Imaging tumour hypoxia with positron emission tomography

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Hypoxia, a hallmark of most solid tumours, is a negative prognostic factor due to its association with an aggressive tumour phenotype and therapeutic resistance. Given its prominent role in oncology, accurate detection of hypoxia is important, as it impacts on prognosis and could influence treatment planning. A variety of approaches have been explored over the years for detecting and monitoring changes in hypoxia in tumours, including biological markers and noninvasive imaging techniques. Positron emission tomography (PET) is the preferred method for imaging tumour hypoxia due to its high specificity and sensitivity to probe physiological processes in vivo, as well as the ability to provide information about intracellular oxygenation levels. This review provides an overview of imaging hypoxia with PET, with an emphasis on the advantages and limitations of the currently available hypoxia radiotracers.

Low oxygen concentration (hypoxia) is associated with many human pathological processes, including ischaemic heart disease, stroke and cancer. In oncology, hypoxic tumours are associated with a poor prognosis, an aggressive phenotype, increased risk of invasion and metastasis, and resistance to chemo and radiation therapy. A practical, robust and reproducible method of detecting and quantifying hypoxia could improve patient outcomes by allowing selection of more appropriate therapies to overcome the effects of hypoxia or allowing stratification of patients for more accurate prognostic information.

Tumour hypoxia has been studied with various techniques: oxygen electrodes; extrinsic (e.g., pimonidazole) and intrinsic (e.g., carbonic anhydrase IX, CAIX) biomarkers; blood oxygen level-dependent (BOLD) and tissue oxygen level-dependent (TOLD) magnetic resonance imaging (MRI); single photon emission computed tomography (SPECT) and positron emission tomography (PET). Each technique interrogates different aspects of the hypoxic microenvironment, as they provide information on hypoxia at different locations: PET, SPECT and extrinsic markers, report on intracellular hypoxia (although not specifically inside cell nuclei and PET/SPECT images quantify data on a macroscopic scale in tumour regions), BOLD-MRI allows assessment of blood oxygenation using deoxy-haemoglobin as an endogenous marker, while oxygen electrodes, OxyLite sampling and electron paramagnetic resonance (EPR) predominantly measure interstitial hypoxia. Indirect methods that report on hypoxia-induced molecular events (e.g., GLUT1, CAIX expression) rather than hypoxia itself have also been employed as markers of tumour oxygenation. Positron emission tomography displays some advantages for studying hypoxia, as it can employ radiotracer probes that

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directly report on oxygen levels, in principle permitting the non-invasive and three-dimensional assessment of intratumour oxygen levels in a more direct manner, and not via hypoxia-mediated changes in phenotype.

Due to the clinical significance of hypoxia imaging, an increasing number of hypoxia PET tracers are being evaluated in the clinic. This review provides a summary and discussion of tumour hypoxia imaging with PET, emphasising the attributes and limitations of the currently available hypoxia radiotracers.

**THE SIGNIFICANCE OF TUMOUR HYPOXIA**

Tissue hypoxia is the result of inadequate tissue oxygenation due to an imbalance between oxygen supply and consumption. Hypoxia in solid tumours is largely due to the decreased delivery of oxygenated blood to meet the increased metabolic demands of the rapidly proliferating tumour cells. Other pathogenetic factors prevalent in the aetiology of tumour hypoxia lie in the chaotic and primitive tumour microvasculature, which exhibits severe structural and functional abnormalities, heterogeneous microcirculation patterns, and an adverse geometry that poses limitations to oxygen diffusion. In addition, the reduced oxygen binding ability and/or transport capacity of haemoglobin, due to rouleaux formation, and the presence of disease- or therapy-related anaemia may also exacerbate hypoxia (Vaupel and Harrison, 2004).

Tumour hypoxia may be broadly classified as chronic and acute. Chronic or diffusion-limited hypoxia primarily arises as a consequence of the disorganised vascular architecture of tumours, where the distances between tumour microvessels are often increased from normal. Consequently, the diffusion distances of oxygen in perivascular space—typically 70–180 μm from the nearest capillary—are often exceeded. In addition, an adverse vascular geometry and prolonged reductions in blood oxygen content due to anaemia can also result in chronic hypoxia. By contrast, acute or perfusion-limited hypoxia is characterised by fluctuations in tumour blood flow that are caused by transient reductions in perfusion. Both chronic and acute hypoxia can concur in tumours, leading to the formation of a highly dynamic microenvironment, where cells are exposed to differential oxygen gradients both spatially and temporally (Vaupel and Harrison, 2004). Owing to the dynamic and heterogeneous character of tumour hypoxia, imaging with PET presents an attractive alternative, as it does not require invasive biopsies, provides information across the entire tumour, and allows repeated and quantifiable measurements.

Hypoxia has been shown to change gene expression to favour survival in a hostile environment (Bristow and Hill, 2008). The cellular response to hypoxia is mainly controlled by the family of hypoxia-inducible factors (HIFs), and may involve regulation of up to 1.5% of the human genome. HIF-1—the best characterised member of the HIF family—is a heterodimeric protein, consisting of an oxygen responsive α-subunit and a constitutively expressed β-subunit. In the presence of oxygen, HIF-1α is continuously synthesised and degraded, but under hypoxic conditions, the protein accumulates, heterodimerises, and acts as a transcription factor to upregulate a multitude of genes, including those involved in glucose metabolism, pH regulation, apoptosis, cell survival under oxidative stress, angiogenesis, and erythropoiesis (Semenza, 2004). These characteristics eventually confer tumours with resistance to chemoradiation therapy and higher degrees of invasiveness. Furthermore, hypoxia itself reduces free radical formation induced by radiation, providing a physical contribution to resistance. Several retrospective immunohistochemical studies have demonstrated that hypoxia-mediated expression of HIF-1α and its downstream genes (e.g., glucose transporter 1, GLUT-1; vascular endothelial factor, VEGF; CAIX) is a negative prognostic indicator for many cancer types (Jubb et al, 2010). Treatment resistance to radio and chemotherapy has also been demonstrated. Radiotherapy relies on the formation of free radicals that cause DNA damage; a mechanism that is enhanced in the presence of oxygen. Chemotherapeutic resistance may also be explained by a multitude of mechanisms, including extracellular acidification, resistance to apoptosis, and increased genomic instability. Consequently, patients with hypoxic tumours often have a poor prognosis and decreased overall survival rate.

**MEASURING TUMOUR HYPOXIA WITH PET**

Radionuclide detection of hypoxia in tumours was first reported in 1981 with 14C-misonidazole autoradiography (Chapman, 1979). Subsequently, two main tracer classes have been developed to specifically study hypoxia with PET: 18F-labelled nitroimidazoles and Cu-labelled diacetyl-bis(N4-methylthiosemicarbazone) analogues (Figure 1).

From a PET imaging perspective, hypoxia markers need to exhibit a number of different properties. The tracer must readily and non-specifically enter cells, sample the intracellular milieu, and leave cells only in the presence of relevant oxygen concentrations. A summary of the attributes of the ideal hypoxia tracer is presented in Table 1. Most PET tracers tested clinically broadly display attributes 1, 4, 5, and 7. The clinical utility of each tracer depends on these key properties, which will influence its distribution in tissues, clearance rate from blood, normoxic and hypoxic cells, metabolism, optimal image acquisition time and ease of synthesis, distribution.

**NITROIMIDAZOLE ANALOGUES**

2-Nitroimidazole compounds were originally developed as hypoxic cell radiosensitisers and were introduced as hypoxia markers in the 1970s (Chapman, 1979). Nitroimidazoles enter cells by passive diffusion, where they undergo reduction forming a reactive intermediate species. Under normoxic conditions, these molecules are re-oxidised into their parent compound and diffuse out of the cell. However, hypoxia causes further reduction of the nitro-radical anion, which eventually becomes irreversibly trapped in the cell at rates that are inversely proportional to the local pO2. As reduction of nitroimidazoles requires the presence of active tissue reductases, these compounds accumulate within viable hypoxic cells, but not apoptotic or necrotic cells.

18F-fluoromisonidazole. Over the years, several fluorinated nitroimidazole-based markers have been developed for PET imaging. Of these, 18F-fluoromisonidazole (18F-FMISO) constitutes the prototype 2-nitroimidazole tracer, and is the most extensively clinically studied PET hypoxia biomarker. The lipophilic nature of this compound ensures facile cell-membrane penetration and diffusion into tissue, and several studies correlating direct oxygen measurements with 18F-FMISO accumulation in vivo demonstrate that a median oxygen level of ≤10 mm Hg is generally required for hypoxia-specific retention. The 18F-FMISO accumulation has been found to reflect hypoxia in gliomas (Valk et al, 1992; Bruehlmeier et al, 2004; Rajendran et al, 2004; Cher et al, 2006; Swanson et al, 2009), head-and-neck (Rasey et al, 1996; Gagel et al, 2004, 2007; Hicks et al, 2005; Thorwarth et al, 2006; Zimny et al, 2006; Mortensen et al, 2010; Abolmaali et al, 2011; Sato et al, 2013), breast (Cheng et al, 2013), lung (Cherk et al, 2006; Vera et al, 2011), and renal tumours (Hugonet et al, 2011). However, 18F-FMISO retention in sarcomas is variable (Rajendran et al, 2003; Mortensen et al, 2010), rectal 18F-FMISO imaging is compromised
by high non-specific tracer accumulation in normoxic tissue (Roels et al, 2008) whereas no retention was observed in pancreatic tumours (Segard et al., 2013). Several clinical studies have shown that a tumour-to-blood activity ratio of $\geq 1.2$ imaged after at least 2 h post injection (p.i.) can be generally considered as indicative of hypoxia (Table 2). Although not commercially available, $^{18}$F-FMISO is produced by a number of institutions, making it available for research purposes.

