microenvironment is being increasingly appreciated as a particularly strong force in driving clonal selection of developing tumors (2). Two critical distinctions between the tumor's microenvironment and that of normal

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tissues are important to this review—the reduction in the level of oxygen, PO_2 , and lower pH, both intracellular and extracellular, pH_i and pH_e . For normal cells, hypoxia and acidosis are major obstacles that generally lead to apoptosis. In tumors the consequences can be quite different, involving a series of adaptive responses that allow for survival and continued proliferation. Furthermore, there is a mechanistic coupling between these two parameters, hypoxia and acidosis, although acidosis is not a valid surrogate for hypoxia.

Gray proposed in 1953 that hypoxic cells were resistant to radiation therapy (3). He interpreted this effect as reflecting the requirement for O_2 as a source of radicals induced by radiation that mediate cell killing. Overcoming the cure-limiting effects of hypoxia has motivated much of research in radiation biology over several decades and has led to numerous approaches to increase oxygenation, albeit with minimal clinical advantage (4).

Although only appreciated in the last decade, hypoxia is an important selection force in the evolution of many cancers (5). It is the primary stimulus for such important aspects of the tumor phenotype as angiogenesis, improved O_2 transport, upregulation of growth factors and other proteins involved in tumor migration. A mechanistic connection between radiation response and the tumor's microvasculature has now developed (6,7). The vasculature is an important target of radiation therapy, as described later.

Cells sense the level of oxygen through the actions of hypoxia-inducible factor (HIF). The HIF family of transcription factors is regulated at the post-translational level through stabilization of the O_2 -labile α subunit. In well oxygenated tissues, HIF-1 α is hydroxylated through proline hydroxylase enzymes and O_2 . Once it is hydroxylated, it is ubiquitinated and targeted to the proteasome for degradation. Under hypoxic conditions this reaction is inhibited and residual HIF-1 α dimerizes with its binding partner, HIF-1 β , and this heterodimer enhances the transcription of a unique group of target genes that promote adaptation to the low-oxygen environment (8).

Many downstream effects of hypoxia are regulated through HIF-1, a transcriptional regulator of more than 70 genes, including ones that switch energy metabolism from oxidative to glycolytic, others that promote angiogenesis and still others that generally increase the survival of the cancer cell through secondary effects that favor cellular migration, invasion and metastasis and promote hemostasis. The latter are particularly important in influencing response to treatment because cancers kill patients through invasion and metastasis.

The role of HIF-1 in modifying energy metabolism is perhaps most well understood. Warburg first observed that tumors exhibit increased glycolytic flux, even under well-oxygenated conditions, and that they export lactate (9). This pioneering observation is a consequence of proteins coded for by the actions of HIF-1. It induces expression of genes that code for glucose transporters (GLUT 1/3) and glycolytic enzymes (HK 1/2) as well as pyruvate dehydrogenase which inactivates the conversion of pyruvate to acetyl CoA and upregulates lactate dehydrogenase (LDH) which is involved in the interconversion of pyruvate to lactate. There is some evidence that it may even lead to decreased activity of electron transport enzymes (10). Pyruvate increases the amount and activity of HIF-1 α by interference with its proteasomal degradation. Due to LDH-coupling, lactate may play an active role in tumor progression. Mueller-Kleiser's lab was able to demonstrate that exogenous lactate and pyruvate increased cellular motility in an in vitro migration assay and they suggest that this mechanism may contribute to metastatic spread of tumors (11).

HIF-1 regulates a gene that codes for carbonic anhydrase IX, CA9, a novel membrane bound enzyme on tumors that regulates acid-base homeostasis. Cancer cells produce large amounts of free protons, H^+ , from hydrolysis of CO_2 and from lactate as a product of glycolysis. CA9 helps export these excess protons to the extracellular milieu. The acid environment protonates

some chemotherapy drugs and leads to poor absorption of otherwise lipophilic drugs such as doxorubicin.

HIF-1 is involved in several ways to support migration and invasion (12). Cell-to-cell adhesion is decreased when E-cadherin is repressed as a result of HIF-1 and this is an important initial step in letting tumor cells break away from their primary site. To the extent that HIF-1 promotes hypervascularization in tumors, it provides a conduit for break-away cells to migrate. HIF-1 also regulates p53 in a way that provides protection against apoptosis. It also activates genes involved in hemostasis, such as MET, which supports tumor seeding and nutrition for new colonies

Evidence is developing that HIF-1 also plays a role in modulating inflammation and immunity. In fact, strategies to increase HIF-1 expression have been suggested as a way to enhance anti-inflammatory pathways. Recent data have clarified the mechanism by which hypoxia promotes the evasion of cancer cells from the immune system through inactivation of anti-tumor T cells secondary to altered metabolism of adenosine (13).

