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Molecular Imaging of Tumor Hypoxia with Positron Emission Tomography

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Abstract

The problem of tumor hypoxia has been recognized and studied by the oncology community for over 60 years. From radiation and chemotherapy resistance to the increased risk of metastasis, the low oxygen concentrations in tumors have caused patients with many types of tumors to respond poorly to conventional cancer therapies. It is clear that patients with high levels of tumor hypoxia have a poorer overall treatment response and that the magnitude of hypoxia is an important prognostic factor. As a result, the development of methods to measure tumor hypoxia using invasive and non-invasive techniques has become desirable to the clinical oncology community. A variety of imaging modalities have been established to visualize hypoxia *in vivo*. Positron Emission Tomography (PET) imaging, in particular, has played a key role for imaging tumor hypoxia because of the development of hypoxia-specific radiolabelled agents. Consequently, this technique is increasingly used in the clinic for a wide variety of cancer types. Following a broad overview of the complexity of tumor hypoxia and measurement techniques to date, this review will focus specifically on the accuracy and reproducibility of PET imaging to quantify tumor hypoxia. Despite numerous advances in the field of PET imaging for hypoxia, we continue to search for the ideal hypoxia tracer to both qualitatively and quantitatively define the tumor hypoxic volume in a clinical setting to optimize treatments and predict response in cancer patients.

Introduction

Tumor hypoxia is a characteristic of solid tumors and has been recognized as a crucial factor impacting cancer treatment effectiveness since its discovery (1–3). Hypoxia occurs because of the high rate of oxygen demanded and consumed by the tumor cells, stroma and endothelial cells exceeds that of the supply which is diminished compared to the blood supply of normal tissue (4). The tumor hypoxic microenvironment impairs response to chemotherapy and radiation treatment for cancer patients resulting in poor prognosis and a reduction in overall survival (5). This is caused by the presence of hypoxia in the tumor and the associated cellular oxygen reduction; as well as hypoxia-induced factors that lead to increased tumor proliferation (tumor invasion and metastases) and a more aggressive tumor phenotype (poor differentiation and reduced apoptosis) (5–7).

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For these reasons, over the last 60 years, invasive and non-invasive techniques have been developed to measure tumor hypoxia. Although no method is ideal, molecular imaging has become the most commonly used non-invasive modality. Positron Emission Tomography (PET), Single-Photon Emission Computed Tomography (SPECT), Magnetic Resonance Imaging (MRI), and Optical Imaging have become useful tools to characterize the magnitude and variability of hypoxia within a tumor and to guide clinical treatment decisions (8,9). PET, in particular, has become a leader in hypoxia imaging. Several PET radiotracers have been developed and continue to be synthesized to visualize the extent of tumor hypoxia on a patient-specific basis (10). It has been shown that several of these tracers, particularly nitroimidazoles, can directly and reproducibly identify the presence of hypoxia (11,12).

Pre-treatment PET imaging for tumor hypoxia combined with quantitative methods for characterizing the hypoxic status of a tumor could allow the clinician to more precisely stratify patients for randomized clinical trials aimed at overcoming the treatment resistance conferred by tumor hypoxia or alter radiation therapy treatment plans. However, a major problem with PET imaging for tumor hypoxia to date is that currently-implemented methods used to quantify the hypoxic fraction do not represent absolute tumor pO_2 values. Moreover, existing techniques are inconsistent between clinics, resulting in highly variable and potentially suboptimal methods for defining hypoxic tumor volumes. Improving methods of quantification would enable more accurate identification of inter- and intra-tumor variability in hypoxia, and help the clinician combat increased tumor aggressiveness and improve poor prognosis for cancer patients. The aim of this review is to discuss the current status and promise of PET imaging for tumor hypoxia measurement in a clinical setting and how it can be used to improve the treatment of oncology patients.

Tumor hypoxia affects cancer therapy in a complex way

Tumor hypoxia results from several tumor-specific traits as illustrated in Figure 1. These include: [1] chronic hypoxia caused by the limited-diffusion distance of oxygen from blood vessels to the tumor cells (~150–200 μm) (2); [2] acute hypoxia due to the perfusion-limited impairment of the vascular vessels by (a) their structural abnormality and (b) high interstitial pressure caused by the combination of fluid in the tumor matrix and the rapidly proliferating tumor cells leading to constriction of the intra-tumor vasculature (13–18); and [3] tumor blood vessel traits such as a reduced vascular density and arteriolar supply, poor blood vessels networks, varied red blood cell flux and high blood viscosity (19).

The adverse impact of tumor hypoxia on cancer treatment response is a result of the reduction in diffusion, perfusion, and delivery of oxygen to cancer cells. Tumor hypoxia has been shown to have a negative impact on the effectiveness of radiation therapy. This resistance is attributed to a reduction in DNA damage under reduced oxygen conditions. There is an intrinsic oxygen dependence of radiation-induced DNA damage which has a higher probability of chemical restoration under hypoxic conditions but may become permanent in the presence of oxygen. (20–22). As a result, the biological effect of megavoltage X-ray radiation is 2–3 times higher in the presence of oxygen (21,23). Reduction in cellular oxygen and nutrients also forms proteomic and genomic changes

creating more mutant, adaptive, and aggressive tumor cells (5,16). However, clinical evidence suggests that hypoxia-induced treatment failure is more likely attributed to radiation resistance and not hypoxia-induced metastasis (24). Consequently, the hypoxic fraction in tumors has been correlated with a negative treatment outcome (25,26) and a reduction in overall survival (6,24,27–30).