Due to its hypoxic selectivity, $^{18}$F-FMISO is the lead candidate in the assessment of hypoxia with PET. However, despite its wide applicability, $^{18}$F-FMISO has not gained general acceptance for routine clinical use due to its slow pharmacokinetic profile: the limited clearance of the tracer from normoxic tissue and blood results in modest hypoxic-to-normoxic tissue ratios (Figure 2) and therefore images with moderate contrast (Figure 3A). The limited hypoxic contrast may potentially impede visual detection of hypoxic regions, and has hampered diagnostic utility in routine practice. Therefore, considerable efforts have been made to develop hypoxia markers with improved pharmacokinetic properties (enhanced clearance of the tracer from normoxic tissues) that are more amenable to clinical use. These are discussed below.

$^{18}$F-fluoroazomycin-arabinofuranoside. $^{18}$F-fluoroazomycin-arabinofuranoside ($^{18}$F-FAZA) is more hydrophilic than $^{18}$F-FMISO. Consequently, there are faster clearance kinetics, resulting in improved tumour-to-reference tissue ratios, and thus hypoxia-to-normoxia contrast. The $^{18}$F-FAZA imaging has been successful in gliomas (Postema et al., 2009), lymphomas (Postema et al., 2009), lung (Postema et al., 2009; Bollineni et al., 2013; Trinkaus et al., 2013), head-and-neck (Grosu et al., 2007; Souvatzoglou et al., 2007; Postema et al., 2009; Mortensen et al., 2012), cervical (Schuetz et al., 2010), and rectal tumours (Havelund et al., 2013), and results have been shown to compare favourably with equivalent $^{18}$F-FMISO data, especially as improved hypoxic-normoxic contrast was obtained at earlier time points. No $^{18}$F-FAZA accumulation has been observed in prostate tumours, although hypoxia may not be a characteristic of this particular tumour type, as in the same study, CAIX immunohistochemistry was also found to be negative in these lesions (Garcia-Parra et al., 2011). High $^{18}$F-FAZA tumour-to-reference tissue values have been associated with reduced disease-free survival and have shown prognostic potential in the detection of hypoxia in head-and-neck patients (Mortensen et al., 2012). Due to the higher tumour-to-reference tissue ratios in comparison with $^{18}$F-FMISO, $^{18}$F-FAZA is gaining popularity for PET imaging of tumour hypoxia. Despite the fact that $^{18}$F-FAZA is not widely available at present, increasing research demand may persuade more sites to produce it.
### Table 2. Clinical hypoxia studies with PET in tumours

