Overview

The Meaning, Measurement and Modification of Hypoxia in the Laboratory and the Clinic

E.M. Hammond *, M.-C. Asselin †, D. Forster ‡, J.P.B. O’Connor §, J.M. Senra *, K.J. Williams ¶

* The Gray Institute for Radiation Oncology and Biology, Department of Oncology, University of Oxford, Oxford, UK
† Wolfson Molecular Imaging Centre, Manchester, UK
‡ Centre for Imaging Sciences, Institute of Population Health, Manchester, UK
§ Manchester Pharmacy School, Cambridge-Manchester Cancer Research UK Comprehensive Imaging Centre, Manchester Academic Health Sciences Centre, The University Manchester, Manchester, UK

Received 6 December 2013; received in revised form 23 January 2014; accepted 4 February 2014

Abstract

Hypoxia was identified as a microenvironmental component of solid tumours over 60 years ago and was immediately recognised as a potential barrier to therapy through the reliance of radiotherapy on oxygen to elicit maximal cytotoxicity. Over the last two decades both clinical and experimental studies have markedly enhanced our understanding of how hypoxia influences cellular behaviour and therapy response. Furthermore, they have confirmed early assumptions that low oxygenation status in tumours is an exploitable target in cancer therapy. Generally such approaches will be more beneficial to patients with hypoxic tumours, necessitating the use of biomarkers that reflect oxygenation status. Tissue biomarkers have shown utility in many studies. Further significant advances have been made in the non-invasive measurement of tumour hypoxia with positron emission tomography, magnetic resonance imaging and other imaging modalities. Here, we describe the complexities of defining and measuring tumour hypoxia and highlight the therapeutic approaches to combat it.

© 2014 The Royal College of Radiologists. Published by Elsevier Ltd. All rights reserved.

Key words: Hypoxia; hypoxia-activated prodrugs; imaging; oxygen; radiation

Statement of Search Strategies Used and Sources of Information

The PUBMED database was interrogated for relevant literature. Due to space constraints, topical review articles rather than primary publications were cited in some instances.

Introduction

The term hypoxia has been extensively used to describe a state of insufficient oxygen, which can be present in tumours as well as normal tissues and wounds (Table 1). Central to this review is how oxygen levels are measured in tumours; the units of oxygen concentration and the relevant conversion factors are shown (Table 2). The normal level of oxygen (tissue normoxia/phsysxia) varies widely between different organs. This should not be confused with hypoxia, as even the lower levels are sufficient to support the tissue in question (Table 3). Hypoxia occurs to some degree in most solid tumours as a result of several factors, including the rapid growth rate of cancer cells and the highly disorganised/inefficient vasculature. Within any tumour, oxygen levels are extremely heterogeneous and can include mild hypoxia (<2% O₂) and severe levels of hypoxia (<0.1% O₂). In addition, due to rapid changes in red cell flux, periods of cycling hypoxia occur, i.e. exposure to hypoxia levels followed by re-oxygenation.

Tumour Hypoxia and Clinical Relevance

The clinical relevance of tumour hypoxia to patient prognosis has been shown in several elegant studies.
The conclusion of these studies is that less oxygenated/hypoxic tumour cells are more resistant to existing anticancer treatments, including chemotherapy, radiotherapy and surgery (Figure 1) [17–19]. Hypoxic cells have been shown to be resistant to chemotherapy for several reasons. These include the distance from functional vessels, which makes the delivery and diffusion of drugs challenging, the low proliferation rates of cells in hypoxic areas and the selection pressure for the loss of p53-dependent apoptosis [20]. About 50% of all cancer patients receive radiotherapy either alone or in combination with other treatment modalities [21]. The efficacy of radiotherapy is determined by a number of factors, including the percentage of cells exposed to radiobiological levels of tumour hypoxia (Table 1) [18]. Many advances have been made in improving radiotherapy outcome and these can be broadly divided into those that increase the effect on the tumour and those that protect the normal tissue [13].

### Table 1

| Definition of terms commonly used to describe oxygen levels in biology | Normoxia | Physoxia/physioxia | Hypoxia | Radiobiological hypoxia |
|---|---|---|---|---|
| Atmospheric oxygen pressure (20.9% O₂) | Low oxygen levels, indicating that the tissue/organ has insufficient oxygen. Associated with the stabilisation of hypoxia inducible factor-1 (HIF-1). | The level of hypoxia where significant resistance to radiation is observed (<0.13% O₂) [1]. | | |
| The pO₂ where most preclinical testing is carried out Also known as ‘tissue normoxia’. | | | | |

The normal oxygen pressure in specific tissues/organs (see Table 3).

### Table 3

Examples of physioxia measurements for specific tissues. See references for details of sample number and method of measurement (recently reviewed in [2]).

| Tissue | pO₂ (mmHg) | % O₂ | Reference |
|---|---|---|---|
| Brain | 35 | 4.6 | [3] |
| Lung | 42.8 | 5.6 | [4] |
| Liver | 31 | 4.08 | [5] |
| Kidney | 72 | 9.5 | [6] |
| Muscle | 25 | 3.25 | [7] |
| Bone marrow | 54.9 | 7.14 | [8] |
| Skin | 8–35 | 1.05–4.61 | [9] |
| Intestine | 61 | 8.03 | [10] |

### Biological Responses to Hypoxia

The biological responses to hypoxia are extensive and well beyond the limits of this review. Some of the key pathways will be covered in brief.

**Hypoxia Inducible Factor-mediated Signalling**

The main players in the hypoxia response are a family of heterodimeric transcription factors named hypoxia inducible factors (HIFs), composed of an oxygen-labile alpha (α) subunit (HIF1α, HIF2α and HIF3α) and a constitutively expressed HIF1β subunit [22,23]. In the presence of oxygen, the HIFα subunits bind to the tumour suppressor protein von Hippel-Lindau, leading to ubiquitination and proteasomal degradation [24]. By contrast, when oxygen is limited, the enzymes that modify HIFα (HIF hydroxylases) become inactive and HIFα is stabilised and translocates to the nucleus. Once nuclear, HIFα heterodimerises with HIF-1β and binds to specific DNA sequences known as HIF-responsive elements. Some of the most well-characterised target genes include the vascular endothelial growth factor A, carbonic anhydrase IX (CA-IX), the glucose transporter (GLUT1) and erythropoietin. Other HIF-regulated genes have roles in glucose metabolism, vasodilation, apoptosis, autophagy, oxygen sensing, invasiveness and metastasis [25,26].