In summary, HIF-1 plays an activating role in the transcription of genes that encode proteins that play critical roles in all aspects to the tumor's growth and spread. Oncogenes and tumor suppressor genes may be the cause of cancer but hypoxia promotes the survival of the cancer cell and is thus an important factor in planning treatment strategies. The reader interested in a more complete review of current perspectives on hypoxia and cancer is referred to the June 2007 special issue of *Cancer and Metastasis Reviews*. The 12 articles in this issue provide considerable detail on the concepts summarized above.

Measuring Tissue Oxygenation with Electrodes

Numerous strategies have been advocated to assay for hypoxia. One of the more widely advocated methods uses miniature O₂ electrodes inserted under CT guidance to measure the regional partial pressure of oxygen, PO₂ (14,15). A commercial device for this assay was developed by Eppendorf (Hamburg, Germany) and is called the PO₂ histograph. Nominally 12 micron electrodes are inside 0.3 mm diameter needles. If the electrodes are calibrated prior to use, this device can be used to generate a histogram of the O₂ partial pressure (mm Hg) and a total of 50 to 100 readings along 5 or 6 electrode tracks can be used to characterize a tumor. Because this instrument can be calibrated in verifiable physical units; its advocates often referred to this technique as a gold standard for hypoxia, although it is more accurately a gold standard for PO₂ at the location of the electrode. Oxygen electrodes are useful tools, but they remain technically challenging because of the need for regular calibration and the OR time, anesthesia and CT requirements. Furthermore, this technique is invasive and can only be readily applied to tumors accessible from the body surface. However, our experience has been that patients are generally willing to undergo this research test. The PO₂ histogram does not distinguish between viable hypoxic cells, nonhypoxic cells, blood vessels and stroma.

Before we accept the Eppendorf PO₂ Histogram as the gold standard for assaying hypoxia, it's useful to consider how the hypoxia information might be used in making clinical decisions. The extent to which hypoxia is measured in the entirety of the tumor and the way it is quantified are both important and may vary for different clinical scenarios. If the goal is selection of one of two treatment strategies, then exact quantification may not be essential and a simple biomarker from a biopsy or even serum could suffice. For example, although we know that on average concomitant chemoradiation regimens result in higher rates of laryngeal preservation, we do not yet have predictors of successful treatment in individual patients (16,17). Hypoxia markers strongly correlated with chemoradiation failure could help identify the subset of patients who could benefit from surgically-based regimens, along the lines of NIH's goal of developing personalized medicine. Other objectives could require quantification of hypoxia,

for example when a tumor is being manipulated to cause reoxygenation or when the clinical objective is a pharmacodynamic assessment of oxygenation status in response to therapy. Still other uses would require a more anatomically exact map of the spatial distribution of hypoxia. The obvious example here is in radiation treatment planning. Image guided radiation therapy (IGRT) could use a three-dimensional map of hypoxia to plan a radiation boost to hypoxic regions in anticipation of achieving better local control of a tumor. Therefore, some treatment decisions can be informed by only the presence or absence of a marker, others might benefit from quantification of oxygen tension and still others could require a complete map of the geography of the hypoxia.

Descriptions of the degree of hypoxia may go one step farther to ask how much of the tumor volume is hypoxic or what is the maximum level of hypoxia in the tumor. Hypoxia images could be characterized in two ways: by volume or fractional hypoxic volume and by the magnitude of the most hypoxic region. We do not yet know which will be more clinically useful. Furthermore, this analysis will be confounded by an anticipated high covariance between the two hypoxia variables. Every detection system integrates data over some volume of interest, the volume of an imaging pixel or the volume of tissue from which O_2 is collected by the electrode. These volumes always involve many cells. Any map of hypoxia has both peak regions of severe hypoxia sufficient to induce genomic changes and valleys of necrosis, dead tissue that, while not oxygenated, should not be confused with hypoxic but potentially viable (quiescent) cells.

Identifying the region with the highest “hypoxic” signal may be the easier of these two variables to quantify. Identifying the volume of the tumor that is hypoxic presents the challenge of setting a threshold between the normal tissue signal and the hypoxic signal and this presents the challenge that PO_2 is a continuous variable. Normal muscle values are in the range of 25–45 mm Hg but in the working myocardium values that transiently reach as low as 15 mm Hg can be tolerated without triggering the oxygen response element and leading to accumulation of HIF-1 (5). For tumors the PO_2 histogram data are often analyzed with 7–10 mm Hg as the cutoff between normoxia and hypoxia, independent of the tumor type (15). To the best of our knowledge, a receiver operating characteristic function to objectively choose the critical value has not been reported and the electrodes’ accuracy drops at low PO_2 values because the electrodes consume a finite amount of O_2 to generate a signal.