| Reference          | Tracer   | Tumour type(s) | N   | Tracer retention (TBR; SUV) | Results                                                                                     |
|--------------------|----------|----------------|-----|----------------------------|----------------------------------------------------------------------------------------------|
| Valk et al (1992)  | $^{18}$F-FMISO | Brain          | 3   | T.P: 0.71–1.49 at 120 min p.i. | $^{18}$F-FMISO-PET is a feasible method for detecting hypoxia in gliomas                  |
| Bruehlmyer et al (2004) | $^{18}$F-FMISO | Brain          | 11  | T.B: 0.96–2.07 at 90 min and ≥ 170 min p.i. | Increased $^{18}$F-FMISO T:B observed in all tumours. T:B independent of tumour perfusion at later imaging times |
| Cher et al (2006)  | $^{18}$F-FMISO | Brain          | 17  | Static scan at 120 min p.i. | $^{18}$F-FMISO uptake in high-grade, but not in low-grade, gliomas. Correlation between $^{18}$F-FDG or $^{18}$F-FMISO uptake with Ki67 and VEGFR-1 expression |
| Swanson et al (2009) | $^{18}$F-FMISO | Brain          | 24  | T:B<sub>max</sub>pre-therapy: 2.7 T:B<sub>max</sub>post-therapy: 1.7 | Hypoxia volume generally straddled outer edge of the T1-Gd abnormality. Correlation between hypoxic volume and T1-Gd abnormality. $^{18}$F-FMISO T:B reduced after therapy |
| Cheng et al (2013) | $^{18}$F-FMISO | Breast         | 20  | T:M<sub>2h</sub>Baseline: 0.72–3.07 T:M<sub>4h</sub>Baseline: 0.8–2.29 (16/20 patients) T:M<sub>2h</sub>Follow-up: 0.27–1.83 T:M<sub>4h</sub>Follow-up: 0.43–2.28 at 120 min and 180 min p.i. | Correlation between FMISO uptake and endocrine therapy outcome. Poor correlation between FMISO uptake and HIF-1α immunostaining |
| Gagel et al (2004) | $^{18}$F-FMISO | H&N            | 16  | T:M: 1.68 (range, 1.23–2.28) Av. SUV<sub>max</sub>: 1.76; Av. SUV<sub>max</sub>: 2.07 at 120 min p.i. | Average to high correlation between oxygen electrode and $^{18}$F-FMISO T:M and SUV. No correlation between tumour oxygenation status and $^{18}$F-FDG uptake |
| Hicks et al (2005) | $^{18}$F-FMISO | H&N            | 15  | SUV<sub>max</sub> Tumour: 2.5 ± 0.5 Nodes: 2.3 ± 0.5 at 120 min p.i. | Positive $^{18}$F-FMISO uptake in 13 patients. Qualitative decrease in $^{18}$F-FMISO and $^{18}$F-FDG uptake induced by therapy |
| Thonwarth et al (2005) | $^{18}$F-FMISO | H&N            | 15  | Median SUV<sub>max</sub>: 2.25 (range, 1.36–4.04) at 120 min and 180 min p.i. | Different types of characteristic hypoxia-perfusion patterns identified in tumours |
| Rajendran et al (2006) | $^{18}$F-FMISO | H&N            | 73  | Mean T:B<sub>max</sub>: 1.6 ± 0.46 | T.B and the presence of nodes were strong independent predictors of survival |
| Rischin et al (2006) | $^{18}$F-FMISO | H&N            | 45  | Independent hypoxic score Static scan at 120 min p.i. | Higher risk of locoregional failure in hypoxic tumours. Patients on trastuzumab had lower risk of locoregional failure |
| Thonwarth et al (2006) | $^{18}$F-FMISO | H&N            | 12  | SUV<sub>max</sub>: 2.20 (range, 1.4–3.22) at 120 min and 240 min p.i. | No correlation between $^{18}$F-FDG and $^{18}$F-FMISO SUV. Maximum $^{18}$F-FMISO SUV showed borderline significance for stratifying patient group |
| Zimny et al (2006) | $^{18}$F-FMISO | H&N            | 24  | Normoxic T:M<sub>mean</sub>: 1.4 Hypoxic T:M<sub>mean</sub>: 1.8 | $^{18}$F-FMISO T:M higher in hypoxic tumours (as detected with oxygen electrode). Moderate correlation between $^{18}$F-FDG and $^{18}$F-FMISO uptake |
| Eschmann et al (2007) | $^{18}$F-FMISO | H&N            | 14  | SUV<sub>mean</sub>, pre-therapy: 2.54 ± 0.81 T:M<sub>pre-therapy</sub>: 1.9 ± 0.64 SUV<sub>mean</sub>, post-therapy: 1.98 ± 0.47, T:M<sub>post-therapy</sub>: 1.49 ± 0.26 at 240 min p.i. | Radiotherapy decreased $^{18}$F-FMISO SUV and T:M ratio |
| Gagel et al (2007) | $^{18}$F-FMISO | H&N            | 38  | SUV<sub>mean</sub>: 1.69 SUV<sub>max</sub>: 1.98 T:M<sub>mean</sub>: 1.57 T:B<sub>max</sub>: 1.13 | Moderate correlation between oxygen measurements and $^{18}$F-FMISO uptake. Low correlation between $^{18}$F-FDG and $^{18}$F-FMISO uptake |
| Lee et al (2008) | $^{18}$F-FMISO | H&N            | 20  | Static scan at 120–150 min p.i. | Variable $^{18}$F-FMISO distribution |
| Nehmeh et al (2008) | $^{18}$F-FMISO | H&N            | 13  | SUV 1.9–4.5 at 117–195 p.i. | Variable $^{18}$F-FMISO distribution |
| Dirix et al (2009) | $^{18}$F-FMISO | H&N            | 15  | Hypoxic volume<sub>pre-therapy</sub>: 4.1 ml T:B<sub>pre-therapy</sub>: 1.5 Hypoxic volume<sub>post-therapy</sub>: 0.3 ml T:B<sub>post-therapy</sub>: 1.2 at 120–160 min p.i | Disease-free survival correlates negatively with baseline T:B<sub>max</sub> and initial hypoxic volume |
| Lee et al (2009) | $^{18}$F-FMISO | H&N            | 28  | Hypoxic volume<sub>pre-therapy</sub>: 4.2 ml T:B<sub>pre-therapy</sub>: 1.46 Hypoxic volume<sub>post-therapy</sub>: 2.4 (range, 1.1–4.4) T:M<sub>max</sub>: 1.6 | Heterogeneous distribution of $^{18}$F-FMISO noted in the primary and/or nodal disease in 90% of patients |
| Abolmaali et al (2011) | $^{18}$F-FMISO | H&N            | 23  | SUV<sub>max</sub>: 2.2 (range, 1.3–3.4) T:M<sub>max</sub>: 1.46 SUV<sub>max</sub>: 2.4 (range, 1.1–4.4) | $^{18}$F-FMISO contrast increases 2–4 h p.i. |
| Reference          | Tracer  | Tumour type(s) | N | Tracer retention (TBR; SUV) | Results                                                                                     |
|--------------------|---------|----------------|---|-----------------------------|---------------------------------------------------------------------------------------------|
| Kikuchi et al (2011) | 18F-FMISO | H&N            | 17 | Median SUV<sub>max</sub>: 2.3 | Disease-specific survival was significantly lower in patient group with high basal 18F-FMISO SUV<sub>max</sub> and T:M<sub>max</sub> |
| Yamane et al (2011)  | 18F-FMISO | H&N            | 13 | SUV<sub>max</sub>: 2.2 (range, 0.7–3.6) | 18F-FMISO SUV<sub>max</sub>, T:M and hypoxic volume significantly decreased after neo-adjuvant chemotherapy |
| Sato et al (2013)   | 18F-FMISO | H&N            | 23 | Median SUV<sub>max</sub>: 1.83 (range, 0.8–2.7) | Weak significant correlation between 18F-FMISO and 18F-FDG SUV<sub>max</sub> was significantly higher in HIF-1α-positive cases than in HIF-1α-negative cases |
| Mortensen et al (2010) | 18F-FMISO | Sarcoma        | 19 | T:M<sub>median</sub>: H&N: 1.68 (range, 0.7–2.38) | No correlation between 18F-FMISO retention and oxygen electrode |
| Koh et al (1995)    | 18F-FMISO | Lung           | 7  | Static scan at 120–180 p.i. | Radiotherapy reduced median fractional hypoxic volume from 58 to 22% |
| Cherk et al (2006)  | 18F-FMISO | Lung           | 21 | SUV: 0.4–2.14; T:N: 1.18–9.73 at 120 min p.i. | Low 18F-FMISO uptake. Poor correlation between 18F-FMISO and 18F-FDG uptake |
| Gagel et al (2006)  | 18F-FMISO | Lung           | 8  | SUV<sub>mean</sub>: pre-therapy: 2.31 ± 0.2 | 18F-FMISO can define hypoxic sub-regions. Changes in FMISO and 18F-FDG PET measure early response to therapy |
| Vera et al (2011)   | 18F-FMISO | Lung           | 5  | SUV<sub>max</sub>, pre-therapy: 1.2–2.5 | 18F-FMISO uptake higher in tumours than in nodes and did not change during therapy |
| Thureau et al (2013) | 18F-FMISO | Lung           | 10 | —                            | Low reproducibility and inter-observer agreement for 18F-FMISO volume measurements on the basis of visual scoring. T:M ≥ 1.4 recommended for hypoxic volume delineation |
| Segard et al (2013) | 18F-FMISO | Pancreatic     | 10 | Mean SUV<sub>max</sub>: 2.3 (range, 1–3.4) | 18F-FMISO accumulation observed in 2/10 patients on the basis of visual analysis. Minimal 18F-FMISO accumulation in pancreatic tumours; correlation with other imaging modalities required to allow tumour localisation and semi-quantitative analysis |
| Hugonnet et al (2011) | 18F-FMISO | Renal          | 53 | Static scan at 120 min p.i. | Reduction in hypoxic volume post-therapy |
| Roels et al (2008)  | 18F-FMISO | Rectal         | 15 | —                           | Mismatch between 18F-FDG and 18F-FMISO scans. 18F-FMISO uptake reduced after therapy |
| Bentzen et al (2003) | 18F-FMISO | Sarcoma        | 13 | T:M ≤ 1–1.6                 | 18F-FMISO accumulation observed in 2/7 malignant tumours. No correlation between 18F-FMISO and pO2 measurements |
| Rajendran et al (2003) | 18F-FMISO | Sarcoma        | 19 | T:B<sub>max</sub>: 1.10–3.46 at 120 min p.i. | 18F-FMISO uptake observed in 14 patients. Poor correlation between tumour grade, hypoxia volume and 18F-FDG T:B |
| Reference            | Tracer     | Tumour type(s) | N  | Tracer retention (TBR; SUV)                                                                 | Results                                                                 |
|----------------------|------------|----------------|----|-------------------------------------------------------------------------------------------|------------------------------------------------------------------------|
| Rajendran et al (2004) | 18F-FMISO  | Brain, Breast, H&N, Sarcoma | 49 | T.Bmax; Brain 2.43 (range, 1.7–2.9)  
Breast 1.52 (range, 0.93–2.6)  
H&N: 1.5 (range, 0.88–2.4)  
Sarcoma: 1.46 (range, 1.1–2.1)  | Hypoxia detected in all tumour types. Low correlation between glucose metabolism and hypoxia |
| Schuetz et al (2010) | 18F-FAZA   | Cervical       | 15 | T.Mmax: 1.2–3.6 at 60 min and 120 min p.i.  
T.Mmax: 1.2 at 240 min p.i. | 5/15 patients had visually identifiable tumours. |
| Grosu et al (2007)   | 18F-FAZA   | H&N            | 18 | T.Mmax: 1.6  
T.Mmax: 2 at 60 min p.i.  | 18F-FAZA uptake located in single confluent region in 11/18 patients and at multiple diffuse regions in 4/18 patients |
| Souvatzeoglou et al (2007) | 18F-FAZA | H&N            | 11 | SUVmax: 2.3 (range, 1.5–3.4)  
SUVmean: 1.4 (range, 1–2)  \  
T.M: 2 (range, 1.6–2.4) | T:M ratio increased 60 min p.i. All tumours had T:M > 1.5. Tumour volume with T:M > 1.5 was highly variable |
| Mortensen et al (2012) | 18F-FAZA   | H&N            | 40 | Median T.Mmax: 1.5 at 120 min p.i. Hypoxia threshold: SUV > 1.4 | High uptake associated with lower disease-free survival. Radiotherapy treatment reduced hypoxic volume |
| Bollineni et al (2013) | 18F-FAZA  | Lung           | 11 | Median T:B: 2.8 (range, 1.8–4.6)  
T:B > 1.2 for hypoxic volume definition | Not significant correlation between 18F-FAZA T:B and 18F-FDG SUVmax or lesion size. Heterogeneous intratumoral distribution for 18F-FAZA-based visual analysis. 18F-FAZA PET is able to detect heterogeneous distributions of hypoxic sub-volumes |
| Trinkhaus et al (2013) | 18F-FAZA  | Lung           | 17 | — | 11/17 patients had baseline hypoxia based on qualitative assessment. 6/8 patients with scans following chemoradiation had resolution of hypoxia on the basis of quantitative analysis |
| Garcia-Parra et al (2011) | 18F-FAZA   | Prostate       | 14 | T.Nmean: 1.21 | 18F-FAZA uptake not increased in tumours. No evidence of hypoxia as assessed by CaIX IHC staining |
| Havelund et al (2013) | 18F-FAZA   | Rectal         | 14 | T.Mmean: 2.83 | 18F-FAZA-PET is feasible for visualisation of hypoxia in rectal cancer |
| Postema et al (2009) | 18F-FAZA   | H&N, Lung, Lymphoma, Glioma | 50 | SUVmax: 1.05–2.35  
Lung TBR: 1.3–3.7;  
Lymphoma TBR: 1.2–3;  
Glioma TBR: 1.9–15.6 At 120–180 min p.i. | High TBR in all 7 gliomas; high TBR, SUVmax observed in 6/9 H&N tumours; moderate TBR, SUVmax in 3/21 lymphomas; increased TBR, SUVmax in 7/11 lung patients |
| Lehtio et al (2001)  | 18F-FETNIM | H&N            | 8  | T.Mmax: 1–4 at 3 h p.i.  | Tumour distribution volume correlated strongly with 18F-FETNIM SUV between 60 and 120 min p.i. and blood flow, but not with 18F-FDG SUV. Values compare favourably with 18F-FMISO data. Late time-point 18F-FETNIM T:M are indicative of hypoxia |
| Lehtio et al (2003)  | 18F-FETNIM | H&N            | 10 | Median T:M: 1.41 (range, 0.86–2)  
Median T.Pmean: 0.96 (range, 0.74–1.1)  
Median T.Pmax: 1.29 (range, 0.91–1.98) | T:P is good estimate of tumour hypoxia |
| Lehtio et al (2004)  | 18F-FETNIM | H&N            | 21 | Median T.Pmax: 1.10 (range, 0.81–1.98)  
T.P>0.93 used for hypoxic volume definition | Patients with higher fractional hypoxic volumes and T:P correlated with poorer survival |
| Hu et al (2013)      | 18F-FETNIM | Lung           | 42 | SUVmax, Tumour: 2.43  
SUVmax, Normal: 0.87  
T.N: 2.48 at 120 min p.i. | SUVmax, higher in tumours than in normal tissue. Similar data observed at 60 and 120 min p.i. |
| Li et al (2010)      | 18F-FETNIM | Lung           | 26 | — | 18F-FETNIM T:B ratio and hypoxic volume were strong predictors for overall survival. No correlation between 18F-FETNIM and 18F-FDG uptake |
| Vercellino et al (2012) | 18F-FETNIM | Cervical       | 16 | T.M: 1.3–5.4 | High uptake associated with lower progression free and overall survival |
| Yue et al (2012)     | 18F-FETNIM | Oesophageal    | 28 | SUVmax, complete response: 3.2  
SUVmean, complete response: 2.1  
SUVmax, partial response: 4.5  
SUVmean, partial response: 2.9  
SUVmax, stable disease: 5.9  
SUVmean, stable disease: 3.2  
Threshold for hypoxia  
SUVmax/SUVmean,pleural: 1.3 | SUVmax and SUVmean are reproducible. High baseline SUVmax associated with poor clinical response |
18F-fluoroerythronitroimidazole. 18F-fluoroerythronitroimidazole (18F-FETNIM) studies in head-and-neck (Lehtio et al, 2001, 2003), lung (Li et al, 2010; Hu et al, 2013), and oesophageal cancer Yue et al (2012) calculated T:M in the range of 1.4–2.48 at 2 h p.i. High tumour-to-muscle values were found to be indicative of reduced progression-free and overall survival in lung (Li et al, 2010; Hu et al, 2013), head-and-neck (Lehtio et al, 2004), oesophageal (Yue et al, 2012), and cervical (Vercellino et al, 2012) tumours. Clinical studies with 18F-FETNIM have been mainly carried out at the University of Turku, Finland. 18F-fluoroerythronitroimidazole is not being used at present in the United Kingdom or in the United States.