**Suppression of DNA Repair, Unfolded Protein Response and DNA Damage Response**

There is increasing evidence that in an attempt to adapt to hypoxic conditions, tumour cells repress cellular processes that involve high-energy consumption. Under hypoxic conditions, many essential components of the DNA repair pathways have been shown to be repressed [27]. Homologous recombination, mismatch repair and non-homologous end-joining have all been shown to be less effective under hypoxic conditions, suggesting that a general response to hypoxia is repression of DNA repair [28–30]. The mechanisms of DNA repair gene/protein repression are varied and include roles for HIF, micro-RNAs and epigenetic modifications [30–32]. For example, the...
repression of the mismatch repair protein MLH1 by hypoxia correlates with increased levels of methylation of a particular histone (H3K9me2), which were attributed to increased levels and activity of the histone methyltransferase in hypoxia [33]. Key components of the homologous recombination pathway, such as RAD51 and BRCA1, have also been shown to be down-regulated under hypoxic conditions. The mechanism for RAD51 and BRCA1 down-regulation has been reported to be dependent on the formation of a repressive E2F4/p130 complex at the E2F site on the gene promoters, and is independent of both HIF-1α and cell cycle phase [34]. Importantly, the hypoxia-mediated repression of DNA repair occurs at a variety of oxygen tensions and not just in regions of almost anoxia, indicating that larger proportions of tumours will have repressed DNA repair pathways.

More severe levels of hypoxia (<0.1% O2) have also been shown to induce specific responses not observed in milder hypoxia. One of the responses includes a reduction in protein synthesis due to ablation of the mRNA translation initiation through the unfolded protein response [35,36]. Under severe hypoxia, two responses (a rapid and a delayed one) affect members of the eukaryotic initiation factors (eIFs) family, which ablate the mRNA translation initiation process. While in the rapid response, the eIF2α is inhibited; in the delayed response, prolonged exposure to severe hypoxia leads to inactivation of eIF4 (reviewed in [35]). An additional specific response observed in severe levels of hypoxia is the DNA damage response (Figure 2) [35,36,41–43]. In the absence of oxygen, the activity of ribonucleotide reductase is compromised, leading to depletion of dNTP levels and replication stress [44]. The accumulation of single-stranded DNA at the replication forks induces ataxia telangiectasia and rad3-related (ATR)-mediated signalling, which includes phosphorylation of H2AX, CHK1 and p53. In addition to the activation of ATR, ataxia telangiectasia mutated (ATM)-mediated signalling is also activated in hypoxia, despite the absence of detectable DNA damage [45,46].

**Targeting Hypoxia with Selective Therapies**

**DNA Damage Response and DNA Repair Inhibitors**

Hypoxia is one of the major physiological differences between a tumour and normal tissue and therefore an attractive therapeutic target [13]. Given the key roles of ATR and ATM during hypoxia-induced replication stress and re-oxygenation, these kinases are regarded as potential targets for therapeutic intervention. The DNA damage response is a complex network of signalling pathways that are activated in response to DNA damage, including the activation of ATM and ATR kinases. The activation of these kinases leads to the phosphorylation of downstream targets, such as CHK1, CHK2, and p53, which are involved in the inhibition of cell cycle progression and the induction of apoptosis or cell cycle arrest. The hypoxia-induced DNA damage response is shown in Figure 2, which illustrates the potential targets for therapeutic intervention.

**Fig 2.** A simplified schematic of the hypoxia-induced DNA damage response. Potential therapeutic targets are in pink [37–40].
therapeutic targets in hypoxic tumour cells (Figure 2). Disruption of these kinases through depletion or expression of kinase-dead proteins sensitises tumour cells to hypoxia/re-oxygenation [42]. Recent studies using potent and specific inhibitors of cellular ATR activity, such as VE-821 and VE-822, showed promise in sensitising a variety of cancer cells to a range of oxygen concentrations [37,47,48]. In particular, VE-821 enhanced the effects of radiation, not only in normoxia, but also in hypoxic conditions [37]. Other ATR inhibitors include NU6027, which was shown to promote the genotoxic effects of several DNA-damaging agents, such as cisplatin, hydroxyurea, radiation and temozolomide [49]. It will be interesting to establish whether these combinations will also be effective under hypoxic conditions. Targeting CHK1, through gene silencing or chemical inhibition, has been shown to be a promising approach in enhancing tumour cell sensitivity to hypoxia/re-oxygenation [50,51]. The CHK1 inhibitor AZD7762, although shown to have no effect under hypoxic conditions, significantly increases cell death after re-oxygenation and radiation treatment in several tumour cell lines [52–54]. Additionally, in xenograft models of lung cancer with brain metastasis, treatment with AZD7762 resulted in a prolonged survival in response to radiation [54]. More recently, ATM was also shown to be a promising target after hypoxia/re-oxygenation. The ATM inhibitor KU-55933 induced DNA damage specifically under severe hypoxic conditions [46].

Moreover, in 2005, studies by Bryant et al. [55] and Farmer et al. [56] revealed that cancer cells with BRCA1 and BRCA2 deficiencies were extremely sensitive to poly ADP ribose polymerase (PARP) inhibitors. The explanation behind this sensitivity lies in the concept of synthetic lethality: when two repair pathways A (e.g. homologous recombination) and B (e.g. non-homologous end-joining) are abrogated alone, the cell remains viable; however, if both pathways are inhibited, this leads to cell death [57]. Recently, the novel concept of context synthetic lethality has emerged, and was shown to occur when one of the pathways is repressed due to the cellular context (e.g. hypoxia). In response to hypoxia, critical factors involved in homologous recombination are repressed, e.g. Rad51 and BRCA1, and this leads to a deficiency in repair and increased sensitivity to PARP inhibitors [58].

**Hypoxia-activated Prodrugs**

An elegant approach is to exploit the low levels of oxygen in hypoxic regions and design compounds that are only activated by enzymatic reduction in such conditions, the so-called hypoxia-activated prodrugs. Several of these agents have been described in the literature, including the well-established tirapazamine, AQ4N, PR-104A, SN30000, RH-1 and TH-302 [59–62]. However, most of these agents have been designed to induce DNA damage once activated under hypoxic conditions. The idea of designing hypoxia-activated prodrugs that can release an active inhibitor of the DNA damage response directly to the hypoxic regions is emerging. Recent proof-of-concept studies include the CHK1 inhibitor (CH-01) and a DNA-PK inhibitor (BCCA621C), each of which is masked by a bioreductive group. Under hypoxic conditions, reduction of the bioreductive group nitro moiety releases the active CHK1 inhibitor CH-01, leading to increased sensitivity of hypoxic cells [63]. In the case of BCCA621C, it was shown to be effectively released under hypoxic conditions and sensitise hypoxic radio-resistant cells.