Measuring Regional Tissue Oxygenation by Imaging

Even though hypoxia is a common tumor phenotype, it is by no means universal and is often heterogeneous within an individual patient. Hypoxia is an important factor contributing to resistance to treatment in a number of tumor types, including head and neck cancer. Treatment strategies are being developed and tested to surmount the cure-limiting consequences of hypoxia, but methods are needed to select patients most likely to benefit from these new treatment strategies.

Our own research program has focused on positron emission tomography to image hypoxia. The first attempt to image hypoxia was reported by Beaney who used $[^{15}O]$ - O_2 imaging in human breast tumors (18). This early report measured the metabolic rate of O_2 in the tumors as well as the oxygen extraction fraction. Normal tumors regulate blood flow and O_2 extraction fraction (OEF) to maintain adequate oxygenation; for example, in stroke oxygen extraction is acutely increased to compensate for reduced flow. Tumors generally lack the regulatory machinery in the capillaries to increase OEF in the presence of hypoxia because of their inadequate and heterogeneous vascular network that is often lacking in receptors that regulate flow, vascular compliance and extraction. In addition hemoglobin levels may be low (< 10 g/

dL) as a consequence of the disease or secondary to treatment (19). Thus the [^{15}O]- O_2 PET approach was not useful for assessing hypoxia and has not been developed.

The imaging objective is to generate a positive picture of what is missing, namely O_2 . This would seem to be a challenging goal but Don Chapman realized that a radiolabeled version of the oxygen-mimetic drugs that the radiation oncology community has tested as radiosensitizers may be useful. They are slowly accumulated in cells that had oxygen levels in the range where the oxygen enhancement ratio was falling from a normoxic value of about three to nothing greater than unity. This critical observation led him to propose a radiolabeled version of this class of drugs as useful for imaging hypoxia (20).

What would be the characteristics of the ideal hypoxia imaging agent? The first is simple; an agent that showed regions of hypoxia as an increased signal over normoxic regions. The second is practical; something that would be simple to prepare, potentially marketable, and therefore with a half life long enough to deliver but short enough to allow minimal radiation absorbed dose. The imaging agent would need to be stable in the vial and while it is circulating in the blood. It should only be metabolized and trapped in hypoxic cells. Additional characteristics are more subtle. Its imaging signal should not reflect delivery because blood flow is often compromised in tumors. Also, it should directly reflect O_2 concentration in the cell at the time it is administered and it should only concentrate in viable cells, not in the necrotic core of a tumor.

The most widely used radiopharmaceutical that meets these criteria, albeit with some compromises, is 1H-1-(3-[^{18}F]-fluoro-2-hydroxy-propyl)-2-nitro-imidazole, commonly referred to as [^{18}F]-fluoromisonidazole or $^{18}\text{FMISO}$. For a historical review of this radiopharmaceutical the reader is referred to reference (21). ^{18}F decays by positron emission with a nuclear decay half-life of 110 minutes. It is readily available in any PET laboratory or commercial radiopharmacy and the synthesis of FMISO is a nucleophilic displacement reaction followed by deprotection and purification so FMISO can be conveniently made by a laboratory skilled in the synthesis in FDG. The radiopharmaceutical product is in 10 mL of 95% isotonic saline : 5% ethanol (v:v). The injectable dose of $^{18}\text{FMISO}$ for most studies will be 7–10 mCi at a specific activity >125 Ci/mmol at the time of injection. The amount of injected drug is <15 μg . FMISO is a small, water-soluble molecule with a molecular weight of 189. It has an octanol : water partition coefficient of 0.41, so that it would be expected to reflect plasma flow as an inert, freely-diffusible tracer immediately after injection, but later images should reflect its tissue partition coefficient in normoxic tissues, a tissue-to-blood ratio of about one. At equilibrium, the volume of distribution is about that of total body water (22). FMISO is less than 5% protein bound, allowing efficient transport from blood into tissues (23).

[^{18}F]-fluoromisonidazole is used for imaging tumor hypoxia with positron emission tomography (PET). Generally only a static image is acquired for a 20-minute interval beginning between 100 and 150 minutes post injection and the image is scaled to a venous blood sample taken during the imaging interval. In hypoxic cells, FMISO is trapped, which is the basis for the use of this tracer to measure hypoxia (21). The selectivity of nitroimidazoles for hypoxic conditions has been demonstrated in rat myocytes (24,25), the gerbil stroke model (26,27), pig livers (28,29), rat livers (30), and dog myocardium (31,32), as well as numerous cancer studies in cell cultures, animals and human trials (21). FMISO is not trapped in necrotic tissue because mitochondrial electron transport is absent so the images only reflect viable tissues. The normal route of elimination for parent FMISO is renal. A small fraction of FMISO is glucuronidated and is also excreted through the kidneys.