In the 1990s, the tumor hypoxic environment (oxygen and delivery imbalance) was connected with many tumor-specific traits (31,32). The main effect of the reduced oxygen level was the increased production of hypoxia-inducible factor 1 (HIF-1), a transcription factor that becomes active under hypoxic conditions and regulates oxygen homeostasis under hypoxic conditions (32). The influence of oxygen concentration on the activity of HIF-1 is mostly a result of a delicate balance between prolyl hydrolyase domain-containing proteins and the crucial role of the von Hippel-Lindau tumor suppressor protein (31). HIF-1 activates genes that upregulate glycolysis, angiogenesis and enhances cell survival under oxidative-stress resulting in a causal relationship between hypoxia and tumor metastasis and growth (33,34). Target genes include vascular endothelial growth factor (VEGF), facilitative glucose transporters (GLUTs), hexokinases (HKs), erythropoietin (EPO), carbonic anhydrase IX (CAIX) and are all targets of hypoxic prevention or identification. Such conditions created by hypoxia, and in turn HIF-1, lead to tumors that are more invasive and resistant to cancer treatments such as chemotherapy (32).

Nearly all solid tumors are positive for some form of hypoxia (severe and intermediate levels) or anoxic (no oxygen present) cells and these areas are often heterogeneously distributed within the tumor (5). The presence of hypoxia does not depend on tumor size, stage, pathology or nodal status (13). Moreover, the prevalence of tumor hypoxia and its traits are not cancer-type specific and have been found in a wide range of human malignancies including cancers of the head and neck (H&N), prostate, rectum, breast, uterine cervix as well as brain tumors, soft tissue sarcomas and malignant melanomas (6,25,26,35–37). Hockel *et al.* (6) showed a drastic decrease in the overall survival in cervical cancer patients with more hypoxic tumors due to hypoxia-induced increased invasiveness and chemoradiation resistance. Brizel *et al.* (37) also clinically demonstrated an increase in hypoxia-induced invasive through metastatic disease in soft tissue sarcoma patients. It is evident based on the biological complexity, prevalence, and negative prognostic impact of tumor hypoxia in cancer patients that a measurement technique with the capability of detecting and quantifying hypoxia would have substantial clinical implications.

Methods of tumor hypoxia measurement

Tumor hypoxia measurement can be divided into two categories, invasive and non-invasive. Invasive measurements are considered to be the ‘gold standard’ as they provide a direct measure of oxygen concentration in tissue. Numerous studies using the Eppendorf pO_2 electrode (38,39) have found that real-time pO_2 measurements are correlated with negative survival in patients with various cancer types (6,25,26,35–37). Although still in use, the Eppendorf electrode is no longer in production or supported by the manufacturer. In 1999, the Oxylite fiber-optic sensor (40) became available to provide a pO_2 measurements but has

not been validated in the clinical setting. Moreover, both methods are user dependent as the invasive technique requires practice and expertise (41). The pO_2 values are also restricted by sampling error as hypoxia is heterogeneous and it is not possible to extract pO_2 measurements for the entire tumor. The invasiveness of the procedure limits its use to measure changes in tumor hypoxia and makes it only useful for superficially-located or easily accessible tumors. Also, the electrode cannot differentiate necrotic and anoxic tissue regions of a tumor.

Immunohistochemical (IHC) staining is another invasive technique used to measure tumor hypoxia. This can be done by administering exogenous bioreductive nitroimidazole compounds, e.g., Pimonidazole or EF5 (42,43), before biopsy that bind to hypoxic regions. IHC staining can also be used to detect hypoxia-specific overexpressed endogenous proteins, e.g., carbonic anhydrase IX (44). However, as the tumor biopsy (even if multiple cores are taken) is not representative of the whole tumor pO_2 , under-sampling is also a problem. Moreover, these methods generally provide information on the presence of the hypoxic regions (as opposed to necrotic ones) but not the location and size. As this technique is invasive, repetitive measurement of changes in the hypoxic fraction after cancer treatment is not realistic in a clinical setting (45).

Molecular imaging techniques can be used to perform non-invasive measurements of tumor hypoxia. These include MRI, SPECT, Optical Imaging, and PET. MRI techniques are based on contrast agents (endogenous or exogenous) and include techniques such as electron paramagnetic resonance (EPR) (46,47), dynamic contrast MRI (DCE-MRI), magnetic resonance spectroscopy (MRS) (48) and blood oxygen-dependent level (BOLD) imaging (49,50). Optical imaging techniques measure the optical absorption, scattering and fluorescence in a tissue (51). Recent developments in PET have allowed it to surpass SPECT in the availability and number of hypoxia imaging agents. These include halogenated nitroimidazole PET agents, such as ^{18}F and ^{124}I , as well as metallic agents, such as $[^{64}Cu]$ -ATSM. The available PET tracers will be discussed below.

PET imaging to identify tumor hypoxia: current status of PET radiotracers

PET radiotracers consist of a radioisotope and a biologically significant molecule that is specific to the functional measurement, e.g., glucose for glucose metabolism (52). ^{11}C , ^{15}O and ^{18}F are popular radioisotopes due to their short half-lives. In 1981, Chapman *et al.* (53) were the first to detect tumor hypoxia with nitroimidazole compounds and molecular imaging. The widespread use of PET for imaging hypoxia is made possible through the use of exogenous markers, hypoxia-specific agents that are reduced and covalently bind to intracellular macromolecules in the tumor in the absence of oxygen (54). Figure 2 shows representative clinical images of PET radiotracers for tumor hypoxia. In nitroimidazoles, a common marker for hypoxia, the relationship between the binding of and oxygen tension, is directly correlated with cell retention of the tracer varying significantly over the same range of oxygen concentration that has the largest impact on radiosensitivity (52).