**Clinical Translation of Hypoxia-selective Therapies**

Of course, a hypoxia-selective therapy is going to be of greater benefit in patients that have hypoxic tumours. The critical importance of integrating measures of tumour hypoxia into therapeutic trials is perhaps best exemplified by the phase III trial of tirapazamine. Substudies conducted within phase II trials in head-and-neck cancer had exemplified that patients with more hypoxic tumours (identified via positron emission tomography [PET] using [18F]-fluoromisonidazole [18F][FMISO]) were more likely to show local regional failure after chemoradiotherapy than non-hypoxic tumours (8/13 versus 1/10). This was countered by the inclusion of tirapazamine into the treatment regimen (local regional failure hypoxic tumours 1/19 [65]). In the phase III trial, tirapazamine did not improve outcome in the overall patient population [66]. However, no measure of hypoxia was undertaken, so it was impossible to define cohorts who may have been showing an enhanced response based on the extent of tumour hypoxia. Additional complexities with the quality assurance of radiotherapy further marred the tirapazamine trial [67]. However, it is now generally recognised that hypoxia biomarkers are crucial for trials of hypoxia-selective therapies.

**Needle Electrodes and Tissue-based Biomarkers**

Oxygenation in tumours can be directly measured using needle electrodes, a technique that was invaluable in first proving the associations with hypoxia and treatment response [67–73]. However, this technique is limited to accessible tumours and, as the equipment went out of production, is available in very few centres. Alternative approaches were therefore needed and there are a number of potential ways of identifying hypoxia in tumour samples. Some exploit the reducing nature of the hypoxic microenvironment, such as the use of pimonidazole, a nitroimidazole that binds to macromolecules in cells under hypoxic conditions. Pimonidazole can be safely given to patients and binding ascertained in biopsies using specific antibodies [74]. It has been used in trials of hypoxia-modifying therapies (e.g. accelerated radiotherapy with carbogen and nicotinamide) and successfully tallies with patients showing the most marked improvements in response [75]. Pimonidazole has also proved invaluable in validating the hypoxic regulation of other potential endogenous tissue markers of hypoxia. From studies using
material from cervical carcinoma patients, expression of the proteins CA-IX and GLUT1 were confirmed as potential surrogates for hypoxia through their coincidental expression with pimonidazole binding [76]. With the advent of array-based technologies, hypoxic signatures have been developed by identifying genes whose expression clusters with known hypoxia-regulated genes, including CA-IX. Such signatures have proven highly prognostic [77,78] and predictive of response to agents that specifically radiosensitise hypoxic cells (nimorazole [79,80]). Clearly, tissue-based biomarkers have extensive utility in clinical studies. However, an obvious issue with these approaches is the need for a biopsy. Biopsies are challenging or impossible in some tumour types and repeat measurements would be restricted. Serological markers, such as osteopontin, have shown some utility [81] and would enable repeat sampling. However, in both cases, these methods do not reveal heterogeneity in hypoxia across a tumour volume, which may be of relevance in terms of overall response and may also be a prerequisite of a therapeutic approach, e.g. if you wanted to increase the radiotherapy dose specifically to hypoxic cells.

Non-invasive Imaging of Tumour Hypoxia

Imaging affords a means of evaluating hypoxia across an entire tumour volume and lends itself well to repeat measurements. The most widely established methods for imaging hypoxia are based on PET. Magnetic resonance imaging (MRI) methods are, in theory, attractive options for serial imaging of tumour hypoxia, as it is widely available and avoids imparting ionising radiation to patients. However, at present, its role is less well established than PET-based methods. Other methods, including bioluminescence and photo-acoustic imaging [82], may play a role in the future, but remain research techniques at present.

Measuring Tumour Hypoxia Using Positron Emission Tomography Imaging

Ideally, a hypoxia PET tracer should target cellular rather than vascular pO2 at clinically relevant oxygen concentrations only in viable cells and possess uniform and rapid cell entry (lipophilic molecule), rapid clearance from normoxic cells (hydrophilic molecule), yielding a high target-to-background ratio independent of perfusion and be resistant to non-hypoxia-dependent metabolism [83]. None of the radiotracers that have been used in clinical studies (Figure 3) fulfil all properties and development of better radiotracers is still on-going. The radiotracers proposed to date for PET imaging fall broadly into two categories, nitroimidazoles and copper-complexed dithiosemicarbazone (Cu-ATSM) derivatives [86], both of which are subject to futile cycling in the presence of oxygen, but enzymatically reduced in hypoxic cells leading to cellular retention. For nitroimidazoles, the mechanism of retention is via the formation of radical anions that bind to intracellular macromolecules, whereas Cu(II)-ATSM undergoes reduction to Cu(I)-ATSM, which is retained in hypoxic cells. However, through in situ metabolism, Cu(I) can become dissociated from ATSM and be washed out from cells, preventing its retention, despite the presence of hypoxia [87]. Nitroimidazoles have been generally labelled with 18F (half-life of 1.83 h) for PET imaging, whereas Cu-ATSM has been radiolabelled with four different positron emitters of copper, two very short-lived isotopes (62Cu and 60Cu with half-lives of 0.16 and 0.40 h, respectively), allowing repeated administrations but limiting the imaging time to less than 1 h, and two longer lived isotopes (61Cu and 64Cu having half-lives of 3.3 and 12.7 h, respectively) for delayed measurement, more likely to reflect hypoxia rather than perfusion, but at the expense of a higher radiation dose to the patient (Table 4) [88].