The mechanism of trapping of FMISO is common to all nitroimidazoles and is based on the chemical reduction that takes place in hypoxic tissue, covalently binding the chemical to

macromolecules in that tissue. The specificity of the reaction is enhanced by the fact that both the reduction and the binding occur within the same cell (20,23). The reduction reaction is reversible at the first step, depending upon the oxygenation status of the tissue, so that some FMISO eventually returns to the circulation and is excreted. The reduction of the nitro group on the imidazole ring is accomplished by tissue nitroreductases that appear to be plentiful and therefore do not represent a rate-limiting factor (33). The 1-electron reduction product may be further reduced to a hydroxylamine or it may competitively transfer its extra electron to O₂ and reform FMISO, a futile cycle. Thus the imaging agent is trapped in cells at a rate that is inversely related to cellular oxygen concentration (25).

A search for articles dealing with the human toxicity of fluoromisonidazole yields no results but an estimate can be made based on similarities among related chemical entities. The octanol/water partition coefficients for MISO and FMISO are 0.43 and 0.41, respectively; the LD₅₀'s in adult male Balb/C mice for MISO and FMISO are 1.8 mg/g (1.3–2.6) and 0.9 mg/g, respectively (34) or in CH3 mice 0.6 mg/g for FMISO (35). Using the relative toxicity factors from Paget (36) of 1.0 for mice and 9.8 for humans, the projected LD₅₀ values are:

LD ₅₀ values	Misonidazole	Fluoromisonidazole
Concentration for human Dose for 70 kg subject	0.184 g/kg 12.86 g	0.06–0.09 g/kg 6.43 g

The MISO values by this calculation are conservative. The serum half-lives of orally administered MISO and FMISO in mice were 2.3 hrs (range 1.87–2.92) and 2.0 hrs (range 1.79–2.24), respectively. The long component of the plasma half-life of FMISO in humans is similar to MISO (8–17 hrs). The maximum dose to humans reported in initial imaging protocols was 1 mg/kg and no adverse events have been reported. Total patient imaging doses of the current radiopharmaceutical formulation contain ≤ 15 µg of FMISO. This is <0.001% of the projected LD₅₀. That the drug has had no toxic effects at these doses can be confidently inferred as a result of the 5400 patients included in MISO studies that were recently reviewed (37).

The uptake of [¹⁸F]FMISO in normal human tissues has been measured and used to estimate the radiation absorbed dose associated with the imaging procedure (38). Calculated total body dose for a 70 kg man injected with 3.7 MBq/kg was 0.013 mGy/MBq; for a 57 Kg woman it was 0.016 mGy/MBq. Effective dose equivalents were 0.013 mSv/MBq for men and 0.014 mSv/MBq for women. Ninety-seven percent of the injected radiation was homogeneously distributed in the body, leaving only 3% for urinary excretion. The radiation exposure from [¹⁸F]FMISO is equal to or lower than from other widely used nuclear medicine studies.

Clinical Experience Using [¹⁸F]-Fluoromisonidazole (FMISO-PET) in HNSCC

Hypoxia imaging in cancer was reviewed in several recent publications (21,39,40,41,42,43). FMISO PET is a robust imaging procedure to quantify regional hypoxia. It is the most commonly used agent for hypoxia PET imaging (21,40,44,45,46,47,48,49,50,51,52). While its biodistribution properties do not result in high contrast images, they result in images at 2–4 hours after injection that unambiguously reflect regional partial pressure of oxygen, PO₂, and hypoxia in the time interval after the radiopharmaceutical was administered. At least 500 patients have been imaged with FMISO and no adverse events have been reported.

The general conclusion from these studies is that FMISO PET identifies hypoxic tissue that is heterogeneously distributed within human tumors (53,54,55) and it promises to help facilitate image-guided radiotherapy and clinical trials of new hypoxia-selective cytotoxins, two ways that might help circumvent the cure-limiting effects of tumor hypoxia. In addition, FMISO has

identified a discrepancy between perfusion, blood-brain barrier disruption, and hypoxia (54) and a lack of correlation between FDG metabolism and hypoxia in several types of malignancies that will be discussed later. Hypoxic tissue does not correlate either with tumor volume or vascular endothelial growth factor (VEGF) expression (40,55). FMISO PET was able to identify post-radiotherapy tumor recurrence by differential uptake of tracer. The mean standardized uptake value (SUV) ratio between recurrent tumor and muscle was >1.6 and between tumor and normal mediastinum was >2.0 (48).