18F-RP-170. More recently, RP-170 (1-2-1-(1H-methyl)ethoxy)-methyl-2-nitroimidazole, another 2-nitroimidazole-based hypoxic radiosensitiser, has also been labelled with 18F. The hypoxic selectivity of 18F-FRP-170 was demonstrated in glioma patients on the basis of significant correlations between uptake, oxygen tension measurements and HIF-1α immunostaining (Beppu et al, 2014). Studies in brain (Shibahara et al, 2010; Beppu et al, 2014) and lung (Kaneta et al, 2007) tumours indicated higher SUV for hypoxic non-hypoxic tumours was 71%, and 28% for those with hypoxic tumours. Overexpression of VEGF, EGFR, COX2, CAIX and increased apoptosis observed in hypoxic tumours.

Table 2. (Continued)

| Reference          | Tracer         | Tumour type(s) | N  | Tracer retention (TBR; SUV) | Results                                                                 |
|--------------------|----------------|----------------|----|----------------------------|-----------------------------------------------------------------------|
| Zegers et al (2013)| 18F-HX4        | Lung           | 15 | SUV<sub>max</sub>, 1.47 ± 0.36 | T:B<sub>max</sub>, >1.4 at 240 min p.i. was observed in 80% of the primary tumours and 60% of lymph-node regions. T:B<sub>max</sub> increased over acquisition time, although pattern stabilised between 120 and 180 min p.i. |
| Kaneta et al (2007)| 18F-FRP170     | Normal lung    | 4/3| T:M<sub>1h</sub>, 1.69     | T:B stable at 60–120 min p.i. Images obtained 60 min p.i. may allow evaluation of tumour accumulation in a clinical setting |
| Shibahara et al (2010)| 18F-FRP170   | Brain          | 8  | SUV<sub>max</sub>, 1.3–2.3 | SUV<sub>max</sub> correlated positively with HIF-1α immunostaining |
| Beppu et al (2014)| 18F-FRP170     | Brain          | 12 | SUV<sub>mean</sub>, Tumour: 1.58 ± 0.35 | Significant correlation between T:N, pO<sub>2</sub>, and strong nuclear immunostaining for HIF-1α in areas of high 18F-FRP-170 accumulation 60 min p.i. in glioblastoma patients |
| Dehdashti et al (2003a, b) | 60Cu-ATSM | Cervical       | 14 | T:M 3.4 ± 2.8 | Tumour uptake of 60Cu-ATSM inversely related to progression-free survival and overall survival. No correlation between FDG and 60Cu-ATSM uptake |
| Grigsby et al (2007) | 60Cu-ATSM      | Cervical       | 15 | —                          | 4 year overall survival estimates were 75% for patients with non-hypoxic tumours and 33% for those with hypoxic tumours. Overexpression of VEGF, EGFR, COX2, CAIX and increased apoptosis observed in hypoxic tumours |
| Dehdashti et al (2008) | 60Cu-ATSM   | Cervical       | 38 | T:M 3.8 ± 2.0 | Tumour uptake of 60Cu-ATSM was inversely related to progression-free survival and cause-specific survival. 3-year progression-free survival of patients with non-hypoxic tumours was 71%, and 28% for those with hypoxic tumours |
| Minagawa et al (2011) | 60Cu-ATSM      | H&N            | 15 | Mean SUV<sub>max</sub>, 5.5 ± 1.7 | All 5 patients with SUV<sub>max</sub> < 5 were complete responders |
| Dehdashti et al (2003a, b) | 60Cu-ATSM | Lung           | 19 | Mean T:M<sub>pre-therapy</sub>, 3.3 ± 1 | Imaging with 60Cu-ATSM feasible in NSCLC. Mean T:M lower in responders than in non-responders. Mean SUV not different between these groups |
| Dietz et al (2008) | 60Cu-ATSM      | Rectal         | 19 | T:M 2.5 ± 0.9 at 30–60 min p.i. | Median tumour-to-muscle activity ratio of 2.6 discriminated those with worse prognosis from those with better prognosis. Overall and progression-free survival worse in hypoxic tumours |
| Lohith et al (2009) | 62Cu-ATSM      | Lung           | 13 | SUV<sub>mean</sub>, SCC: 1.95 ± 0.88 | 18F-FDG and 60Cu-ATSM had spatially similar distributions in adenocarcinomas |

Abbreviations: CAIX = carbonic anhydrase IX, EGFR = epidermal growth factor, H&N = head and neck cancer, N = number of patients; NSCLC = non-small cell lung cancer, pO<sub>2</sub> = partial oxygen pressure; p.i. = post injection; RT = radiotherapy; SUV = standardised uptake value; T:B = tumour-to-plasma ratio; T:B<sub>max</sub> = tumour-to-plasma ratio at maximum SUV; T:M = tumour-to-muscle ratio; T:N = tumour-to-normal tissue ratio; VEGFR = vascular endothelial growth factor.
Imaging tumour hypoxia with PET

For nitromidazole-based analogues (FMISO, FAZA, FETNIM, HX4, FRP-170) values are given for acquisitions performed at 120 min post tracer administration. For Cu-ATSM, values are presented for scans conducted 60 min. (2-4 h p.i.) can further enhance the hypoxic-to-normoxic signal. In all of the above tracers, the more accurate hypoxic measure is made at least 2 h p.i., but the trade-off is the reduced radioactivity and noisier data.

**CU-DIACETYL-BIS(N4-METHYLTHIOSEMICARBAZONE)**

An alternative class of agents for the study of hypoxia with PET is based on a complex of Cu with diacetyl-bis(N\(^4\)-methylthiosemicarbazone) (ATSM) ligands, among which ATSM is the prototype. Due to its lipophilicity and low molecular weight, Cu-ATSM is characterised by high membrane permeability and therefore rapid diffusion into cells. The hypoxic specificity of Cu-ATSM is thought to be partly imparted by the intracellular reduction of Cu(II) to Cu(I) combined with re-oxidation by intracellular molecular oxygen. Under hypoxic conditions, the unstable Cu(I)-ATSM complex may further dissociate into Cu(I) and ATSM, leading to the intracellular trapping of the Cu(I) ion. In the presence of oxygen, the [Cu(I)-ATSM\(^-\)] can be re-oxidised to its parent compound, allowing efflux from the cell (Dearling and Packard, 2010).

Tumour-specific Cu-ATSM retention has been demonstrated for head-and-neck (Minagawa *et al*, 2011; Nyfölt *et al*, 2012) (Figure 3B), lung (Takahashi *et al*, 2000; Dehdashi *et al*, 2003a, b; Lohith *et al*, 2009), cervical (Dehdashi *et al*, 2003a, b; Grigsby *et al*, 2007; Lewis *et al*, 2008; Dehdashi *et al*, 2008), rectal tumours (Dietz *et al*, 2008) and gliomas (Tateishi *et al*, 2013). Hypoxia specificity may be dependent on tumour type: preclinical studies showed good correlation in the intratumour distribution of Cu-ATSM and \(^{18}\)F-FMISO in a DaFu squamous carcinoma model but not at early time points in an R3327-AT anaplastic rat prostate tumour (O’Donoghue *et al*, 2005). A recent study has raised concerns about the hypoxic specificity of Cu-ATSM, as hepatic metabolism of the compound results in images that reflect the behaviour of ionic Cu (uptake of which may itself be hypoxia-related) rather than Cu-ATSM itself, especially at later time points (1-24 h) (Hueting *et al*, 2014). Of concern is also the fact that while some preclinical studies show that tumour uptake of hypoxia-selective Cu-ATSM analogues (e.g., Cu-ATSE) decreases with increased oxygenation (McQuade *et al*, 2005), another report showed that increased oxygenation resulted in a decrease in uptake of FMISO, but not of Cu-ATSM (Matsumoto *et al*, 2007). Nevertheless, \(^{64}\)Cu-ATSM retention has been shown to correlate clinically with poor prognosis (Dehdashi *et al*, 2003a, b; 2008; Grigsby *et al*, 2007; Dietz *et al*, 2008). Attempts to investigate the relationship between the intratumoural distribution of Cu-ATSM with histological and other hypoxia markers have also yielded both positive and negative correlations. Although it appears to be premature to reject Cu-ATSM on the grounds of hypoxic non-specificity, further studies are required to elucidate the in vivo behaviour of this tracer to allow for better interpretation of the imaging information. The development of second-generation Cu-ATSM analogues, with reduced lipophilicity and improved hypoxia selectivity and sensitivity, appears to be a promising alternative to Cu-ATSM (Handley *et al*, 2014). Cu-ATSM has several potential advantages relative to other tracers for the imaging of tumour hypoxia, including simpler synthesis/radiolabelling methodology and faster clearance from normoxic tissues, which allows shorter intervals between injection and imaging and higher hypoxic-to-normoxic contrast. Notwithstanding the limited availability of Cu isotopes, \(^{64}\)Cu-ATSM is currently being produced at a few research sites, and due to the 12-h half-life could potentially be utilised for clinical studies.