Selection of Radiotracers

PET tracers are at varying stages of validation and evaluation, with [18F]FMISO being the most established and [18F]FAZA increasingly being used. A comparison of radiotracers (Table 4) is hampered by the use of tumour models and tumour types, with varying degrees of hypoxia and radiotracer uptake being quantified using different methods. For the radiotracers used in clinical studies, validation in rodents showed tumour uptake above background level that correlates with pO2 electrode measurements and/or pimonidazole immunohistochemistry and that is sensitive to changes in oxygen tissue concentration [84,89]. Few direct comparisons have been made between existing radiotracers. Overall, fluorinated nitroimidazoles have a low tumour uptake relative to surrounding tissue. Preclinically [18F]FAZA [90,91], and not [18F]FETNIM [92], has been shown to be superior to [18F]FMISO, whereas [18F]HX4 was found to be comparable with [18F]FMISO in clinical head and neck cancer [93], but the imaging time of both radiotracers was suboptimal in this study [94,95]. There are also limited reproducibility data that have been collected only for [18F]FMISO in head and neck cancer [96,97]. Inter-tumoural differences in hypoxia selectivity of Cu-ATSM [98,99] have prevented its widespread acceptance as a hypoxia PET tracer.

Quantification of Tumour Hypoxia

Radiotracer uptake in tumours is heterogeneous in time and in space (Figure 4). The time window for static imaging should be selected to decouple radiotracer retention from perfusion, which differ between tumour types and may vary in different parts of a tumour. High uptake is assumed to indicate retention in hypoxic cells, but could also result from slow clearance from normoxic cells. Conversely, low uptake is assumed to result from rapid washout from normoxic cells, but may also arise from slow entry into hypoxic cells, especially those poorly perfused. Tissue uptake will be further confounded by the presence of radiolabelled metabolites, which can differ between species (Table 4), between individuals and with treatment. Alternatively,
dynamic imaging with different modelling approaches can potentially characterise both perfusion and retention and allow hypoxia perfusion patterns to be identified [100,101].

Hypoxia in PET images is most often quantified using the tumour to background ratio at a given time and requires estimation of first the tumour uptake, which can be done using the maximum value or an average over a given volume, and second the background uptake, which can be taken from a neighbouring muscle, the contralateral side (brain) or blood (sampled or extracted from a blood pool in PET image). The hypoxic volume is the volume of the tumour that is hypoxic and its measurement from PET images requires selection of an uptake threshold, which depends on the radiotracer, the tumour type and the imaging time, e.g. tumour to background ratio >1.2–1.4 for \(^{18}\text{F}\)FMISO [102,103]. The hypoxic fraction is the fraction of the tumour that is hypoxic and its derivation requires that the whole tumour volume be delineated in addition to the estimation of the hypoxic volume and this is typically extracted from other images, either computed tomography or \(^{18}\text{F}\) 2-fluoro-2-deoxy-glucose PET [104].

**Application to Hypoxia-modifying Treatments**

PET imaging of hypoxia can be used to identify patients with hypoxic tumours who could benefit most from additional treatment, either by prescribing hypoxia-modifying drugs or by increasing the radiation dose to the tumour [105]. PET imaging with fluorinated nitroimidazoles is being integrated into clinical trials of hypoxia-modifying treatments. Significantly fewer head and neck cancer patients with hypoxic tumours as measured with \(^{18}\text{F}\)FMISO had locoregional failure after radiochemotherapy with tirapazamine compared with those who were not given the hypoxic cell cytotoxin [65]. Less convincing results were reported with \(^{18}\text{F}\)FAZA in a similar clinical imaging study [106], despite promising results in mice bearing a murine breast cancer xenograft (EMT6) [107]. \(^{18}\text{F}\)EF5 uptake in a human non-small cell lung cancer xenograft model (H460) was reduced in rats treated with the hypoxia-activated prodrug SN30000 alone or with radiotherapy and the changes correlated with baseline hypoxia levels [108]. The distribution of hypoxia in the images could also be used to boost the radiation dose to hypoxic subvolumes of the tumour (called dose painting), exploiting the conformal and accurate radiation delivery made possible with the development of intensity-modulated radiotherapy and image-guided radiotherapy. Clinical implementation and evaluation await clarification in the treatment dose delivery [105].

**Magnetic Resonance Imaging and Measurement of Hypoxia**

Most MRI studies to evaluate tumour hypoxia to date have used either dynamic contrast-enhanced MRI (DCE-MRI) or blood oxygen level-dependent (BOLD) imaging. In DCE-MRI, a gadolinium-based contrast agent bolus is injected into a vein and the passage of the contrast agent is tracked through the tumour microvasculature. The presence of gadolinium species increases the longitudinal
| Table 4                                                                 |
|----------------------------------------------------------------------|
| Comparison of radiotracers for uptake in different human tumours, peripheral metabolism and dosimetry |

|                      | [\(^{18}\text{F}\)]EF5 | [\(^{18}\text{F}\)]FMISO | [\(^{18}\text{F}\)]FETNIM | [\(^{18}\text{F}\)]FAZA | [\(^{18}\text{F}\)]HX4 | Cu-ATSM |
|----------------------|------------------------|--------------------------|---------------------------|-------------------------|-------------------------|---------|
| **Tumour to background ratio (TBR\(_{\text{max}}\)) for various cancer (imaging time)** |                         |                          |                           |                         |                         |         |
| Head/neck            | 1.5\(^*\)/1.9 (170–180 min) | 2.2/3.0 (240–250 min)   | 1.1 (90–120 min)           | 1.8 (150 min)            | 1.8 (90–99 min)         | 5.5 [\(^{62}\text{Cu}\)] (10–12 min) |
| Lung                 | n/a                    | 2.0/2.7 (120/240 min)    | 2.0 (120–160 min)          | 2.3 (150 min)            | 1.6/2.0 (120/240 min)   | 2.3 [\(^{60}\text{Cu}\)] (30–60 min) |
| Brain\(^\dagger\)    | 1.3\(^*\) (210 min)    | n/a                      | n/a                       | 5.3 (150 min)            | n/a                     | 2.2 [\(^{60}\text{Cu}\)] (30–40 min) |
| Cervix               | n/a                    | 1.6 (150–170 min)        | n/a                       | >1 in 5/15 (60–75 min)   | n/a                     | 5.9 [\(^{60}\text{Cu}\)]/7.3 [\(^{64}\text{Cu}\)] (30–60 min) |
| Colon/rectum         | n/a                    | >1 in 4/6 (120 min)      | n/a                       | 1.9 (120–135 min)        | n/a                     | 2.5 [\(^{60}\text{Cu}\)] (30–60 min) |
| Prostate             | n/a                    | >1.4 (120–160 min)       | n/a                       | 2.2 \(^*\) (180 min)     | n/a                     | n/a     |
| Breast               | n/a                    | 1.15/1.22 (120/240 min)  | n/a                       | n/a                     | n/a                     | n/a     |
| Pancreas             | n/a                    | 2.0 (120–150 min)        | n/a                       | n/a                     | n/a                     | n/a     |
| Kidneys              | n/a                    | >1.2 (180 min)           | n/a                       | n/a                     | n/a                     | n/a     |
| Oesophagus           | n/a                    | >1.3\(^\ddagger\) (120 min) | n/a | n/a | n/a | n/a |
| **Metabolism (measurement time)** |                         |                          |                           |                         |                         |         |
| Mouse                | 0.37 (120 min)         | 0.50 (120 min)           | n/a                       | n/a                     | n/a                     | n/a     |
| Rat                  | 0.80 (120 min)         | 0.80 (120 min)           | n/a                       | n/a                     | n/a                     | n/a     |
| Man                  | 0.95 (120 min)         | 0.94 (90 min)            | 0.92 (180 min)            | >0.90 (70 min)           | 0.82 (120 min)          | n/a     |
| **Effective dose**   | (\(\mu\text{Sv}/\text{MBq}\)) | 18                       | 13 (total body)           | 15–19 (2–4 h voiding)   | 27                      | 3 [\(^{62}\text{Cu}\)], 11 [\(^{60}\text{Cu}\)], 29 [\(^{61}\text{Cu}\)], 36 [\(^{64}\text{Cu}\)] |