In addition to FMISO imaging studies, alternative nitroimidazoles have been evaluated as imaging agents in single-center pilot studies. A 2001 study from Finland used [^{18}F]-fluoroerythro-nitroimidazole (^{18}F -FETNIM) to evaluate 8 patients with head and neck squamous cell carcinoma at doses of ~ 370 MBq (54). A derivative that is more hydrophilic than FMISO, [^{18}F]-fluoroazomycin-arabinofuranoside (FAZA) had been recommended for further study (57,58) and shows considerable clinical promise.

The remainder of this section will focus on FMISO imaging of head and neck cancer and will include, wherever possible, comparative studies with PO_2 electrodes and FDG-PET. Several studies have compared FMISO images with PO_2 histography. In one study, sixteen patients with metastatic neck lymph nodes were studied with the needle electrode placed under CT guidance without anesthesia (51). The FMISO image was a single static scan after 2 hours and was reported as a tumor-to-muscle ratio, T/M. Average to high correlations were found between T/M and the fraction of electrode measurements with $\text{PO}_2 < 10$ mm. Coincidentally, this study found no correlation between FDG PET and PO_2 status. Other studies have found no relationship between increased MISO uptake and decreased PO_2 (14). The cutoff value of 10 mm Hg was based on a detailed study in pig livers which showed that FMISO preferentially bound when PO_2 was below this value (28,29). Even though there is hepatic metabolism of 2-nitroimidazoles and the liver functions at a significantly lower oxygen level than other normally oxygenated tissues (59), this study is probably the most detailed attempt to correlate FMISO and electrode measurements in animals.

A significant correlation was found between hypoxic tissue identified by FMISO and by immunohistochemical staining for both pimonidazole, another nitroimidazole derivative, and carbonic anhydrase IX, a membrane enzyme that is a regulator of proton flux (60). A more recent animal study used immunohistochemistry to evaluate the dynamics of tumor hypoxia in mice with human colorectal cancer xenografts (61). Oxygenation status was modified by carbogen and hydralazine. In animals with normal breathing, CA9 and pimonidazole showed similar spatial distributions. Mice treated with hydralazine to exacerbate hypoxia showed increase uptake of pimonidazole but CA9 was unchanged. In animals treated with carbogen (95% O_2) to improve tumor oxygenation, the pimonidazole uptake decreased but, again, CA9 was not altered. These results showed that CA9 is not a direct measure of hypoxia.

The rationale for using a T/ B ratio of 1.2 to separate normoxia from hypoxia is based on the fact that FMISO distribution in normoxic tissue reflects partition coefficient (21) or the water space (22) and so all tissue/blood values should be about one. This hypothesis has been supported by both human and animal data. The initial animal results showed that normoxic myocardium ratios were nearly unity over a wide range of flows. In numerous other organs of normal mice, rats, rabbits and dogs, the mean of the distribution histogram was 1.035, median 0.96, for 1342 samples (62). Therefore, a cutoff of 1.2 was selected, with confidence that any T/B above that value was indicative of hypoxic tissue. This conclusion is further justified by the human study in figure 2, which is similar to results in figure 5 of Sorensen (19).

In this patient with a squamous cell carcinoma in the base of the tongue, the FDG image was co-registered with the FMISO image (left panel). In regions far from the tumor, the uptake

ratios for FMISO were uniformly less than 1.2, as depicted by the blue and green dots in the right panel, even though FDG SUV spanned a range from about 3 to above 20. In the tumor area, a substantial fraction of the pixels were still in the normal range, but many values exceeded the cut-off as shown by the red pixels in the scatter plot. A distribution histogram of the red data points shows a continuous but skewed distribution, reflecting the fact that the level of oxygenation is a continuum from normoxic to hypoxic. One consequence of this continuous scale is that FMISO images exhibit only modest contrast and are not useful for defining the total extent of tumor. However, the evidence that uptake is independent of blood flow and numerous other physiologic parameters, as described above, provides confidence that FMISO images uniquely identify tumors with prognostically significant levels of hypoxia and for that reason they have clinical importance.

Several clinical studies have compared FDG PET and FMISO PET. The rationale is simply that increased glycolysis is one of the major consequences of hypoxia. Minn showed that FDG accumulated in hypoxic more than in normoxic cells (63). Two cell lines from patients with HNSCC were exposed to graded levels of O₂ and FDG uptake increased about 2-fold. We have compared the uptake of FDG and FMISO in patients with sarcoma (64), breast cancer (40), and head and neck cancer (39) and concluded that there was no correlation. There were even some patients with a reverse correlation, namely low FDG SUV and high FMISO.