Table 1 provides an overview of all of PET radiotracer human studies published to date that have been used in human patients to image tumor hypoxia, their clinical application and

conclusions. These include several nitroimidazole radiotracers (10), such as 2-(2-nitro-(1H-imidazol-1-yl)-N-(2,2,3,3,3-pentafluoropropyl)-acetamide ($[^{18}\text{F}]$ -EF5) (55), $[^{18}\text{F}]$ -Fluoroazomycin ($[^{18}\text{F}]$ -FAZA) (56), $[^{18}\text{F}]$ -Fluoromisonidazole ($[^{18}\text{F}]$ -FMISO) (57,58), 3- $[^{18}\text{F}]$ fluoro-2-(4-((2-nitro-1H-imidazol-1-yl)methyl)-1H-1,2,3-triazol-1-yl)propan-1-ol ($[^{18}\text{F}]$ -HX4) (59), (^{18}F) -fluoroerythronitroimidazole ($[^{18}\text{F}]$ -FETNIM) (60), as well as non-nitroimidazole compounds such as $[^{64}\text{Cu}]$ diacetyl-bis(*N*4-methylthiosemicarbazone) ($[^{64}\text{Cu}]$ -ATSM) (61). $[^{18}\text{F}]$ -EF5 is a promising agent stemming from etanidazole radiosensitizer. With higher lipophilicity than $[^{18}\text{F}]$ -FMISO, the tracer has shown good tumor diffusion (62). Similarly, $[^{18}\text{F}]$ -FAZA is becoming a more popular PET imaging radiotracer for tumor hypoxia as its difference in chemical structure to $[^{18}\text{F}]$ -FMISO increases its hydrophilicity allowing for faster clearance and higher tumor-to-background ratio (63). $[^{18}\text{F}]$ -FAZA has also been successfully correlated with Pimonidazole immunohistochemical staining in animal models and shown to be a promising clinical marker of hypoxia (64). However, it still requires more clinical testing. $[^{64}\text{Cu}]$ -ATSM is a non-nitroimidazole compound that can be trapped intracellularly though the exact hypoxia retention mechanism for $[^{64}\text{Cu}]$ -ATSM has yet to be fully elucidated (65). *In vitro* experiments did not demonstrate hypoxic selectivity with $[^{64}\text{Cu}]$ -ATSM (66,67). Moreover, a direct comparison between $[^{64}\text{Cu}]$ -ATSM and $[^{18}\text{F}]$ -FMISO showed that an increase in oxygenation only resulted in a decrease in uptake of $[^{18}\text{F}]$ -FMISO and not $[^{64}\text{Cu}]$ -ATSM (68). So despite some clinical use with this agent, more studies are necessary to confirm if it is truly a hypoxia-specific marker (69).

Since its development (70), numerous studies, both pre-clinical and clinical, consider $[^{18}\text{F}]$ -FMISO and PET imaging to be the most promising method for hypoxia quantification since the tracer binds in hypoxic cells selectively (57,58,71,72). As result, it is a lead contender in the *in vivo* and clinical assessment of hypoxia and is the most extensively studied PET hypoxia tracer (73,74). Koh *et al.* (57) and Valk *et al.* (58) first demonstrated that $[^{18}\text{F}]$ -FMISO could detect hypoxia in human tumors. Rasey *et al.* (71) further validated the tracer sensitivity as a hypoxic marker in 37 patients and confirmed the prevalence, presence, and variability of hypoxia in human tumors. Bruehlmeier *et al.* (75) also supported the use of $[^{18}\text{F}]$ -FMISO for hypoxia quantification and showed the lack of influence of perfusion and the blood-brain-barrier on tracer hypoxia detection. This implies tracer differentiation and specificity. Only Bentzen *et al.* (76) has shown an inability for $[^{18}\text{F}]$ -FMISO to detect hypoxia in human tumors. However, this discrepancy could be attributed to the trial protocol design, as $[^{18}\text{F}]$ -FMISO was used to characterize tumors, not to explicitly detect hypoxia. Most significantly, Gagel *et al.* (77) showed the tracer was representative of intracellular $p\text{O}_2$ as $[^{18}\text{F}]$ -FMISO uptake correlated with Eppendorf $p\text{O}_2$ probe measurements, while this was not the case for $[^{18}\text{F}]$ -FDG (78). Statistically significant correlations have also been found between $[^{18}\text{F}]$ -FMISO uptake and immunohistochemistry staining (79–81) and the HIF1- α hypoxic-inducible factor (76). Okamoto *et al.* (82) showed the $[^{18}\text{F}]$ -FMISO PET could provide reproducible hypoxic volumes in H&N cancer patients. In the clinic, $[^{18}\text{F}]$ -FMISO has been shown to detect hypoxia in a variety of tumor types from soft-tissue sarcoma, H&N cancer, non-small cell lung cancer, breast cancer, and brain tumors (71,77,83–86).