HIF, hypoxia inducible factor; EF5, \(^{18}\text{F}\)-2-(2-nitro-1H-imidazol-1-yl)-N-(2,2,3,3,3,-penta-fluoropropyl)-acetamide; FMISO, \(^{18}\text{F}\)fluoromisonidazole; FETNIM, \(^{18}\text{F}\)fluoroerythonitrimidazole; FAZA, \(^{18}\text{F}\)fluorozomycin-arabinofuranoside; HX4, 3-[\(^{18}\text{F}\)]fluoro-2-(4-((2-nitro-1H-imidazol-1-yl)methyl)-1H-1,2,3-triazol-1-yl)propan-1-ol; Cu-ATSM, \(^{60,61,62,64}\text{Cu}\)copper(II)-diacetyl-bis(N\(^4\)-methylthiosemicarbazone).

* Tumour mean SUV reported instead of maximum SUV.
\(^\dagger\) Blood as background also reported (FMISO). Blood used as background for FMISO (prostate) and background derived from left ventricle for FMISO (kidneys). Plasma and blood used as background for FETNIM (head/neck and lung, respectively).
\(^\ddagger\) Different background regions used: cerebellum (FMISO), contralateral side (FAZA, Cu-ATSM), normal brain (EF5). Note that only lipophilic radiotracers cross the blood–brain barrier.
\(^\ddagger\) Significant difference between responders and non-responders (1.5 versus 3.4 for lung) and significant difference between HIF-1\(\alpha\) negative and HIF-1\(\alpha\) positive (1.2 versus 2.9 for brain).
\(^\ddagger\) Uptake in benign prostate used as background region (FAZA) and uptake in spleen used as background region (FETNIM).
\(^\dagger\) Fraction of intact tracer in plasma.
radiation rate (termed $R_1$) of protons, causing signal change, from which estimates of perfusion can be derived [109]. Several DCE-MRI studies have related such measurements to genetic readouts of hypoxia [110], oxygen tension [111] and immunohistochemical measurement of hypoxic fraction [112,113]. However, the strength of these relationships is inconsistent. This is explained partially by the fact that different studies measure slightly different MRI parameters, all of which have an incomplete and varying relationship to perfusion. Furthermore, chronic hypoxia and acute transient variations in oxygen tension are affected not only by oxygen delivery (perfusion), but also by diverse factors, including haemoglobin saturation, vascular geometry and consumption (oxidative phosphorylation). Therefore, DCE-MRI estimates of perfusion are at best indirect estimates of hypoxia and in some circumstances bare little relationship to hypoxia at all.

BOLD techniques have been developed to exploit the differences in magnetic resonance transverse relaxation (termed $R_2^*$) induced by regional differences in deoxygenated haemoglobin levels [114]. BOLD measurements are typically carried out in combination with gas challenge, designed to alter the oxy- and deoxygenated haemoglobin ratio dynamically and therefore help map hypoxia. However, as BOLD measurements are sensitive to changes in vessel calibre they are subject to ambiguities in interpretation [115,116]. Although the technique has been carried out in cancer patients for nearly two decades [117], substantial artefact is found in some body regions, the extent of which depends on organ site, the control of visceral motion and the precise protocol adopted. This method has some success in mapping chronic hypoxic regions (delineated by pimonidazole adduct formation) in pelvic tumours such as prostatic carcinoma [118], but signal changes do not correlate well with absolute $pO_2$ levels [116]. Despite some initial encouraging data, there is little current evidence that either DCE-MRI or BOLD will provide validated, translational biomarkers of hypoxia in the near future. There is, therefore, a need to develop MRI methods of quantifying hypoxia that are more closely coupled to tissue chronic low oxygen tension and acute fluctuations in $pO_2$ and are free from the technical difficulties found with BOLD.

One promising method is oxygen-enhanced MRI (O2-MRI) using a $T_1$-weighted contrast mechanism. It has long been recognised that oxygen dissolved in blood and tissue plasma increases $R_1$ in a similar mechanism to gadolinium [115,116], but the signal change is very small and so this contrast mechanism has only been exploited more recently, enabled by improvements in MRI scanner hardware and analysis methods. Human studies have shown that signal changes of up to 20% can be measured reliably in a range of well-vascularised normal tissues and that the technique is well tolerated [119]. Preclinical experiments have shown significant increases in tumour $R_1$ in a variety of tumour models [120,121] that relate to hypoxic fraction and vessel density [122]. A handful of clinical studies across a range of solid tumours have shown that this method is translational [122,123], but further studies are required to determine whether this or other MRI methods provide useful biomarkers for monitoring therapy and/or determining clinical outcome for patients undergoing radiotherapy and hypoxia-modifying therapy.

**Conclusion**

We have appreciated for many years that tumour hypoxia poses a significant problem for effective therapy. This knowledge has led to the development of strategies to target tumour hypoxia and equally importantly the preclinical testing of novel therapies in conditions that mimic the tumour microenvironment. It is clear that in order to enhance our ability to target hypoxic tumour cells we need to know where they are and how they respond to therapy. The advances in application of tissue biomarkers and hypoxia imaging discussed here highlight this real and exciting possibility.