A more thorough comparison was recently reported involving voxel-by-voxel analysis in twelve patients who underwent FDG PET and FMISO PET prior to RT (52). Scatter plots from each of the patients were presented to show a diverse spectrum of relative distributions and several parameters from both imaging studies were tested for their ability to stratify the patient groups with respect to treatment outcome. There was no general correlation of increased FDG SUV and FMISO uptake, although the correlation was often better in primary tumor where there was very little hypoxia than it was in lymph nodes with more hypoxia. These authors suggested the intriguing hypothesis that a good correlation of FDG and FMISO might hint at a good treatment response but they did not have enough cases to test their hypothesis. The maximum uptake of FMISO was the only parameter that was significant for stratifying this relatively small patient group. The authors concluded that these two radiopharmaceuticals provide independent information about tumors.

FMISO and FDG have been compared in other cancers. In a study of 21 patients with non-small cell lung cancer there was no correlation ($r=0.26$) (65). Another report of 17 patients with suspected primary gliomas found a general relationship of no FMISO uptake in hypometabolic tumors although the inverse was not true and the pattern of uptake showed substantial differences between the two radiopharmaceuticals (66).

Taken together, these imaging studies show that FMISO PET is able to identify a unique feature of malignant and endangered tissues, hypoxia, thereby adding to the armamentarium of specific markers used to image tumors and potentially impact treatment for the benefit of individual patients. The relationship between the low oxygenation status that is phenotypic of tumor and the often poor response of hypoxic tumors to therapy justifies extensive investigation with agents like FMISO to improve the specificity of treatment regimens directed at hypoxic tumors.

The fact that FDG was not a strong correlate of FMISO should not be a surprise. While HIF1 results in transcriptional activation of GLUT1 and many enzymes that support glycolysis, there are other factors in the biodistribution of FDG. It may be that further comparisons of FMISO PET and markers related to glycolysis (FDG as well as sampled biomarkers) will lead to complementary clinical roles. Tumor measurements related to lactate are likely to provide a useful assay even though lactate can come from either aerobic or anaerobic glycolysis. For example, there is a correlation of high lactate levels in head and neck tumors with incidence

of metastasis (67). There is certainly a dynamic change in the metabolic status of cancers during tumor progression and there is probably a time before metastases occur when the metabolic milieu deteriorates and lactate levels start to increase. Thus, the increase in intratumor lactate may precede metastatic seeding and may be an early indicator of tumor progression.

There are laboratory data and a solid mechanistic argument to support this hypothesis. Hypoxia is known to enhance the metastatic efficiency of murine tumor cells lines, whereas long term hypoxia usually induces programmed cell death in normal tissues. Cell shedding from the spheroid surface into the medium within spinner flasks was drastically enhanced at high versus low lactate levels (67). E-cadherin is down-regulated in hypoxia, leading to cell detachment and development of distant metastases (68). Some tumors are characterized by a loss of the p53 tumor suppressor gene and an over-expression of the bcl-2 apoptosis inhibitor gene. Such tumor cells are able to sustain hypoxic stress for a period of time. Therefore, hypoxia could select for cells with defects in apoptosis; p53 deficient cells will overgrow wild-type cells (69).

Brizel has studied elevated tumor lactate concentration as a predictor of increased risk of metastases in head and neck cancer (70). The assay involved quantitative bioluminescent imaging (11) of cryosections from shock-frozen surgical specimens for measuring lactate, glucose and ATP in 34 patients with HNSCC, all treated with curative intent. A Kaplan-Meier analysis with patients divided at the median showed a much worse metastasis-free survival for those with high lactate, presumably associated with hypoxia-mediated radioresistance and the up-regulation of metastasis-associated genes. Repeated hypoxia-reoxygenation injury as may occur in tumors as a result of blood-flow instability may lead to an up-regulation of genes and gene products associated with angiogenesis and metastasis. It may be important to determine whether oxygenation status and lactate levels provide independent prognostic information. The importance of this work is that MRI/MRS provides a convenient tool for noninvasive measurement of regional lactate levels (71,72).

How Would a Positive FMISO-PET Study Impact Treatment?

In order for a new imaging procedure to gain acceptance in the medical community, it needs to provide information that adds to the clinicopathologic information already available to the oncologist. To appreciate the extent to which FMISO PET might do this, we will first consider the standard imaging methods that are available as well as recent developments in genomics and proteomics. Earlier sections described the unique value of hypoxia as an important variable for stratifying patients but it is important to evaluate whether FMISO PET competes favorably with other ways that evaluate the oxygenation status of patients.

Several authors have reviewed head and neck imaging in general (73) and others have focused on PET imaging (74). One review focused on functional imaging of hypoxia (75). Hermans recently provided a critical review of how imaging predicts treatment outcome in patients with head and neck cancer, building from the standard TNM classification to anatomic imaging with CT and MRI and including molecular imaging with MRI contrast agents and FDG PET as well as FMISO PET (76). The general hypothesis in this review was that pretreatment imaging findings have predictive value for patient outcome that is independent of the current TNM classification and may be used to tailor treatment to the individual patient. It even advocates per-treatment imaging and re-planning during radiotherapy. The importance of excellent anatomic imaging cannot be over-emphasized because, even in advanced HNSCC, concomitant chemoradiotherapy can achieve relatively high locoregional control and survival (77). However, the cure rates reported in the literature are variable and prognosis is not sufficiently explained by TNM staging alone. Furthermore, more sophisticated radiation treatment planning and equipment requires more accurate delineation of the tumor volume.