PET imaging of tumor hypoxia: Predicting treatment response and developing more optimal radiotherapy treatments

The schematic in figure 3 shows two potential therapeutic uses of hypoxia imaging in the clinical setting. It is widely accepted that PET imaging can identify tumor hypoxia in cancer patients to predict prognosis to treatment (85,87–95) to stratify them into responding and non-responding groups or provide more targeted treatments for the poor responders (86,96–100). Such a process could also prevent some patients from receiving unnecessary treatments and consequential side-effects or indicate the use of hypoxia-selective drugs such as tirapazamine (98) or the hypoxic radiosensitizer nimorazole (100). However, a potential limitation of this application is that a single pre-therapy scan (used to define hypoxic patient group) may be an inadequate measure of tumor hypoxia as oxygenation is a dynamic process and one single pre-treatment imaging time point may not be adequate.

Lee *et al.* (11) reviewed the clinical data of over 300 patients and concluded [^{18}F]-FMISO is a predictor of treatment response and prognosis for cancer patients. Direct comparisons between [^{18}F]-FDG and [^{18}F]-FMISO show the latter to be a similar or stronger predictor of outcome (77,101). This predictive information is vital as hypoxia is correlated with poor local control and a high incidence of metastasis (102). Eschmann *et al.* (92) and other groups (87–91,93,95) reported that [^{18}F]-FMISO could predict local recurrence in H&N cancer and non-small cell lung cancer (NSCLC) patients. Zips *et al.* (87) also confirmed that [^{18}F]-FMISO PET could predict local recurrence in 25 patients. Additional studies in various cancer types have confirmed that [^{18}F]-FMISO is a prognostic factor in glioma (85) and breast (86) cancer patients.

PET imaging of tumor hypoxia is also used to modify radiation therapy treatment to improve therapy outcomes, such as accelerated radiotherapy with carbogen and nicotinamide (ARCON) (103) and dose escalation (104,105). Lee *et al.* (106) showed in an 11 patient treatment planning feasibility study that boosting the hypoxic volume (GTV_H) was feasible based on [^{18}F]-FMISO scans and provided a theoretical improved local tumour control without exceeding the normal tissue tolerance. Both Eschmann *et al.* (107) and Popple *et al.* (104) dose escalated [^{18}F]-FMISO-defined hypoxic volumes and predicted a theoretical increase in tumour control. This is true for [^{64}Cu]-ATSM treatment planning studies that showed an IMRT dose boost was feasible with respect to normal tissue constraints (108). However, most of the studies (105,106,109–113) on dose escalation are *in silico* and are not actually delivered to patients. Temporal changes in the spatial distribution of hypoxia on [^{18}F]-FMISO may also be problematic and lead to insufficient dose coverage of the tumor hypoxic region if hypoxic volumes change significantly during treatment (114). Practical dose escalation strategies may therefore require serial imaging and adaptive planning which would be more feasible in a hypofractionation paradigm (115). In addition, tumor hypoxia is diffusely distributed and thus heterogeneity can restrict the feasibility of defining a hypoxic subvolume (110).

Some studies implemented dose painting by numbers (116,117), however, the results yielded clinically implausible boost volumes to achieve the desired tumor-control probability. Clinical implementation of dose painting is dependent on accurate quantification of the

hypoxic region and the capability of delivering large dose gradients on small spatial scales which may be challenging with current techniques (118). This challenge in radiation delivery is of great concern during hypofractionated treatments due to increased radioresistance of the hypoxic fraction in these cases (119). Moreover, simulations found that less dose was required for cell kill in a given PET voxel when based on oxygen concentration and not treating the voxel as a homogenous level of hypoxia (120). This suggests dose painting by numbers may be useful (121), but can we get a reliable image of hypoxia? In a recent review, Geets *et al.* (122) agree that, although dose painting is an attractive concept, it is restricted by low contrast, high noise, and poor spatial resolution of the PET image as well as degradation by errors in treatment delivery (set-up error and patient motion). Animal studies may make further investigation possible to perhaps confirm the benefit of dose painting or provide evidence for or against uniform target boosting or redistribution of dose to increase local control.

PET imaging for tumor hypoxia – where to go from here?

PET imaging for tumor hypoxia is an essential part of radiation therapy treatment. However, there are still a number of important challenges that need to be overcome.

The ideal PET tracer for tumor hypoxia

The criterion for a clinical PET radiotracer is outlined in figure 4. The first hurdle is the choice of PET tracer. It is evident that each PET tracer for hypoxia has positive and negative attributes. Even [^{18}F]-FMISO has its limitations despite wide clinical implementation. A PET tracer should be capable of providing both qualitative and quantitative measurements. The PET image should show hypoxia only, not regions of necrosis or normoxia and be representative of tumor cell $p\text{O}_2$ levels within the clinically-relevant hypoxic range (e.g., 0–10 mmHg) irrespective of the tumor grade or cell type (10). Often tracers are only able to detect very low $p\text{O}_2$ levels, but the cells with ‘intermediate’ levels of hypoxia can be more important than the maximally resistant cells in determining tumor response to fractionated radiotherapy (123). As radiotracers require a biologically-significant molecule, e.g., a nitroimidazole combined with a radioactive component such as ^{18}F , it is crucial that the radioisotope does not interfere with the biological properties of the exogenous marker. For example, the lipophilic and hydrophilic balance of the marker is responsible for tracer distribution and clearance of the unbound tracer from the tumor to provide a better tumor to background ratio. Much of radiotracer selection stems from the availability of the tracer, ease of synthesis and tumor type/model.