**CLINICAL APPLICATIONS OF PET HYPOXIA IMAGING**

Identification of tumour hypoxia and prediction of prognosis/response to treatment. Identifying individuals with poor prognosis and those likely to benefit from hypoxia-targeted therapy are important objectives of PET hypoxia research. Several studies have shown that PET hypoxia imaging can provide information on prognosis. High \(^{18}\)F-FMISO retention has been associated with higher risk of loco-regional failure and shorter progression-free survival in head-and-neck (Rischin *et al*, 2006; Rajendran *et al*, 2006; Thorwarth *et al*, 2006; Dirix *et al*, 2009; Lee *et al*, 2009; Kikuchi *et al*, 2011) and renal cancer (Hugonet *et al*, 2011). Furthermore, a meta-review of the clinical data of over 300 patients concluded that FMISO is a predictor of poor treatment response and prognosis (Lee and Scott, 2007). Similar results have been reported for \(^{18}\)F-FETNIM in lung (Li *et al*, 2010), head-and-neck (Lehtio* et al*, 2004), and oesophageal cancer (Yue *et al*, 2012), where high tumour-to-reference tissue values were also associated with poor patient outcomes. Studies conducted with \(^{18}\)F-FAZA in...
squamous cell carcinomas of the head and the neck (Mortensen et al., 2012) and Cu-ATSM in patients with cervical (Dehdashti et al., 2003a, b; Grigsby et al., 2007), lung (Dehdashti et al., 2003a, b), and rectal cancer (Dietz et al., 2008) have also demonstrated that lower tumour-to-muscle ratios are indicative of better prognosis, progression-free and overall survival. A meta-analysis of published PET hypoxia studies has demonstrated a common tendency towards poorer outcome in tumours showing higher tracer accumulation (Horsman et al., 2012). Decreased $^{18}$F-FMISO uptake in response to radio- or chemotherapy has been reported in brain (Swanson et al., 2009), head-and-neck (Yamane et al., 2011; Eschmann et al., 2007), lung (Koh et al., 1995; Gagel et al., 2006), and renal tumours (Hugonet et al., 2011); although some studies did not observe an analogous decrease with response to therapy (Thorwarth et al., 2006; Vera et al., 2011). Decreased tumour-to-muscle ratios signifying full or partial response to chemotherapy have also been obtained with Cu-ATSM in lung (Dehdashti et al., 2003a, b) and head-and-neck tumours (Minagawa et al., 2011), and $^{18}$F-FAZA in lung cancer (Trinkaus et al., 2013).

### Table 3. Matrix summarising clinical imaging findings with leading hypoxia tracers

| Tumour type       | $^{18}$F-FMISO | $^{18}$F-HX4 | $^{18}$F-FAZA | $^{18}$F-FETNIM | $^{18}$F-EFS | $^{18}$F-FRP170 | Cu-ATSM |
|-------------------|----------------|--------------|--------------|----------------|-------------|---------------|---------|
| Brain             | Yes            | Not recommended | Yes          | Yes            | Yes         | Yes           | Yes     |
| Head & Neck       | Yes            | Yes          | Yes          | Yes            | Yes         | Yes           | Yes     |
| Breast            | Yes            | Yes          | Yes          | Yes            | Yes         | Yes           | Yes     |
| Sarcoma           | Variable data  |              |              |                |             |               |         |
| Lung              | Yes            | Yes          | Yes          | Yes            | Yes         | Yes           | Yes     |
| Lymphoma          |                |              |              |                |             |               |         |
| Renal             | Variable data  | Not recommended | Not recommended | Not recommended | Not recommended | Not recommended | Recommended |
| Liver             | Not recommended | Recommended | Not recommended | Not recommended | Not recommended | Not recommended | Yes     |
| Colorectal        | Not recommended | Yes          | Not recommended | Not recommended | Not recommended | Not recommended | Yes     |
| Bladder           | Not recommended | Not recommended | Not recommended | Not recommended | Not recommended | Recommended   |         |
| Cervical          | Yes            | Yes          | Yes          | Yes            |             |               |         |
| Prostate          | No             |              |              |                |             |               | Not recommended |

Note: Yes — good clinical data obtained. No — poor clinical data obtained. Not recommended — preclinical/metabolic data unfavourable. Recommended — preclinical/metabolic data favourable.

### RADIOThERAPY PLANNING

In oncology, there is interest in the identification of intratumoural areas with hypoxia to guide radiation dose escalation to radio-resistant sub-volumes. Despite possible limitations associated with the reproducibility of hypoxic volume measurements (temporal changes and/or heterogeneity in the spatial distribution of intratumoural hypoxia), the biological information from PET hypoxia scans is being explored for the identification and delineation of hypoxic areas within the tumour mass for dose escalation. Modern radiation techniques, such as intensity modulated radiotherapy (IMRT) or image-guided radiotherapy (IGRT) can help with radiotherapy planning (Horsmann et al., 2012). ‘Dose painting’ by numbers, where a higher radiation dose is selectively delivered to areas of biological resistance identified either before or during the treatment course, has also been suggested (Geets et al., 2013). The feasibility of dose escalation to hypoxic sub-volumes has been primarily investigated in cancers of the head and neck, lung, and brain, and demonstrated with Cu-ATSM (Chao et al., 2001), $^{18}$F-FMISO (Lee et al., 2008), and $^{18}$F-FAZA (Grosu et al., 2007). Despite the fact that the majority of the aforementioned studies have not been conducted on actual patients, but on anthropomorphic phantoms (in silico) (Rischin et al., 2006; Grosu et al., 2007; Lee et al., 2008), dose escalation on the basis of PET hypoxia imaging appears to be feasible, and further studies are required to investigate whether this can translate into clinical benefit.

### HYPOXIA THERAPEUTICS

As the hypoxic microenvironment constitutes a unique characteristic of tumours, hypoxia can also be harnessed as a therapeutic target. The main strategies for targeting hypoxia involve hypoxic cell radiosensitisers (e.g., nimorazole), hypoxic cell cytotoxins (e.g., tirapazamine, TH-302, and PR-104A); and altering oxygen delivery (e.g., carbogen plus nicotinamide). Other approaches being investigated include hypoxia-selective gene therapy, altering metabolic pathways essential for survival under stress, and inhibitors of molecular targets activated in hypoxia (e.g., HIF-1) (Wilson and Hay, 2011). Imaging hypoxia with PET could facilitate the development of therapeutic agents by identifying patients with hypoxic tumours, and measuring response to hypoxia-modifying treatments providing a basis for individualising hypoxia-specific treatment, and/or assessing drug efficacy. Furthermore, it will allow development of new predictors and answer key questions, such as the relation of baseline or induced hypoxia to response to anti-angiogenic drugs and the relation of baseline hypoxia to response to hypoxic-activated toxins. Such studies should be incorporated into trials of these agents routinely, to develop the necessary validation for their utility. This would greatly help the personalised and economic use of such therapies, which will be even more important if used in combination, for example, anti-angiogenics and hypoxia-activated toxins. The potential of PET hypoxia imaging in directing hypoxia therapeutics has been clinically demonstrated with tirapazamine with $^{18}$F-FMISO in head and neck tumours, whereby only those with hypoxia benefited from bioreductive drugs (Rischin et al., 2006; Overgaard, 2011).

### CONSIDERATIONS

The ‘ideal’ PET tracer for tumour hypoxia. Table 3 presents a summary of clinical imaging findings with the hypoxia tracers discussed in this review. None of the currently available tracers have all the properties that constitute the ideal PET hypoxia tracer, and therefore none is optimal for imaging hypoxia in all...
cancer types. Nevertheless, the feasibility of imaging hypoxia with PET has been clinically demonstrated in various tumour entities using several of the existing radiotracers. Much of the radiotracer selection stems from the availability of the tracer, ease of synthesis, and the tumour type.

The magnitude of the challenge of PET hypoxia imaging. A challenging aspect of PET hypoxia imaging is the fact that hypoxic tumours are often hypoperfused. Limited perfusion will restrict effective delivery of tracer into the tissue often, influencing tracer accumulation in regions of normal or tumour tissue, and often yielding results that are complex to interpret. Several studies have compared tumour perfusion with dynamic PET to ascertain whether tracer accumulation reflects blood flow during imaging. 18F-FMISO (Bruehlmeier et al, 2004), 18F-FETNIM (Lehtiö et al, 2001), and 18F-FAZA (Shi et al, 2010) exhibited similar distribution patterns to [15O]-H2O PET (reflecting blood flow) up to 15 min p.i., while different patterns were observed at later imaging times, consistent with tracer accumulation in hypoxic regions. Pharmacokinetic analysis of 18F-FMISO data suggests that different hypoxia-perfusion profiles can be identified in tumours (Thorwarth et al, 2005), the latter perhaps corresponding with the heterogeneity observed in tumour hypoxia distribution patterns (Grosa et al, 2007). The significant heterogeneity of the tumour microenvironment in terms of perfusion and hypoxia necessitates further clinical studies, not only to evaluate hypoxia-perfusion patterns, but also their relationship to clinical outcome.