Identification of lymphatic tumor spread is crucial, as lymphatic metastasis is the most important mechanism of tumor spread. The presence of cervical metastases strongly influences the likelihood of locoregional control, the risk for distant metastasis, and overall survival. None of the current imaging methods, including FDG-PET, can reliably detect very small tumor deposits in lymph nodes or differentiate reactively enlarged nodes from metastatic lymphadenopathy (78). In patients with positive neck nodes, imaging of extranodal tumor spread and/or involvement of critical structures such as the carotid arteries has prognostic and therapeutic relevance. In a multivariate analysis, extranodal spread visible by MRI was the only independent predictor of distant metastasis (79).

Investigators at the Catholic University in Belgium have reported a careful comparison of CT, MRI and FDG PET validated against surgical specimens that were co-registered in 3D (80). They imaged 29 patients with stage II to IV HNSCC using a customized thermoplastic mask; nine of these patients had total laryngectomy with rigorous marking for aligning the surgical material with the images. GTVs contoured using CT or MR exceeded those by FDG PET to a large extent and the PET images most closely matched the surgical specimen. Furthermore, in this small group FDG alone was more specific than was CT or MR imaging. Both of the anatomic imaging modalities failed to depict a significant percentage of GTV inferred from the surgical material. Of course none of the imaging methods detect superficial or microscopic material and so a security margin was required to define the clinical target volume (78).

A recent study from Australia was designed to study prospectively the incremental value of FDG PET over conventional clinical assessment and CT/MRI (81). Seventy-six patients with HNSCC were included in the study. As this study was to evaluate the incremental rather than the independent value of FDG PET/CT, the results were read in concert with all available information available at the time of the interpretation. FDG PET/CT led to an altered TNM classification in 34% of cases and a change in RT planning in 29%. The specificity of FMISO uptake adds to the imaging evaluation of HNSCC.

CT and MR imaging can provide more than structural information. Dynamic contrast enhancement (DCE) can be used to assess tumor vascularity and perfusion and, by inference, oxygenation. In a series of 105 patients with advanced head and neck cancer, tumor perfusion by DCE-CT was an independent predictor of local outcome after radiotherapy (82). A similar DCE study can be done using MR imaging. Several other MRI approaches to characterizing HNSCC have been evaluated. Blood oxygenation level-dependent (BOLD) contrast depends on the endogenous switch from paramagnetic deoxyHb to diamagnetic O₂Hb, a conversion that is reflected in changes in the MR signal. However, BOLD suffers from magnetic susceptibility artifacts that are a serious practical limitation when imaging the neck. Diffusion-weighted (DW) MRI is a useful technique for measuring changes in the microscopic motion of water after therapy. Because it does not require a contrast agent, DW MRI may have some important practical advantages for evaluating tumors. DWI may offer an approach to identifying acute (transient) hypoxia that is not otherwise identified by the electrode, PET or IHC methods described in this review (41).

There have been some reports of hypoxia-related proteins that are upregulated in head and neck cancer, but we are not aware of any reliable serum tests of hypoxia and its consequences. As much as 1.5% of the human genome is thought to be transcriptionally responsive to hypoxia (83). Tissue array profiling of proteins in head and neck cancer specimens have identified a hypoxia signature that was a significant prognostic factor for survival independent of clinicopathologic risk factors (84,85). In addition to the well-known hypoxia-induced genes related to glycolysis, angiogenesis and metastasis, recent work has identified many post-transcriptionally mediated proteins. Perhaps the more significant result is that IKK β has been identified as associated with hypoxia (84). IKK β is a regulator of the nuclear factor- κ B

transcription factor, thus coupling two important factors in cancer biology and identifying IKK β as a potential new target for anticancer therapy. The hypoxia-related markers that have been studied the most are HIF1, CA9 and GLUT1 but these markers have not proved sufficiently specific to be robust markers for selecting patients for hypoxia-directed therapy (86,87). One important advantage of protein immunohistochemical assays is that they can be applied to archival tissue blocks so that it should be possible to do retrospective assays of hypoxia.

Molecular imaging has the potential of providing objective regional information on angiogenesis, tumor receptors and apoptosis as well as hypoxia. This abundance of biological characterization has lead Cliff Ling to propose the concept of a biological target volume to be integrated with the overall treatment plan (88). Hypoxia in solid tumors reduces their sensitivity to conventional treatment modalities, modulating response to ionizing radiation or certain chemotherapeutic agents.