The second challenge is developing a reliable method for tracer validation. PET tracers are often validated against an accepted standard of hypoxia measurement. However, no ‘true’ gold standard exists. While the Eppendorf $p\text{O}_2$ electrode is often considered the gold standard, it is limited by user-dependence and sampling error (41). Comparisons have been made against immunohistochemical staining; however, the spatial registration can be challenging. On imaging, necrotic and normoxic regions appear to be the same (no uptake) while some immunohistochemical stains can differentiate between these regions. (124). Moreover, this highlights one of the fundamental limitations of PET imaging – the

discrepancy between the microscopic scale of hypoxia and the macroscopic resolution of the PET voxel. Thus, through imaging, we are missing much of heterogeneity of hypoxia via the partial volume effect and large voxel sizes. If we compare the PET voxel to Eppendorf pO_2 values or microscopic immunohistochemistry, it is essential to average the measurement values. We also do not know the accuracy of these comparisons at low or intermediate pO_2 values that show the most resistance to treatment (125).

The third obstacle is assessing tracer uptake and the tumor hypoxic fraction in a quantitative and reproducible way. The most commonly used metric is the tumor-to-blood ratio (TBR) which is dependent on an arbitrary threshold value. So what threshold should be used? Monnich *et al.* (126) correlated the tumor voxel pO_2 as determined by the tissue oxygenation and tracer diffusion dynamic simulations to attempt to identify an [^{18}F]-FMISO parameter that was representative of or correlated with a pO_2 value for the same voxel (127). The main limitation is that the pO_2 value is cycling while a static [^{18}F]-FMISO scan only represents a 'snapshot' of hypoxia. Moreover, when the tracer is compared to a pO_2 value such as in Chang *et al.* (128), the correlative [^{18}F]-FMISO values may be low for pO_2 due to the sampling error of the electrode. Furthermore, in a situation where hemoglobin levels are low (low pO_2 gradient), but perfusion is high, [^{18}F]-FMISO retention will be high.

What is realistic in a clinical setting?

Considering all of these challenges, is it realistic to quantify tumor hypoxia with PET in a clinical setting? [^{18}F]-FMISO uptake, for example, appears to vary across tumor types and sites within the same patient (71,77,83). This inconsistency reduces the hypoxia differentiation of the tracer but may be attributed to the hypoxia definition used, e.g., the maximum Standard uptake value (SUV_{max}) or TBR. Differentiating between hypoxic levels based on TBR can be ambiguous as ratios can vary across patients and tumor types. However, dynamic as opposed to static scanning further elucidates the movement of the tracer from the blood into the tumor and the hypoxic regions. This method provides both temporal and spatial information for the tracer and hypoxia and is more reliable and accurate than the SUV measurement. As a result, static scans are insufficient as a dynamic or early scan is needed to provide information about the input function and the perfusion of the tumor vasculature within the tumor voxels (94,126). The Thorwarth model (129), which has been clinically validated (94), accounts for perfusion, diffusion, and binding of the tracer and can distinguish between necrosis and severely hypoxic regions. Monnich *et al.* (126) proposed a scan at an early time-point, 15 minutes post-injection, and a late scan at 4 hours. Ideally, the tracer kinetic model would be sensitive to patient specific tracer pharmacokinetics, e.g., clearance and metabolite formation. In addition, models must be robust enough to account for large voxel sizes (3–4 mm in each dimension), noise, organ motion, and technical challenges associated with image registration. Despite the introduction of kinetic modeling techniques to analyze dynamic PET data, several challenges remain before this analysis technique can become a clinical reality. Perhaps a model that could accommodate a clinically realistic imaging schedule would be better, i.e., to image at an early and late time point and use the model to calculate the hypoxic fraction. Such a model could provide more information on the best imaging time points during radiotherapy

(87,107). Alternatively, the [^{18}F]-FMISO imaging data could be predictive of response to RT instead of being used to provide adaptive information (94).

Conclusions and Outlook

It is evident that PET imaging is a powerful tool for visualizing tumor hypoxia in patients in a clinical setting. The feasibility of imaging hypoxia with PET has been clinically demonstrated in a number of cancer types using several existing radiotracers. PET has allowed the prediction of treatment response and demonstrated the potential for optimizing radiotherapy plans. However, due to the complexity of tumor hypoxia, technological advances to combat low image contrast, high noise, and poor spatial resolution are needed to improve our ability to routinely quantify the hypoxic fraction in human tumors. Improvements in spatial resolution with PET, MRI, or bioluminescent imaging may bring us closer to clinically incorporating the hypoxic fraction of the patient tumor in routine cancer therapy. Moreover, the development of improved tracers and image analysis techniques are essential to provide better hypoxia specificity and quantification metrics. A systematic review of randomized clinical trials demonstrates a clinical benefit of adding hypoxic modification to radiotherapy for H&N squamous cell carcinomas (130). This, combined with the advancement of PET imaging techniques and tracer development coupled with more *in vivo* studies, PET imaging could make hypoxic modification a standard of care in the clinical setting for many cancer types.

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Figure 1.

Tumor hypoxia is caused by several tumor-specific traits that result in chronic (diffusion-limited), acute (perfusion-limited) hypoxia. In combination with tumor blood vessel abnormalities, tumor hypoxia is a highly complex biological mechanism, making it difficult to counteract with current treatment techniques.

Figure 2.

