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Review

# New ways to image and target tumour hypoxia and its molecular responses

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ABSTRACT

Tumour hypoxia and its molecular responses have been shown to be associated with poor prognosis. Detection of hypoxia, preferably in a non-invasive manner, could therefore predict treatment outcome and serve as a tool to individualize treatment. This review gives an overview of recent literature on hypoxia imaging markers currently used in clinical trials. Furthermore, recent progress made in targeting hypoxia (hypoxia-activated prodrugs) or hypoxia response (carbonic anhydrase IX inhibitors) is summarized. Last, window-of-opportunity trials implementing non-invasive imaging are proposed as an important tool to prove anti-tumour efficacy of experimental drugs early during drug development.


It is well established that tumours are not a collection of relatively homogeneous cancer cells, but act as organs with a complexity that might even exceed that of healthy tissues. Therefore to understand the biology of a tumour both the different individual cell types within a tumour as well as its microenvironment need to be studied [1]. Within this review, we will focus on hypoxia, a common characteristic of solid tumours, which has been associated with poor prognosis [2]. Detection of hypoxia, preferably in a non-invasive manner, could predict treatment outcome and serve as a tool to support treatment decisions. Such non-invasive imaging approaches that are routinely available in clinical practice including positron emission tomography (PET), magnetic resonance imaging (MRI) and perfusion computed tomography (CT) are able to accurately and reliably image hypoxia in tumours. Over the last decade, these diagnostic techniques are developing into versatile tools integrated in treatment monitoring, outcome prediction and treatment targeting. A meta-analysis evaluating the relationship between hypoxia imaging and outcome after radiation treatment demonstrated a uniform tendency for poor response when tumours were hypoxic. This was not only observed for widely used hypoxic PET tracers, but also when hypoxia was indirectly evaluated using perfusion-CT or DCE-MRI [3].

While the prognostic significance of tumoural hypoxia on outcome has been established more than two decades ago only recently compounds are being tested in clinical trials that enable monitoring and selective elimination of hypoxic tumours cells. Here we will provide an update on the current status of hypoxia imaging agents and strategies to combat tumour hypoxia.

## Hypoxia PET imaging tracers

Multiple PET tracers suitable for the detection of hypoxia have been developed, validated and shown to exhibit different characteristics. The ideal hypoxia tracer has complete clearance of unbound tracer at time of imaging, thus only bound in oxygen deprived tissues resulting in high signal to noise ratios [3]. We recently reviewed the PET hypoxia tracers that were validated in preclinical and clinical studies and reported accurate quantification methods and clinical applications [4]. The most investigated PET hypoxia tracer is fluoromisonidazole (FMISO). However, due to concerns regarding FMISO stability, metabolite formation and slow clearance properties [5,6], alternative hypoxia PET tracers with different clearance and hydrophilicity characteristics have been developed trying to overcome these limitations:

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fluoroazomycin arabinoside (FAZA), fluoroerythronitroimidazole (FETNIM) and fluorinated etanidazole derivatives (EF1, EF3, EF5), which all have been extensively reviewed previously [3,4,7].

More recently, the hydrophilic flortanidazole (HX4), with preferred pharmacokinetics and clearance properties, has been synthesized through click-chemistry [8] showing 82% intact and 84% unmetabolized tracer at 2 h post injection (h p.i.) in plasma and urine respectively [9]. HX4 has been evaluated in a preclinical rhabdomyosarcoma rat tumour model, where binding was causally dependent on tumoural oxygenation status. Furthermore, a significant spatial relationship at tumour-microregional level between HX4 distribution and the exogenous hypoxia marker pimonidazole staining was observed [10,11]. Studies in primates and healthy volunteers [9] and in patients with histologically proven solid cancer [12] provided evidence for a good safety profile. Recently, in non-small cell lung cancer patients, image contrast was found to be superior 4 h p.i. compared with earlier time points and uptake patterns were strongly correlated between two scans [13]. Overlap studies between HX4 and the metabolism tracer FDG indicated that on average 24% of the hypoxic volume is outside the FDG volume [14]. Similar results have been obtained for head and neck cancer patients [15], suggesting that hypoxia PET imaging provides complementary information to FDG imaging.