There are several different ways in which patient management might be modified in response to information about the oxygenation status of head and neck cancer. As discussed above, if oxygenation status is associated with differential treatment-specific responsiveness, oxygenation status can be used to guide selection of treatment modalities. Furthermore, specific treatment modalities can be tailored to be more aggressive in hypoxic regions. FMISO could form the basis for dose painting (39,88,89). Patients with hypoxia should be the preferred candidates for adjuvant drug treatments that are designed to increase the oxygenation status of tumors during RT. For example, there are some data suggesting that outcome can be improved by having the patient breath carbogen (95% O₂) rather than air during radiation therapy. The effect is improved if nicotinamide, which reduces intermittent fluctuations in perfusion, is administered an hour prior to each radiation treatment. This protocol is called ARCON (accelerated radiotherapy with carbogen and nicotinamide) and it yields high local and regional controls rates in advance HNSCC and needs to be tested in a randomized trial with accelerated RT (90). These trials will need to follow patients for sufficient time to identify long-term toxicities and quality of life.

One group has even suggested a strategy for cancer gene therapy that takes advantage of acute hypoxia as a valid therapeutic target with the goal of destroying aberrant tumor vasculature as a strategy to eliminate tumors (91). Durand has made the case that acute hypoxia is an under-appreciated challenge because it protects cells against standard therapy and spares them for tumor progression when the hypoxia resolves (92).

Hypoxia imaging will also play an important role in the evaluation of hypoxia-selective cytotoxins. Because many solid tumors are hypoxic, investigators have been developing pro-drugs designed to turn hypoxia into a therapeutic target. Rather than acting as anti-proliferative agents, these molecules target a unique feature of the cancer cell for preferential cytotoxicity. Tumor hypoxia is a good therapeutic target because normoxic cells would be spared. Furthermore, the hypoxic cells are the ones that are more resistant to current therapy. The first drug tested in the clinic that was designed to be hypoxia-selective is tirapazamine, TPZ (93). TPZ undergoes 1-electron reduction to form a radical that abstracts protons from molecules, including DNA, and produces single and double strand breaks. If O₂ is present, the TPZ radical is oxidized back to its nontoxic parent TPZ, a mechanism that is analogous to the mechanism for retention of 2-nitroimidazole imaging agents in hypoxia cells. A phase III multicenter trial of TPZ has been reported in patients with advanced head and neck cancer and randomized to two arms, 70 Gy RT with TPZ plus cisplatin versus 70 Gy with 5FU plus cisplatin. The results were disappointing when patients were randomized to the two arms. However, when a subset of 45 patients who had been imaged with FMISO before treatment was analyzed, it was clear that the locoregional failure rate, LRF, for the TPZ arm was much less for patients with positive

versus negative FMISO scans, 1/19 versus 2/3 (45). For the 5FU arm patients without hypoxia did much better, LRF of 1/10 for FMISO negative versus 8/13 for FMISO positive. These results need to be confirmed but they suggest that FMISO PET will be an effective way to stratify patients to those who are most likely to benefit from a hypoxia-targeted treatment. And this is the goal of molecular imaging—to select the best treatment for the individual patient.

In conclusion, abundant evidence supports the role of hypoxia as a negative prognostic factor in a substantial fraction of patients with head and neck cancer. The consequences of hypoxia are many, including up regulation of proteins that promote new vessel growth, glycolytic metabolism and metastatic spread, all factors that enable tumor cells to survive and even escape the hypoxic microenvironment. Invasive methods to measure regional oxygen levels are now being replaced by noninvasive imaging methods using radionuclides or magnetic resonance detection. The most widely studied imaging procedure is FMISO PET, which has been shown to have independent predictive value for outcome. The eventual role of hypoxia imaging will probably be less to detect disease and more to improve radiation treatment planning and select the appropriate patients for treatment with hypoxia-selective cytotoxins.

Acknowledgements

Supported by P01 CA42045 from the National Cancer Institute.

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Figure 1.

Panel A depicts the structure of fluoromisonidazole and location of the PET label. Panel B shows the mechanism by which a steady-state concentration of radical anion accumulates in live cells and is either reduced further to become trapped or gives up its electron to O₂ and returns to its original chemical form. Panel C is a dark-field autoradiograph of a multicellular spheroid exposed to tritiated fluoromisonidazole. The tritium shows up as white dots that are inside the viable and well-oxygenated outer surface (about 200 μm thick). The necrotic core does not trap tritiated FMISO. Panel D summarizes the radiochemical synthesis and purification of FMSIO.

Figure 2.
Spatial correlation of FMISO and FDG in a patient with squamous cell carcinoma at the base of the tongue, T1/1. N2C, M0.