Representative clinical images of PET radiotracers for tumor hypoxia. (A) ^{64}Cu -ATSM axial image (arbitrary scale) from a static PET scan 30–60 min post-injection in a patient with primary cervical tumor of uterine cervix patients with a tumor-to-muscle activity ratio of 10.3, adapted with permission from Lewis *et al.* (61). (B) ^{18}F -FMISO axial image from a static 120–160 min PET scan in a non-small cell lung cancer patient with a maximum tumor-to-blood ratio of 2.8, adapted with permission from Koh *et al.*(131).

Figure 3.

A schematic illustrating two potential uses of hypoxia PET imaging in a clinical setting in an effort to (a) develop more optimal radiotherapy treatments and to (b) use hypoxia status to stratify patients for a drug trial.

Figure 4.

The characteristics of the 'ideal' PET radiotracer for imaging hypoxia to allow for both qualitative and quantitative measurement of the tumor hypoxic fraction and be available in a clinical setting.

Table 1

PET radiotracer imaging studies to visualize tumor hypoxia in humans subjects

PET Tracer	Study	Cancer type	No. of patients (n)	Type and duration of scan + tracer uptake metric	Study conclusion
[¹⁸ F]-FMISO	Koh <i>et al.</i> 1992 (57)	Non-small cell lung cancer	8	Static scan at 120 mins TBR 1.4	[¹⁸ F]-FMISO imaging may help select patients for trials targeting radioresistant cancers
[¹⁸ F]-FMISO	Valk <i>et al.</i> 1992 (58)	Glioma	3	Static scan at 120–180 mins Tumor-to-plasma No threshold	[¹⁸ F]-FMISO imaging is feasible to detect hypoxia in human gliomas
[¹⁸ F]-FMISO	Koh <i>et al.</i> 1995 (131)	Non-small cell lung cancer	7	Static scan at 120–160 mins TBR 1.4	[¹⁸ F]-FMISO imaging elucidated the changes in tumor oxygenation during radiotherapy
[¹⁸ F]-FMISO	Rajendran <i>et al.</i> 2003 (83)	Soft Tissue Sarcomas	19	Static scan at 120 mins TBR 1.2	Tumor hypoxia as defined by [¹⁸ F]-FMISO PET imaging does not correlate with metabolism via [¹⁸ F]-FDG imaging
[¹⁸ F]-FMISO	Gagel <i>et al.</i> 2004 (77)	Head & Neck	16	Static scan at 120 mins TMR. No threshold	[¹⁸ F]-FMISO PET provides non-invasive measurement of tumor hypoxia in H&N cancer patients
[¹⁸ F]-FMISO	Bruehlmeier <i>et al.</i> 2004 (75)	Glioblastoma	11	Dynamic scan for 90 mins ad Static scan at 150–170 mins Distribution Volume >1	[¹⁸ F]-FMISO shows hypoxia in brain tumors
[¹⁸ F]-FMISO	Eschmann <i>et al.</i> 2005 (92)	Head & Neck and NSCLC	40	Static scan at 240 mins SUV TMR > 2	Prediction of radiotherapy outcome is feasible based on [¹⁸ F]-FMISO uptake in tumor
[¹⁸ F]-FMISO	Loi <i>et al.</i> 2005 (99)	Rectal cancer	16	Static scan at 120 mins SUV No threshold	Difficult to use [¹⁸ F]-FMISO PET imaging to stratify patients for this site but hypoxia was detectable
[¹⁸ F]-FMISO	Thorwarth <i>et al.</i> 2005 (94)	Head & Neck	16	Dynamic scan from 15–60 mins and static scan at 120 mins and 180 mins SUV _{max} HRP SUV _{max} No threshold	Local control information for H&N cancer patients may be derived from the perfusion and diffusion of [¹⁸ F]-FMISO PET imaging
[¹⁸ F]-FMISO	Hicks <i>et al.</i> 2005 (93)	Head & Neck	15	Static scan at 120mins SUV _{max} Independent scoring system No threshold	High prevalence of [¹⁸ F]-FMISO uptake in patient cohort may support prediction of adverse prognosis through PET imaging
[¹⁸ F]-FMISO	Cher <i>et al.</i> 2006 (85)	Glioma	17	Static scan at 120 mins SPM99 value	[¹⁸ F]-FMISO PET imaging provides prognostic information for glioma based on hypoxia status
[¹⁸ F]-FMISO	Cherk <i>et al.</i> 2006 (84)	Non-small cell lung cancer	21	Static scan at 120 mins SUV _{max} No threshold	[¹⁸ F]-FMISO and [¹⁸ F]-FDG imaging did not show a significant correlation between hypoxia and glucose metabolism
[¹⁸ F]-FMISO	Gagel <i>et al.</i> 2006 (91)	Non-small cell lung cancer	8	Static scan at 180 mins SUV _{max}	[¹⁸ F]-FMISO PET allowed the definition of hypoxic sub-regions that may correspond to areas of local recurrence