**Acknowledgments**

The authors would like to thank Alison Smigova for her support with preclinical PET imaging. EMH and JMS are supported by Cancer Research UK (CRUK, awarded to EMH). Work within KJWs group is supported by EU FP7 Metoxia Grant agreement no. 222741 (awarded to KJW). JPBOC is supported by a personal CRUK Clinician Scientist Fellowship (reference C19221/A15267). The Cambridge-Manchester Cancer Research UK Comprehensive Imaging Centre is funded by CRUK and Engineering and Physical Sciences Research Council (reference C8742/A018097).

**References**

[1] Hall EJ, Giaccia AJ. *Radiobiology for the radiologist*, 6th ed. Philadelphia, USA: Lippincott Williams & Wilkins; 2006.
[2] Carreau A, El Hafny-Rabhi B, Matejuk A, Grillon C, Kieda C. Why is the partial oxygen pressure of human tissues a crucial
parameter? Small molecules and hypoxia. *J Cell Mol Med* 2011;15(6):1239–1253.

[3] Dings J, Meixensberger J, Jager A, Roosen K. Clinical experience with 118 brain tissue oxygen partial pressure catheter probes. *Neurosurgery* 1998;43(5):1082–1095.

[4] Le QT, Chen E, Salim A, et al. An evaluation of tumor oxygenation and gene expression in patients with early stage non-small cell lung cancers. *Clin Cancer Res* 2006;12(5):1507–1514.

[5] Brooks AJ, Eastwood J, Beckham J, Girling KJ. Liver tissue partial pressure of oxygen and carbon dioxide during partial hepatectomy. *Br J Anaesth* 2004;95(5):735–737.

[6] Muller M, Padberg W, Schindler E, et al. Renocortical tissue oxygen pressure measurements in patients undergoing living donor kidney transplantation. *Anesth Analg* 1998;87(2):474–476.

[7] Boekstegers P, Riessen R, Seyde W. Oxygen partial pressure distribution within skeletal muscle: indicator of whole body oxygen delivery in patients? *Adv Exp Med Biol* 1997;434:507–514.

[8] Harrison JS, Rameshwar P, Chang V, Bandari P. Oxygen saturation in the bone marrow of healthy volunteers. *Blood* 2002;98(1):394.

[9] Wang W, Winlove CP, Michel CC. Oxygen partial pressure in outer layers of skin of human finger nail folds. *J Physiol* 2003;549(Pt 3):855–863.

[10] Thermmann J, Jostardt L, Eberhard F, Richter H, Sass W. Oxygen supply of the human small intestine in mechanical ileus. *Langenbecks Arch Chir* 1985;363(3):179–184.

[11] Gray LH, Conger AD, Ebert M, Hornsey S, Scott OC. The concentration of oxygen dissolved in tissues at the time of irradiation as a factor in radiotherapy. *Br J Radiol* 1953;26(312):638–648.

[12] Thomlinson RH, Gray LH. The histological structure of some human lung cancers and the possible implications for radiotherapy. *Br J Cancer* 1955;9(4):539–549.

[13] Begg AC, Stewart FA, Vens C. Strategies to improve radiotherapy with targeted drugs. *Nat Rev Cancer* 2011;11(4):239–253.

[14] Cosse JP, Michiels C. Tumour hypoxia affects the responsiveness of cancer cells to chemotherapy and promotes cancer progression. *Anticancer Agents Med Chem* 2008;8(7):790–797.

[15] Barker HE, Cox TR, Erler JT. The rationale for targeting the LOX family in cancer. *Nat Rev Cancer* 2012;12(8):540–552.

[16] Fokas E, McKenna WG, Muscler RJ. The impact of tumor microenvironment on cancer treatment and its modulation by direct and indirect antiangiogenic strategies. *Cancer Metastasis Rev* 2012;31(3–4):823–842.

[17] Brown JM, Giaccia AJ. The unique physiology of solid tumors: opportunities (and problems) for cancer therapy. *Cancer Res* 1998;58(7):1408–1416.

[18] Nordmark M, Bentzen SM, Rudat V, et al. Prognostic value of tumor oxygenation in 397 head and neck tumors after primary radiation therapy. An international multi-center study. *Radiother Oncol* 2005;77(1):18–24.

[19] Vaupel P, Harrison L. Tumor hypoxia: causative factors, compensatory mechanisms, and cellular response. *Oncoologist* 2004;9(Suppl.5):4–9.

[20] Brown JM, Wilson WR. Exploiting tumour hypoxia in cancer treatment. *Nat Rev Cancer* 2004;4(6):437–447.

[21] Delaney G, Jacob S, Featherstone C, Barton M. The role of radiotherapy in cancer treatment: estimating optimal utilization from a review of evidence-based clinical guidelines. *Cancer* 2005;104(6):1129–1137.
recombination and recombination through inhibition of Chk1 and Aurora A. ACS Chem Biol 2013;8(7):1451–1459.

[64] Lindquist KE, Cran JD, Kordic K, et al. Selective radio-sensitization of hypoxic cells using BCCA621C: a novel hypoxic activated prodrug targeting DNA-dependent protein kinase. Tumor Microenviron Ther 2013;1:46–55.

[65] Rischin D, Hicks RJ, Fisher R, et al. Prognostic significance of [18F]-misonidazole positron emission tomography-detected tumor hypoxia in patients with advanced head and neck cancer randomly assigned to chemoradiation with or without tirapazamine: a substudy of Trans-Tasman Radiation Oncology Group Study 98.02. J Clin Oncol 2006;24(13):2098–2104.

[66] Rischin D, Peters LJ, O’Sullivan B, et al. Tirapazamine, cisplatin, and radiation versus cisplatin and radiation for advanced squamous cell carcinoma of the head and neck (TROG 02.02, HeadSTART): a phase III trial of the Trans-Tasman Radiation Oncology Group. J Clin Oncol 2010;28(18):2989–2995.

[67] Peters J, O’Sullivan B, Giralt J, et al. Critical impact of radiotherapy protocol compliance and quality in the treatment of advanced head and neck cancer: results from TROG 02.02. J Clin Oncol 2010;28(18):2996–2001.

[68] Brizel DM, Sibley GS, Rossin L, Scher RL, Dewhirst MW. Tumor hypoxia adversely affects the prognosis of carcinoma of the head and neck. Int J Radiat Oncol Biol Phys 1997;38(2):285–289.