Validation of PET hypoxia measurements. Validation of PET tracers as indicators of regional hypoxia is extremely challenging and attempts to correlate PET images with other accepted hypoxia markers have produced mixed and contradictory results. While oxygen electrodes are considered to be the gold standard against which PET hypoxia measurements are authenticated, comparisons may yield several discrepancies due to the sampling limitations of oxygen probes and the fact that it measures hypoxia in a different location (interstitial for oxygen probes vs intracellular for PET), as well as the fact that this technique will fail to distinguish between necrotic and viable hypoxic tissue (Höckel et al, 1993). This may partly explain results from several studies that have reported mixed correlations between tracer uptake and oxygen electrode measurements in various tumour types (Bentzen et al, 2003; Gagel et al, 2004, 2007; Zimny et al, 2006; Mortensen et al, 2010). Indirect immunohistochemical methods based on the detection of exogenous (e.g., pimonidazole and EF5) or endogenous hypoxia markers (e.g., CAIX and HIF-1) have also been employed (Dehdashti et al, 2003a, b; Jubb et al, 2010), albeit with limited success. This is primarily due to the fact that comparisons as such rely on reproducible staining, and several representative biopsies (which are not always available), and may often require a technically challenging spatial co-registration between PET images with immunohistochemistry photographs for analogies to be drawn. Of note is the fact that although tracer accumulation has been widely compared with pimonidazole staining preclinically (Dubois et al, 2004), equivalent clinical comparisons have not yet been performed. The differential detection of acute and chronic hypoxia and the discrepancy between hypoxia at the microscopic level and the macroscopic resolution of the PET voxel are factors that will also limit the accuracy of such comparisons (Mortensen et al, 2010).

Reproducibility of PET hypoxia measurements. Validation of the reproducibility of PET hypoxia measurements is also particularly important for clinical applications. There are limited clinical data available on scan reproducibility with PET hypoxia biomarkers. Studies with 18F-FMISO in head-and-neck cancer reported reproducible hypoxic volumes in PET scans performed 3 days apart, but a considerable degree of intratumoural spatial variability in tracer accumulation (Nehmeh et al, 2008). Another study with 18F-FMISO in lung cancer showed good inter-observer reproducibility on the basis of visual analysis, but low inter-observer agreement with respect to hypoxic volume measurements (Thureau et al, 2013). A more recent 18F-FMISO study in head-and-neck cancer reported high reproducibility in SUV and tumour-to-reference tissue measurements in scans acquired 2 days apart (Okamoto et al, 2013). Other than 18F-FMISO, a study with 18F-FETNIM in oesophageal cancer patients observed similar uptake values between scans performed on separate days before concurrent chemoradiotherapy, but a shift in the geographical location of hypoxic regions (Yue et al, 2012). These heterogeneous findings can be partly explained by the dynamic character of hypoxia that will limit scan reproducibility. Although acute hypoxia has been shown to minimally influence 18F-FMISO PET imaging in simulations (Mönnich et al, 2012), a study in head-and-neck tumours that used sequential 18F-FMISO scans to distinguish between regions of acute and chronic hypoxia, accounted for 14–52% of acute hypoxia (Wang et al, 2009); a percentage that is comparable to the proportion of acute hypoxia measured in rodent tumours. Methodological discrepancies (scan setup and image acquisition protocol), the selection of hypoxic-to-normoxic thresholds for the definition of hypoxic regions, the temporal variability in intratumoural PO2 levels between consecutive measurements, as well as the small number of patients in the majority of the studies may also account for the observed disparities in reproducibility. Further studies addressing the variability of PET hypoxia measurements are warranted, so as to clarify uncertainties in tumour hypoxia quantification.

CONCLUSIONS

As a number of PET hypoxia tracers have now been evaluated in cancer patients, it is apparent that PET imaging can be a powerful tool to identify hypoxia in the clinical setting. Although none of the currently available tracers exhibit all of the properties of the ‘ideal’ hypoxia tracer or are optimal for imaging hypoxia in all tumour types, studies have demonstrated the feasibility for imaging hypoxia in various cancers. As the clinical utility and limitations of PET hypoxia biomarkers are now being elucidated the process will be facilitated by performing larger studies with these tracers using standardised protocols and hypoxia definitions so as to improve comparison between tracers in various tumour types. This may be best achieved via inter-institutional collaborations that should help to advance study designs and homogeneous data reporting. Equally important are the performance of test–retest studies, harmonisation of data reporting, and clinical validation of hypoxia tracers. These key objectives must be addressed before PET hypoxia tracers can be used to their full clinical utility.

Search strategy and selection criteria. We searched PubMed and Scopus using combinations of the following search terms: ‘tumor hypoxia’, ‘oncology’, ‘PET’, ‘positron emission tomography’, radiotherapy’, ‘nitroimidazoles’, ‘fluoromisonidazole’, ‘pimonidazole’, ‘FMISO’, ‘FAZA’, ‘FETNIM’, ‘FRP-170’, ‘HX4’, ‘Cu-ATSM’. The search results were screened for relevance and the reference lists of relevant publications were also surveyed. PubMed and Scopus article recommendations were also examined for relevance. Only papers published in English were considered. The final reference list was compiled by considering papers published between January 1973 and May 2014.
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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

INF contributed organisation of the hypoxia workshop, literature search and wrote core manuscript and edited various versions of manuscript, approved final version of the manuscript. RM contributed to literature search, edited manuscript, prepared Figure 2, approved final version of the manuscript. PJB attended the hypoxia workshop, wrote Cu-ATSM section and edited various versions of manuscript, approved final version of the manuscript. CW attended the hypoxia workshop, wrote radiotherapy section, approved final version of the manuscript. SL attended the hypoxia workshop, approved final version of the manuscript. KJW attended the hypoxia workshop, wrote therapeutics section, approved final version of the manuscript. ALH contributed organisation of the hypoxia workshop, literature search, wrote hypoxia section, approved final version of the manuscript. FJG attended the hypoxia workshop, wrote Cu-ATSM section and edited various versions of the manuscript. RM is also supported by the Chief Scientific Office. ALH is supported by Cancer Research UK and the Breast Cancer Research Foundation. RM is also supported by the Chief Scientific Office.

REFERENCES

Abolmaali N, Haase R, Koch A, Zips D, Steinbach J, Baumann M, Kotzerke J, Zöphel K (2011) Two or four hour [18F]FMISO-PET in HNSCC: When is the contrast best? Nuklearmedizin 50(1): 22–27.
Bentzen L, Keiding S, Nordmark M, Falborg L, Hansen SB, Keller J, Nielsen OS, Overgaard J (2003) Tumour oxygenation assessed by 18F-fluoromisonidazole PET and polarographic needle electrodes in human soft tissue tumours. Radiother Oncol 67(3): 339–344.
Beppu T, Terasaki K, Sasaki T, Fujii T, Matsura H, Ogasawara K, Sera K, Yamada N, Uesagi N, Sugai T, Kudo K, Sasaki M, Ehara S, Iwata R, Takai Y (2014) Standardized uptake value in high uptake area on positron emission tomography with 18F–HX4 as a predictor of primary endocrine therapy resistance in breast cancer. J Nucl Med 54(3): 333–340.
Chao KSC, Bosch WR, Muts S, Lewis JS, Dehdashi F, Mintun MA, Dempsey JF, Perez CA, Purdy JA, Welch MJ (2001) A novel approach to overcome hypoxic tumor resistance: Cu-ATSM-guided intensity-modulated radiation therapy. Int J Radiat Oncol Biol Phys 49(4): 1171–1182.
Chapman JD (1979) Hypoxia sensitisers – Implications for radiation therapy. N Engl J Med 301(26): 1429–1432.
Chen L, Zhang Z, Kolb HC, Walsh JC, Zhang J, Guan Y (2012) 18F–FMISO hypoxia imaging with PET/CT in head and neck cancer: A comparison with 15O-PET. Nucl Med Commun 33(10): 1096–1102.
Cheng J, Lei L, Xu J, Sun L, Wang X, Pan L, Shao Z, Zhang Y, Liu G (2013) 18F-fluoromisonidazole (FMISO)-PET/CT: a potential tool for predicting primary endocrine therapy resistance in breast cancer. J Nucl Med 54(3): 333–340.
Cher LM, Murone C, Lawrentschuk N, Ramsdell S, Papenfuss A, Anhanna A, O’Keefe GJ, Sachinidis JI, Berlangieri SU, Fabinyi G, Scott AM (2006) Correlation of hypoxic cell fraction and angiogenesis with glucose metabolic rate in gliomas using 18F-fluoromisonidazole, 18F-FDG PET, and immunohistochimical studies. J Nucl Med 47(3): 410–418.
Cherkez MH, Foo SS, Poon AMT, Knight SR, Murone C, Papenfuss AT, Sachinidis JI, Sauder TH, O’Keefe GJ, Scott AM (2006) Lack of correlation of hypoxic cell fraction and angiogenesis with glucose metabolic rate in non-small cell lung cancer assessed by 18F-fluoromisonidazole and 18F-FDG PET. J Nucl Med 47(12): 1921–1926.
Dearling ILJ, Packard AB (2010) Some thoughts on the mechanism of cellular trapping of Cu(II)-ATSM. Nucl Med Biol 37(3): 237–243.
Dehdashi F, Grigoby PW, Mintun MA, Lewis JS, Siegel BA, Welch MJ (2003a) Assessing tumor hypoxia in cervical cancer by positron emission tomography with 60Cu-ATSM: Relationship to therapeutic response – a preliminary report. Int J Radiat Oncol Biol Phys 55(5): 1233–1238.
Dehdashi F, Mintun MA, Lewis JS, Bradley J, Govindan R, Laforest R, Welch MJ, Siegel BA (2003b) In vivo assessment of tumor hypoxia in lung cancer with 60Cu-ATSM. Eur J Nucl Med Mol Imaging 30(6): 844–850.
Dehdashi F, Grigoby PW, Lewis JS, Siegel BA, Welch MJ (2008) Assessing tumor hypoxia in cervical cancer by PET with 60Cu-labeled diacyetyl–bis(N4-methylthiosemicarbazone). J Nucl Med 49(2): 201–205.
Dietz DW, Dehdashi F, Grigoby PW, Malyapa RS, Myerson RJ, Picus J, Ritter J, Lewis JS, Welch MJ, Siegel BA (2008) Tumor hypoxia detected by positron emission tomography with 60Cu-ATSM as a predictor of response and survival in patients undergoing neoadjuvant chemoradiotherapy for rectal carcinoma: a pilot study. Dis Colon Rectum 51(11): 1641–1648.
Dirix P, Vandeveeye V, De Keyzer F, Stroobants S, Hermans R, Nuyts S (2009) Dose painting in radiotherapy for head and neck squamous cell carcinoma: value of repeated functional imaging with 18F-FDG PET, 18F-fluoromisonidazole PET, diffusion-weighted MRI, and dynamic contrast-enhanced MRI. J Nucl Med 50(7): 1020–1027.
Dubois L, Landuyt W, Haustermans K, Verbeken E, Mortelmans L (2004) Evaluation of hypoxia in an experimental rat tumour model by [18F]-fluoromisonidazole and [18F]-fluorodeoxyglucose: an appraisal of radiotherapeutically relevant hypoxia. Strahlenther Onkol 180(10): 616–622.
Gagel B, Reinartz P, DiMartino E, Zimmny M, Pinkawa M, Stanisland S, Hamacher K, Coenen HH, Buehl U, Eble MJ (2004) pO2 polarography versus positron emission tomography. [18F] fluoroquinolone. [18F]-2-fluoro-2-deoxyglucose: an appraisal of radiotherapeutically relevant hypoxia. Strahlenther Onkol 180(10): 616–622.
Gabel B, Reinartz P, Demirel C, Kaiser HJ, Zimmny M, Piroth M, Pinkawa M, Stanisland S, Asadpour B, Hamacher K, Coenen HH, Buehl U, Eble MJ (2006) 18F-fluoromisonidazole and 18F fluorodeoxyglucose positron emission tomography in response evaluation after chemo-radiotherapy of non-small-cell lung cancer: a feasibility study. BMC Cancer 6: 51.
Gabel B, Piroth M, Pinkawa M, Reinartz P, Zimmny M, Kaiser HJ, Stanisland S, Asadpour B, Demirel C, Hamacher K, Coenen HH, Scholbach T, Maneschi P, DiMartino E, Eble MJ (2007) pO2 polarography, contrast enhanced color duplex sonography (CDS), [18F] fluoromisonidazole and [18F] fluorodeoxyglucose positron emission tomography: validated methods for the evaluation of therapy-relevant tumor oxygenation or only bricks in the puzzle of tumor hypoxia? BMC Cancer 7: 113.
Grigsby PW, Malyapa RS, Higashikubo R, Schwarz JK, Welch MJ, Geets X, Gre´goire V, Lee JA (2013) Implementation of hypoxia PET imaging in resectable primary prostate cancer as demonstrated by 18F-FPAZET/CT utilizing multimodality fusion techniques. *Eu J Nucl Med Mol Imaging* 2011: 1–8.