PET Tracer	Study	Cancer type	No. of patients (n)	Type and duration of scan + tracer uptake metric	Study conclusion
				SUV_{mean} TMR No threshold	
[¹⁸ F]-FMISO	Rischin <i>et al.</i> 2006 (98)	Head & Neck	45	Static scan at 120 mins SUV_{max} Independent hypoxic score (93)	Showed the prognostic value of [¹⁸ F]-FMISO detection of hypoxia in patients stratified to chemoradiation with or without tirapazamine
[¹⁸ F]-FMISO	Thorwarth <i>et al.</i> 2006 (95)	Head & Neck	12	Static scans at 120, 240 mins $SUV > 1.4$	[¹⁸ F]FMISO and [¹⁸ F]FDG PET provide independent tumor information
[¹⁸ F]-FMISO	Eschamnn <i>et al.</i> 2007 (107)	Head & Neck	14	Static scan at 240 mins SUV TMR No threshold	Changes in [¹⁸ F]-FMISO PET uptake during radiation therapy are indicative of reoxygenation
[¹⁸ F]-FMISO	Lee <i>et al.</i> 2008 (106)	Head & Neck	10	Static scan at 120–150 mins TBR 1.3	In silico dose escalation is possible in H&N cancer patients based on [¹⁸ F]-FMISO PET imaging
[¹⁸ F]-FMISO	Lin <i>et al.</i> 2008 (114)	Head & Neck	7	Static scan at 120–150 mins TBR 1.3	Changes in tumor hypoxia spatial distribution as shown by [¹⁸ F]-FMISO PET indicate that coverage of volumes by dose-painting may not be achievable
[¹⁸ F]-FMISO	Nehmeh <i>et al.</i> 2008 (132)	Head & Neck	20	Static scan at 117–195 mins SUV TBR > 1.2	Changes in [¹⁸ F]-FMISO PET tumor uptake between scans in the same patient can occur
[¹⁸ F]-FMISO	Dirix <i>et al.</i> 2009 (133)	Head & Neck	15	Static scan at 120–160 mins TBR > 1.2	Imaging with [¹⁸ F]-FMISO PET provides added value to radiotherapy planning in H&N patients
[¹⁸ F]-FMISO	Yamane <i>et al.</i> 2011 (90)	Head & Neck	13	Static scan at 150mins SUV_{max} TMR No threshold	A reduction in [¹⁸ F]-FMISO uptake after neoadjuvant chemotherapy was seen in H&N patients
[¹⁸ F]-FMISO	Kawai <i>et al.</i> 2011 (134)	Glioblastoma	10	Static scan at 15mins, 130–160mins TBR > 1.2	[¹⁸ F]-FMISO PET detected hypoxia can be related to the neovascularization shown on by gadolinium-enhanced MRI
[¹⁸ F]-FMISO	Kikuchi <i>et al.</i> 2011 (89)	Head & Neck	17	Static scan at 150 mins SUV_{max} TBR > 1.3	[¹⁸ F]-FMISO PET may predict radiotherapy and survival outcomes in H&N patients
[¹⁸ F]-FMISO	Hugonnet <i>et al.</i> 2011 (135)	Metastatic renal cell carcinoma	53	Static scan at 120–240 mins SUV_{max} TBR > 1.2	Sunitinib reduced hypoxia in metastases and did not induce significant hypoxia in non-hypoxic metastases
[¹⁸ F]-FMISO	Hirata <i>et al.</i> 2012 (96)	Glioblastoma	23	Static scan at 240 mins $SUV_{max} > 1.3$ x mean SUV	[¹⁸ F]-FMISO PET may distinguish glioblastoma from lower grade gliomas based on tumor hypoxia status
[¹⁸ F]-FMISO	Zips <i>et al.</i> 2012 (87)	Head & Neck	25	Static scan at 120 mins SUV_{max} TBR No threshold	[¹⁸ F]-FMISO PET imaging provides strong prognostic information for treatment

PET Tracer	Study	Cancer type	No. of patients (n)	Type and duration of scan + tracer uptake metric	Study conclusion
[¹⁸ F]-FMISO	Toma-Dasu <i>et al.</i> 2012 (117)	Head & Neck	7	Static scan at 120–160 mins Uptake values converted to TCP	Incorporation of [¹⁸ F]-FMISO PET imaging with this approach to treatment planning may provide improved treatment results for H&N cancer patients
[¹⁸ F]-FMISO	Mammar <i>et al.</i> 2012 (136)	Chordoma	7	Static scan at 120 mins SUV _{max} Tumor-to-cerebellum >1	[¹⁸ F]-FMISO PET/CT enables imaging of the tumor hypoxia in residual chordomas
[¹⁸ F]-FMISO	Askoxylakis <i>et al.</i> 2012 (88)	Non-small cell lung cancer	15	Dynamic scan for 60 mins Tracer kinetic parameter (K1, k2, k3 and k4) No threshold	Correlated functional MRI and [¹⁸ F]-FMISO PET tumor hypoxic regions and evaluated tumor local response using RECIST criteria
[¹⁸ F]-FMISO	McKeage <i>et al.</i> 2012 (97)	Advanced solid tumors	42	Static scan at 90–120 mins TBR > 1.2	The presence or absence of [¹⁸ F]-FMISO defined tumor hypoxia did not correlate with response to PR-104-based combination chemotherapy
[¹⁸ F]-FMISO	Okamoto <i>et al.</i> 2013 (82)	Head & Neck	11	Static scan at 240 mins SUV _{max} TBR 1.5 TMR > 1.25	[¹⁸ F]-FMISO PET defined tumor hypoxic regions were reproducible in subsequent scans
[¹⁸ F]-FMISO	Segard <i>et al.</i> 2013 (137)	Pancreatic	10	Static scan at 120 mins SUV _{max} TBR No threshold	Hypoxia was minimally detected using [¹⁸ F]-FMISO PET imaging in pancreatic cancer
[¹⁸ F]-FMISO	Chang <i>et al.</i> 2013 (112)	Head & Neck	8	Static scan at 120 mins TMR > 1.5 Threshold = 1.5	Hypoxia dose-painting in H&N patients using [¹⁸ F]-FMISO PET imaging is feasible
[¹⁸ F]-FMISO	Cheng <i>et al.</i> 2013 (86)	Breast cancer	11	Static scan at 120, 240 mins SUV 2.1 TBR 1.2	In breast cancer patients [¹⁸ F]-FMISO PET imaging can predict endocrine resistance
[¹⁸ F]-FAZA	Grosu <i>et al.</i> 2007 (110)	Head & Neck	18	Static scan at 120 mins SUV 1.5 TMR No threshold	[¹⁸ F]-FAZA PET imaging has the potential to be used for hypoxia-driven radiotherapy treatment planning
[¹⁸ F]-FAZA	Postema <i>et al.</i> 2009 (138)	Head & Neck, Lung, glioma and lymphoma	50	Static scan at 120–180mins SUV _{max} TBR No threshold	Imaging with [¹⁸ F]-FAZA PET can visualize hypoxic lesions in patients with glioma
[¹⁸ F]-FAZA	Schuetz <i>et al.</i> 2010 (139)	Uterine cervix	15	Dynamic scan 60, 120 mins SUV _{max} TMR No threshold	[¹⁸ F]-FAZA PET imaging is feasible in uterine cervix cancer patients
[¹⁸ F]-FAZA	Le <i>et al.</i> 2012 (140)	Head & Neck	39	Static scan at 120 mins SUV _{max} TBR No threshold	Circulating hepatocyte growth factor correlated with [¹⁸ F]-FAZA tumor uptake values
[¹⁸ F]-FAZA	Mortensen <i>et al.</i> 2012 (64)	Head & Neck	40	Static scan at 120 mins TMR 1.4	[¹⁸ F]-FAZA provides suitable prognostic detection in H&N cancer patients with hypoxic tumors