[69] Hockel M, Schlenker K, Aral B, Mitze M, Schaffer U, Vaupel P. Association between tumor hypoxia and malignant progression in advanced cancer of the uterine cervix. Cancer Res 1996;56(19):4509–4515.

[70] Okunieff P, Hoeckel M, Dunphy EP, Schlenker K, Knoop C, Vaupel P. Oxygen tension distributions are sufficient to explain the local response of human breast tumors treated with radiation alone. Int J Radiat Oncol Biol Phys 1993;26(4):631–636.

[71] Gatenby RA, Kessler HB, Rosenblum JS, et al. Oxygen distribution in squamous cell carcinoma metastases and its relationship to outcome of radiation therapy. Int J Radiat Oncol Biol Phys 1988;14(5):831–838.

[72] Fyles AW, Milosevic M, Pintilie M, Hill RP. Cervix cancer oxygenation measured following external radiation therapy. Int J Radiat Oncol Biol Phys 1998;42(4):751–753.

[73] Nordsmark M, Overgaard J. A confirmatory prognostic study on oxygenation status and loco-regional control in advanced head and neck squamous cell carcinoma treated with radiotherapy alone. Int J Radiat Oncol Biol Phys 1993;26(4):631–636.

[74] Janjigian YY, Kociura RA, Kronenbarg IJ, et al. Identification of a novel hypoxia marker for complementary study of tumor hypoxia and cell proliferation in cervical carcinoma. Gynecol Oncol 1998;71(2):270–277.

[75] Janssens GO, Rademakers SE, Terhaard CH, et al. Accelerated radiotherapy with carbogen and nicotinamide for laryngeal cancer: results of a phase III randomized trial. J Clin Oncol 2012;30(15):1777–1783.

[76] Airley RE, Loncaster J, Raleigh JA, et al. GLUT-1 and CAIX as intrinsic markers of hypoxia in carcinoma of the cervix: relationship to pimonidazole binding. Int J Cancer 2003;104(1):85–91.

[77] Winter SC, Buffa FM, Silva P, et al. Relation of a hypoxia metagene derived from head and neck cancer to prognosis of multiple cancers. Cancer Res 2007;67(7):3441–3449.

[78] Buffa FM, Harris AL, West CM, Miller CJ. Large meta-analysis of multiple cancers reveals a common, compact and highly prognostic hypoxia metagene. Br J Cancer 2010;102(2):428–435.
Mees G, Dierckx R, Vangestel C, Van de Wiele C. Molecular Laforest R, Dehdashti F, Lewis JS, Schwarz SW. Dosimetry of Burgman P, O'sullivan M, Alers J, Horsman MR. Plasma osteopontin, hypoxia, and response to the hypoxia sensitiser nimorazole in radiotherapy of head and neck cancer: results from the DAHANCA 5 randomised double-blind placebocontrolled trial. Lancet Oncol 2005;6(10):757764.

Shao Q, Morgounova E, Jiang C, Choi J, Bischof J, Ashkenazi S. In vivo photoacoustic lifetime imaging of tumor hypoxia in small animals. J Biomed Opt 2013;18(7):076019.

Chia K, Fleming IN, Blower PJ. Hypoxia imaging with PET: which tracers and why? Nucl Med Commun 2012;33(3):217222.

Dubois LJ, Lieuwees NG, Janssen MH, et al. Preclinical evaluation and validation of [18F]HX4, a promising hypoxia marker for PET imaging. Proc Natl Acad Sci USA 2011;108(35):1462014625.

Vavere AL, Lewis JS. Cu-ATSM: a radiopharmaceutical for the PET imaging of hypoxia. Dalton Trans 2007;21(43):48934902.

Krohn KA, Link JM, Mason RP. Molecular imaging of hypoxia. J Nucl Med 2008;49(Suppl 2):1291485.

Burgman P, O'Donoghue JA, Lewis JS, Welch MJ, Humm JL, Ling CC. Cell line-dependent differences in uptake and retention of the hypoxia-selective nuclear imaging agent Cu-ATSM. Nucl Med Biol 2005;32(6):623630.

Laforest R, Dehdashti F, Lewis JS, Schwarz SW. Dosimetry of 60/61/62/64Cu-ATSM: a hypoxia imaging agent for PET. Eur J Nucl Med Mol Imaging 2005;32(7):764770.

Mees G, Dierckx R, Vangestel C, Van de Wiele C. Molecular imaging of hypoxia with radiolabelled agents. Eur J Nucl Med Mol Imaging 2009;36(10):16741686.

Sorger D, Patt M, Kumar P, et al. [18F]Fluoroazomycin-arabinofuranoside (18FZAFA) and [18F]Fluoromisonidazole (18FMISO): a comparative study of their selective uptake in hypoxic cells and PET imaging in experimental rat tumors. Nucl Med Biol 2003;30(3):317326.

Piert M, Machulla HJ, Picchio M, et al. Hypoxia-specific tumor imaging with 18F-fluoroazomycin arabinoside. J Nucl Med 2005;46(1):106113.

Gronroos T, Bentzen L, Marjamaki P, et al. Comparison of the biodistribution of two hypoxia markers [18F]EF5NIM and [18F]FMISO in an experimental mammary carcinoma. Eur J Nucl Med Mol Imaging 2004;31(4):513520.

Chen L, Zhang Z, Kolb HC, Walsh JC, Zhang J, Guan Y. [1]8F-HX4 hypoxia imaging with PET/CT in head and neck cancer: a comparison with [1]8F-FMISO. Nucl Med Commun 2012;33(10):10961102.

Zegers CM, van Elmpt W, Wierts R, et al. Hypoxia imaging with [18F]HX4 PET in NSCLC patients: defining optimal imaging parameters. Radiother Oncol 2013;109(1):5864.

Abolmaali N, Haase R, Koch A, et al. Two or four hour [(1)8F] FMISO-PET in HNSCC. When is the contrast best? Nuklearmedizin 2011;50(1):2227.

Okamoto S, Shiga T, Yasuda K, et al. High reproducibility of tumor hypoxia evaluated by 18F-fluoromisonidazole PET for head and neck cancer. J Nucl Med 2013;54(2):201207.

Nehmeh SA, Lee NY, Schroder H, et al. Reproducibility of intratumor distribution of [(1)8F]fluoromisonidazole in head and neck cancer. Int J Radiat Oncol Biol Phys 2008;70(1):235242.