Geets X, Grégoire V, Lee JA (2013) Implementation of hypoxia PET imaging in radiation therapy planning. Q J Nucl Med Mol Imaging 57(3): 271–282.

Grigsby PW, Malyapa RS, Higashikubo R, Schwarz JK, Welch MJ, Huettner PC, Dehdashti F (2007) Comparison of molecular markers of hypoxia and imaging with 60Cu-ATSM in cancer of the uterine cervix. Mol Imaging Biol 9(5): 227–283.

Grosu AL, Souvatzoglou M, Röper B, Dobritz M, Wiedemann N, Jacob V, Wiedenmann N, Jacob V (2008) Hypoxia imaging with FAPET/CT and theoretical considerations with regard to dose painting for individualization of radiotherapy in patients with head and neck cancer. *Int J Radiat Oncol Biol Phys* 69(2): 541–551.

Handley MG, Medina RA, Mariotti E, Mariotti E, Kenny GD, Shaw KP, Garcia–Parra R, Wood D, Shah RB, Siddiqui J, Hussain H, Park H, Desmond T, Meyer C, Piert M, McQuade P, Martin KE, Castle TC, Went MJ, Blower PJ, Welch MJ, Lewis JS (2005) Investigation into 64Cu-labeled Bis(selenocarcabzam) and Bis(thios erbicabzam) complexes as hypoxia imaging agents. *Nucl Med Biol* 32(2): 147–156.

Havelund BM, Holgaard PC, Rafelsen SR, Mortensen LS, Theil J, Bender D, Plen E, Spindler KL, Jakobsen A (2013) Tumour hypoxia imaging with 18F-fluorozymcinarabinofuranoside PET/CT in patients with locally advanced rectal cancer. *Nucl Med Commun* 34(2): 155–161.

Hicks RJ, Rischin D, Fisher R, Birns D, Scott AM, Peters J (2005) Utility of FMISO PET in advanced head and neck cancer treated with chemoradiation incorporating a hypoxia-targeting chemotherapy agent. *Eu J Nucl Med Mol Imaging* 32(12): 1384–1391.

Hickel M, Knoop C, Schleenger K, Vordran D, Baumann E, Mitze M, Knaapstein PG, Vaupel P (1993) Intratumoral pO2 predicts survival in advanced cancer of the uterine cervix. *Radiother Oncol* 26(1): 45–50.

Horsman MR, Mortensen LS, Petersen JB, Busk M, Overgaard J (2012) Imaging hypoxia to improve radiotherapy outcome. *Nat Rev Clin Oncol* 9(12): 674–687.

Hu M, Xing L, Mu D, Yang W, Yang G, Kong L, Yu J (2013) Hypoxia imaging with 18F-fluoromisonidazole integrated PET/CT and theoretical considerations with regard to dose painting for individualization of radiotherapy in patients with head and neck cancer. *Int J Radiat Oncol Biol Phys* 75(1): 101–108.

Lee ST, Scott AM (2007) Hypoxia positron emission tomography imaging with 18F-fluoromisonidazole. *Semin Nucl Med* 37(6): 451–461.

Lehtio K, Oikonen V, Grönroos T, Eskola O, Kallikoski K, Bergman J, Solin O, Grénman R, Nuutila P, Menn H (2001) Imaging of blood flow and hypoxia in head and neck cancer: initial evaluation with 15O(H2)O and 13O]Fluoroerythronitroimidazole PET. *J Nucl Med* 42(11): 1643–1652.

Lehtio K, Oikonen V, Nyman S, Grönroos T, Rovainen A, Eskola O, Menn H (2003) Quantification of tumour hypoxia with fluorine-18 fluorochinoylhydrazine, -fluoroerythronitroimidazole ([18F]FETNIM) and PET using the tumour plasma ratio. *Eu J Nucl Med Mol Imaging* 30(1): 101–108.

Lehtio K, Eskola O, Viljanen T, Oikonen V, Grönroos T, Sillanmäki I, Grénman R, Menn H (2004) Imaging perfusion and hypoxia with PET to predict radiotherapy response in head- and–neck cancer. *Int J Radiat Oncol Biol Phys* 59(4): 971–982.

Lewis JS, Laforest R, Dehdashti F, Grigsby PW, Welch MJ, Siegel BA (2008) An imaging comparison of 64Cu-ATSM and 64Cu in cancer of the uterine cervix. *J Nucl Med* 49(7): 1177–1182.

Li L, Hu M, Zhu H, Zhao W, Yang G, Yu J (2010) Comparison of 18F-fluoromisonidazole positron emission tomography and prognostic value in locally advanced non-small-cell lung cancer. *Clin Lung Cancer* 11(5): 335–340.

Lohith TG, Kudo T, Demura U, Umeda Y, Kiyyono Y, Fujibayashi Y, Okazawa H (2009) Pathophysiological correlation between 64Cu-ATSM and 68FDG in lung cancer. *J Nucl Med* 50(12): 1948–1953.

Matsumoto K, Sazrek L, Krishna MC, Cook JA, Seidel J, Grimes K, Carson J, Sowers AL, English S, Green MV, Bacharach SL, Eckelman WC, Mitchell JB (2007) The influence of tumor oxygenation on hypoxia imaging in murine squamous cell carcinoma using [6Cu]Cu-ATSM or [18F]Fluoromisonidazole positron emission tomography. *Int J Radiat Oncol Biol Phys* 70(3): 871–881.

McQuade P, Martin KE, Castle TC, Went MJ, Blower PJ, Welch MJ, Lewis JS (2005) Investigation into 64Cu-labeled Bis(selenocarcabzam) and Bis(thios erbicabzam) complexes as hypoxia imaging agents. *Nucl Med Biol* 32(2): 147–156.

Minagawa Y, Shizukubashi K, Kikoe I, Horiuchi C, Watanuki K, Hata M, Omura M, Odagiri K, Tohnoi I, Inoue T, Tateishi U (2011) Assessment of tumor hypoxia by 64Cu-ATSM PET/CT as a predictor of response in head and neck cancer: a pilot study. *Ann Nucl Med* 25(5): 339–345.

Mönich D, Troost EGC, Kaanders JHAM, Oyen WJG, Alber M, Thorwarth D (2012) Modelling and simulation of the influence of acute and chronic hypoxia on [18F]fluoromisonidazole PET imaging. *Phys Med Biol* 57(6): 1675–1684.

Mortensen LS, Buus S, Nordsmark M, Bentzen L, Munk OL, Keiding S, Overgaard J (2010) Identifying hypoxia in human tumors: a correlation study between 18F-FMISO PET and the Eppendorf oxygen-sensitive electrode. *Acta Oncol* 49(7): 934–940.

Mortensen LS, Johansen J, Kallehaug E, Primdahl H, Busk M, Lassen P, Alnser J, Sørensen BS, Tostrup K, Jakobsen S, Petersen J, Petersen H, Theil J, Nordsmark M, Overgaard J (2012) FAZA PET/CT hypoxia imaging in patients with squamous cell carcinoma of the head and neck treated with radiotherapy: results from the DAHANCA 24 trial. *Radiother Oncol* 105(1): 14–20.