PET Tracer	Study	Cancer type	No. of patients (n)	Type and duration of scan + tracer uptake metric	Study conclusion
⁶⁰ Cu]-ATSM	Dietz <i>et al.</i> 2008 (141)	Rectal carcinoma	19	Dynamic scan for 60mins TMR > 2.6	[⁶⁰ Cu]-ATSM-PET may predict survival and tumor response to neoadjuvant chemoradiotherapy for rectal cancer patients
⁶⁴ Cu]-ATSM	Lewis <i>et al.</i> 2008 (61)	Uterine cervix	10	Static 30–60 mins scan TMR No threshold	[⁶⁴ Cu]-ATSM can be used to image tumor hypoxia in uterine cervix patients
⁶² Cu]-ATSM	Lohith <i>et al.</i> 2009 (142)	Lung (adenocarcinoma and SCC)	8	Dynamic scan and static at 30mins and 66 mins SUV _{mean} No threshold	Pathohistologic lung cancer type may result in different [⁶² Cu]-ATSM tumor uptake
[¹⁸ F]-HX4	van Loon <i>et al.</i> 2010 (59)	Solid cancer	12	Static scan at 30, 60 and 120mins SUV _{max} TMR No threshold	Detecting tumor hypoxia with [¹⁸ F]-HX4 is not associated with patient toxicity
[¹⁸ F]-HX4	Zegers <i>et al.</i> 2013 (143)	Non-small cell lung cancer	15	Static scan at 120 and 240 mins SUV _{max} TBR > 1.5	Most of the NSCLC lesions exhibited [¹⁸ F]-HX4 uptake and the 4 hour imaging timepoint provided better contrast
[¹⁸ F]-EF3	Mahy <i>et al.</i> 2008 (144)	Head & Neck	10	Static scans at 30, 65, 115, 180, 245 and 310 and 375mins % of injected dose per gram of tissue No threshold	[¹⁸ F]EF3 PET imaging in head & neck cancer patients is feasible and hypoxic tumor regions could be visualized
[¹⁸ F]-EF5	Komar <i>et al.</i> 2008 (62)	Head & Neck	15	Dynamic scan for 60mins and static scans at 120, 180 and 240 mins TMR > 1.5	[¹⁸ F]-EF5 PET imaging can be used to identify hypoxia in H&N patients
[¹⁸ F]-EF5	Koch <i>et al.</i> 2010 (145)	Glioblastoma	10	3 dynamic PET scans 210mins SUV	[¹⁸ F]-EF5 is safe to use in patients to identify tumor hypoxia
[¹⁸ F]-FETNIM	Lehtio <i>et al.</i> 2003 (146)	Head & Neck	10	Dynamic scan for 20 and 80–120 mins DV TMR No threshold	[¹⁸ F]-FETNIM PET imaging is a hypoxia marker in oncological patients and uptake is better understood using dynamic image analysis
[¹⁸ F]-FRP-170	Shibahara <i>et al.</i> 2010 (147)	Brain cancers	8	Static scan at 120 mins SUV _{max} No threshold	FRP-170 PET imaging can identify tumor hypoxia lesions in patients with various brain cancers as verified by histology

Abbreviations: TMR = tumor-to-muscle ratio, TBR = tumor-to-blood ratio, SUV = standard uptake value, SUV_{max} = maximum standard uptake value, SUV_{mean} = mean standard uptake value, HRP = Tracer-retention-potential-to-perfusion ratio, DV = Distribution volume, SPM99 = Statistical Parametric Mapping value