Yuan H, Schroeder T, Bowsher JE, Hedlund LW, Wong T, Dewhirst MW. Intertumoral differences in hypoxia selectivity of the PET imaging agent 64Cu(Il)-diacetyl-bis(N4-methylthiosemicarbazone). J Nucl Med 2006;47(6):989998.

Vaitorta S, Belloli S, Sanvito F, et al. Comparison of 18F-fluoroazomycin-arabinofuranoside and 64Cu-diacetyl-bis(N4-methylthiosemicarbazone) in preclinical models of cancer. J Nucl Med 2013;54(7):11061112.

Busk M, Munk OL, Jakobsen S, et al. Assessing hypoxia in animal tumor models based on pharmacokinetic analysis of dynamic FAZA PET. Acta Oncol 2010;49(7):922933.

Thorwarth D, Eschmann SM, Scheiderbauer J, Paulsen F, Alber M. Kinetic analysis of dynamic 18F-fluoromisonidazole PET correlates with radiation treatment outcome in headandneck cancer. BMC Cancer 2005;5:152.

Koh WJ, Bergman KS, Rasey JS, et al. Evaluation of oxygenation status during fractionated radiotherapy in human nonsmall cell lung cancers using [F-18] fluoromisonidazole positron emission tomography. Int J Radiat Oncol Biol Phys 1995;33(2):391398.

Kikuchi M, Yamane T, Shinozaka S, et al. 18F-fluoromisonidazole positron emission tomography before treatment is a predictor of radiotherapy outcome and survival prognosis in patients with head and neck squamous cell carcinoma. Ann Nucl Med 2011;25(9):625633.

Komar G, Seppanen M, Eskola O, et al. 18F-EF5: a new PET tracer for imaging hypoxia in head and neck cancer. J Nucl Med 2008;49(12):19441951.

Horsman MR, Mortensen LS, Petersen JB, Busk M, Overgaard J. Imaging hypoxia to improve radiotherapy outcome. Nat Rev Clin Oncol 2012;9(12):674687.

Le QT, Fisher R, Oliner KS, et al. Prognostic and predictive significance of plasma HGF and IL-8 in a phase III trial of chemoradiation with or without tirapazamine in locoregionally advanced head and neck cancer. Clin Cancer Res 2012;18(6):17981807.

Beck R, Roper B, Carlsem JM, et al. Pretreatment 18F-FAZA PET predicts success of hypoxia-directed radiochemotherapy using tirapazamine. J Nucl Med 2007;48(6):973980.

Chitneni SK, Bida GT, Yuan H, et al. 18F-EF5 PET imaging as an early response biomarker for the hypoxia-activated prodrug SN30000 combined with radiation treatment in a non-small cell lung cancer xenograft model. J Nucl Med 2013;54(8):13391346.

O’Connor JP, Jackson A, Parker GJ, Roberts C, Jayson GC. Dynamic contrast-enhanced MRI in clinical trials of antiangiogenic therapies. Nat Rev Clin Oncol 2012;9(3):167177.

Halle C, Andersen E, Lando M, et al. Hypoxia-induced gene expression in chemoradiosensitive cervical cancer revealed by dynamic contrast-enhanced MRI. Cancer Res 2012;72(20):52855295.

Egeland TA, Simonsen TG, Gaustad JV, Gulliksrud K, Ellingsen C, Roфтad EK. Dynamic contrast-enhanced magnetic resonance imagingof tumors: preclinical validation of parametric images. Radiat Res 2009;172(3):339347.

Gulliksrud K, Mathiesen B, Galappathi K, Roftad EK. Quantitative assessment of hypoxia in melanoma xenografts by dynamic contrast-enhanced magnetic resonance imaging: intradermal versus intramuscular tumors. Radiother Oncol 2010;97(2):233238.
Donaldson SB, Betts G, Bonington SC, et al. Perfusion estimated with rapid dynamic contrast-enhanced magnetic resonance imaging correlates inversely with vascular endothelial growth factor expression and pimonidazole staining in head-and-neck cancer: a pilot study. *Int J Radiat Oncol Biol Phys* 2011;81(4):1176–1183.

Ogawa S, Menon RS, Tank DW, et al. Functional brain mapping by blood oxygenation level-dependent contrast magnetic resonance imaging. A comparison of signal characteristics with a biophysical model. *Biophys J* 1993;64(3):803–812.

Howe FA, Robinson SP, McIntyre DJ, Stubbs M, Griffiths JR. Issues in flow and oxygenation dependent contrast (FLOOD) imaging of tumours. *NMR Biomed* 2001;14(7–8):497–506.

Baudelet C, Gallez B. How does blood oxygen level-dependent (BOLD) contrast correlate with oxygen partial pressure (pO2) inside tumors? *Magn Reson Med* 2002;48(6):980–986.

Griffiths JR, Taylor NJ, Howe FA, et al. The response of human tumors to carbogen breathing, monitored by Gradient-Recalled Echo Magnetic Resonance Imaging. *Int J Radiat Oncol Biol Phys* 1997;39(3):697–701.

Hoskin PJ, Carnell DM, Taylor NJ, et al. Hypoxia in prostate cancer: correlation of BOLD-MRI with pimonidazole immunohistochemistry-initial observations. *Int J Radiat Oncol Biol Phys* 2007;68(4):1065–1071.

O’Connor JPB, Jackson A, Buonaccorsi GA, et al. Organ-specific effects of oxygen and carbogen gas inhalation on tissue longitudinal relaxation times. *Magn Reson Med* 2007;58(3):490–496.

Matsumoto K, Bernardo M, Subramanian S, et al. MR assessment of changes of tumor in response to hyperbaric oxygen treatment. *Magn Reson Med* 2006;56(2):240–246.

Burrell JS, Walker-Samuel S, Baker LC, et al. Exploring DeltaR(2) * and DeltaR(1) as imaging biomarkers of tumor oxygenation. *J Magn Reson Imaging* 2013;38(2):429–434.

Linnik IV, Scott MLS, Holliday K, et al. Non-invasive tumor hypoxia measurement using magnetic resonance imaging in U87 murine model of human glioma and in patients with glioblastoma. *Magn Reson Med* 2013 Jun 24. http://dx.doi.org/10.1002/mrm.24826. [Epub ahead of print].

O’Connor JP, Naish JH, Parker GJ, et al. Preliminary study of oxygen-enhanced longitudinal relaxation in MRI: a potential novel biomarker of oxygenation changes in solid tumors. *Int J Radiat Oncol Biol Phys* 2009;75(4):1209–1215.