Nehme SA, Lee NY, Schröder H, Squire O, Zanonzonic PB, Erdi YE, Greco C, Mageras G, Pharm HS, Larson SM, Ling CC, Humm JL (2008) Reproducibility of tumor distribution of 18F-fluoromisonidazole in head and neck cancer. *Int J Radiat Oncol Biol Phys* 70(1): 235–242.

Nyfflot MJ, Harari MP, Yip S, Perlman SB, Jera R (2012) Correlation of PET images of metabolism, proliferation and hypoxia to characterize tumor phenotype in patients with cancer of the oropharynx. *Radiother Oncol* 105(1): 36–40.

O'Donoghue JA, Zanonzonic P, Pugachew A, Wen B, Smith-Jones P, Cai S, Burniazi E, Finn RD, Burgman P, Ruan S, Lewis JS, Welch MJ, Ling CC, Humm JL (2005) Assessment of regional tumor hypoxia using 18F-fluoromisonidazole and 64Cu(II)-diacetyl-bis([4-N- methylthiosemicarbazone]) positron emission tomography: comparative study featuring microPET imaging, pO2 probe measurement, autoradiography, and fluorescent microscopy in the R3327–AT and FaDu murine squamous cell carcinoma models. *Int J Radiat Oncol Biol Phys* 70(1): 2–13.

Lee N, Nehme S, Schneider H, Fury M, Chan K, Ling CC, Humm J (2009) Prospective trial incorporating pre-/mid-treatment [18F]-misonidazole positron emission tomography for head-and-neck cancer patients undergoing concurrent chemoradiotherapy. *Int J Radiat Oncol Biol Phys* 75(1): 101–108.
evaluated by [18F]-fluoromisonidazole PET for head and neck cancer. J Nucl Med 54(2): 201–207.
Overgaard J (2011) Hypoxic modification of radiotherapy in squamous cell carcinoma of the head and neck – a systematic review and meta-analysis. Radiother Oncol 100(1): 22–32.
Postema EC, McEwan AJB, Riauka TA, Kumar P, Richmond DA, Abrams DN, Wiebe LI (2009) Initial results of hypoxia imaging using 1-\(\alpha\)-d-(5-deoxy-5\)-
[18F]-fluoroorbifurcanonolours-2-nitroimidazole (\[^{18}\text{F}^{\text{F}}\text{FAZA}\]). Eur J Nucl Med Mol Imaging 36(10): 1563–1573.
Rajendran JG, Wilson DC, Conrad EU, Peterson LM, Bruckner JD, Rasey JS, Chin LK, Hofstrand PD, Grierson JR, Eary JF, Krohn KA (2006) \[^{18}\text{F}^{\text{F}}\text{FMISO}\] and \[^{18}\text{F}^{\text{F}}\text{FDG}\] PET imaging in soft tissue sarcomas: correlation of hypoxia, metabolism and VEGF expression. Eur J Nucl Med Mol Imaging 30(5): 695–704.
Rajendran JG, Mankoff DA, Hiller US, Schwartz DL, Conrad EU, Spence AM, Muzi M, Farwell DG, Krohn KA (2004) Hypoxia and glucose metabolism in malignant tumors: evaluation by \[^{18}\text{F}^{\text{F}}\text{FMISO}\] and \[^{18}\text{F}^{\text{F}}\text{FDG}\] positron emission tomography (PET) imaging. Clin Cancer Res 10(7): 2245–2252.
Sato J, Kitagawa Y, Yamazaki Y, Hata H, Okamoto S, Shiga T, Shindoh M, Rajendran JG, Schwartz DL, O'Sullivan J, Peterson LM, Schwartz DL, Schuetz M, Schmid MP, Potter R, Kommata S, Georg D, Lukic D, Dudczak R, Segard T, Robins PD, Yusoff IF, Ee H, Morandeau L, Campbell EM, Francis RJ, Postema EJ, McEwan AJB, Riauka TA, Kumar P, Richmond DA, Abrams DN, Adamsen TC, Krohn KA, Spence AM (2009) Complementary but distinct roles for MRI and \[^{18}\text{F}^{\text{F}}\text{FMISO}\] PET in the assessment of human glioblastomas. J Nucl Med 50(1): 36–44.
Takahashi N, Fujibayashi Y, Yonekura Y, Welch MJ, Waki A, Tsuchida T, Sadato N, Sugimoto K, Itoh H (2000) Evaluation of \[^{18}\text{F}^{\text{F}}\text{FAZA}\] labeled diacetyl-bis(N4-methylthiosemicarbazone) as a hypoxic tissue tracer in patients with lung cancer. Ann Nucl Med 14(5): 323–328.
Tateishi K, Tateishi U, Sato M, Yamanaka S, Kanno H, Murata H, Inoue T, Kawahara N (2013) Application of \[^{18}\text{F}^{\text{F}}\text{FAZA}\] PET imaging to predict highly malignant tumor grades and hypoxia-inducible factor-1a expression in patients with glioma. Am J Neuroradiol 34(1): 92–99.
Thorwarth D, Eschmann SM, Scheiderbauer J, Paulsen F, Alber M (2005) Kinetic analysis of dynamic \[^{18}\text{F}^{\text{F}}\text{FMISO}\] PET correlates with radiation treatment outcome in head-and-neck cancer. BMC Cancer 5: 152.
Thorwarth D, Eschmann SM, Holzner F, Paulsen F, Alber M (2006) Combined uptake of \[^{18}\text{F}^{\text{F}}\text{FDG}\] and \[^{18}\text{F}^{\text{F}}\text{FMISO}\] correlates with radiation therapy outcome in head and neck cancer patients. Radiother Oncol 80(2): 151–156.
Thureau S, Chaumet-Riffaud P, Modzelewski R, Fernandez P, Tessonnier L, Vervueren L, Cachin F, Berriolo-Riedinger A, Olivier P, Kolesnikov-Gauthier H, Blagosklonov O, Bridi B, Devillers A, Collombier L, Courtbon F, Gremillet E, Houzard C, Caignon JM, Roux J, Aide N, Brotetz-Rossi L, Doyeux K, Dubray B, Vera P (2013) Interobserver agreement of qualitative analysis and tumor delineation of \[^{18}\text{F}^{\text{F}}\text{FAZA}\] and \[^{18}\text{O}^{\text{2}}\] PET images in lung cancer. J Nucl Med 54(9): 1543–1550.
Trinkaus ME, Blum R, Rischin D, Callahan J, Bressel M, Rosebolt E, Bu P, Birns D, MacManus MP, Ball D, Hicks RJ (2013) Imaging of hypoxia with \[^{18}\text{F}^{\text{F}}\text{FAZA}\] PET in patients with locally advanced non-small cell lung cancer treated with definitive chemoradiotherapy. J Med Imaging Radiat Oncol 57(4): 475–481.
Valk PE, Mathis CA, Prados MD, Gilbert JT, Buderding TF (1992) Hypoxia in human gliomas: demonstration by PET with fluorne-18-fluoromisonidazole. J Nucl Med 33(12): 2133–2137.
Vaulp P, Harrison L (2004) Tumor hypoxia: causative factors, compensatory mechanisms, and cellular response. Oncologist 9(Suppl 5): 4–9.
Vera P, Bohn P, Edet-Sanson A, Salles A, Hapday S, Gardin I, Ménard JF, Modzelewski R, Thiberville L, Dubray B (2011) Simultaneous postion emission tomography (PET) assessment of metabolism with \[^{18}\text{F}^{\text{F}}\text{FDG}\] and hypoxia with \[^{18}\text{F}^{\text{F}}\text{FMISO}\] before and during radiotherapy in patients with non-small-cell lung cancer (NSCLC): a pilot study. Radiother Oncol 98(1): 109–116.
Vercellino L, Groheux D, Thouy A, Delord M, Schlager MH, Delpech Y, Barré E, Baruch-Hennequin V, Tylski P, Homyda I, Walker F, Barranger E, Hindle E (2012) Hypoxia imaging of uterine cervix carcinoma with \[^{18}\text{F}^{\text{F}}\text{FAZA}\] PET/CT. Clin Nucl Med 37(11): 1065–1068.
Wang K, Yorke E, Nehmeh SA, Humm JL, Ling CC (2009) Modeling acute and chronic hypoxia using serial images of \[^{18}\text{F}^{\text{F}}\text{FMISO}\] PET. Med Phys 36(10): 4400–4408.
Wilson WR, Hay MP (2011) Targeting hypoxia in cancer therapy. Nat Rev Cancer 11(6): 393–410.
Yamane T, Kikuchi M, Shinohara S, Senda M (2011) Reduction of \[^{18}\text{F}^{\text{F}}\text{FMISO}\] uptake after neoadjuvant chemotherapy for head and neck squamous cell carcinoma. Mol Imaging Biol 13(2): 227–231.
Yue J, Yang Y, Cabrera AR, Sun X, Zhao S, Xie P, Zheng J, Ma L, Fu Z, Yu J (2010) Hypoxia imaging of head and neck squamous cell carcinoma – a systematic review and meta-analysis. Eur J Nucl Med Mol Imaging 37(12): 1426–1431.
Zimny M, Gagel B, Krause BJ, Beck R, Reischl G, Eble M, Buell U, Reintz P (2006) FDG – A marker of tumour hypoxia? A comparison with \[^{18}\text{F}^{\text{F}}\text{FMISO}\] and pO2-polarography in metastatic head and neck cancer. Eur J Nucl Med Mol Imaging 33(12): 1426–1431.