Due to the large heterogeneity in uptake, differences in tumour and animal models, different time points of imaging and anaesthesia observed in the literature, it is difficult to compare different hypoxia markers. Although characterization of new hypoxia markers should be preferably performed in multiple cancer models, highly additive data can be expected from comparisons of different tracers within the same tumour models [3,11]. Recently, we performed a comparative study characterizing the clinically approved hypoxia markers FMISO, FAZA and HX4 on tumour to blood ratio (TBR), reproducibility and reversibility within a rat rhabdomyosarcoma model [16]. Blood clearance for FAZA and HX4 became similar 3 h p.i., while for FMISO as expected clearance from normal tissues was significantly lower. Differences in tumour uptake resulted in significantly higher TBR for HX4 compared to the other tracers. Reproducibility and spatial overlap between two PET acquisitions over a 48 h time period was high for both FMISO and HX4. Furthermore, decreasing the hypoxic fraction using carbogen resulted in loss of FMISO uptake, while increased hypoxia achieved by breathing 7% oxygen, further enhanced FAZA and HX4 uptake. Another study performed a similar comparison in a SQ20b head and neck xenograft mouse model and found similar tumour to muscle ratios for FMISO, FAZA and HX4 [10]. However, these results were obtained at 80–90 min p.i., a time point which is probably too early for evaluation since normal tissue clearance is still ongoing. A comparative study in head and neck cancer patients found similar tumour to muscle ratios for HX4 imaging at 1.5 h p.i. and FMISO imaging at 2 h p.i. [17]. For HX4 higher uptake and increasing ratios would be expected at later time points based on our clinical results [13]. Recently a simulation study, comparing FMISO, FAZA and HX4 based on their respective physical and chemical properties, revealed that tracer clearance and diffusion are the major parameters influencing image contrast. Highest clearance and image contrast was observed for HX4, but also the largest patient-to-patient variation, which might be a concern for clinical imaging to define tumour hypoxia based on a reliable threshold value [18].

Current available tracers have proven to be reliable for evaluation of tumour hypoxia, although with inherent problems resulting in clinical limitations. Alternative tracers, such as HX4, are promising with respect to deliver higher contrast images, whereas FMISO remains a robust reproducible hypoxia marker. It is not inconceivable that more tracers will be developed; but currently existing PET tracers should rather be used in clinic with standardized protocols enabling comparisons between different institutes. Furthermore, applicability and clinical validation should be proven in multiple cancer types and tracers need be tested with respect to their prognostic and predictive value.

## Hypoxia targeting

The compelling evidence for hypoxia in tumour tissue and its therapeutic importance makes hypoxia a high priority target for cancer therapy. Bioreductive prodrugs selectively activated under hypoxia and drugs that inhibit molecular targets in hypoxic cells (vide infra) are currently extensively investigated. A recent overview described the challenges and opportunities of these strategies [19]. The clinically most advanced hypoxia-activated prodrug is tirapazamine (TPZ). Although promising results have been reported in a number of Phase 2 trials, TPZ failed in several Phase 3 clinical trials since no survival benefit was observed when incorporated into standard therapy regimens. Possible explanations are its poor tumour penetration, low *in vivo* potency at tolerable doses and unacceptable toxicity levels and lack of patient selection with high levels of tumour hypoxia [20]. A more potent hypoxia-activated prodrug currently undergoing early clinical testing is TH-302. It is a 2-nitroimidazole conjugated to bromo-isophosphoramide mustard, which is released and activated upon very low levels of oxygen [21] and diffuses to surrounding cells creating a cytotoxic bystander effect [19]. TH-302 displayed clinical activity when used as single agent, which makes it unique compared to earlier generation hypoxia-activated cytotoxins which demonstrate anti-tumour activity only when used in combination with radiation or chemotherapy [22]. Furthermore, TH-302 efficacy was correlated with the hypoxic fraction across different tumour models [23–26]. Phase 1 trials have proved TH-302 safety with nausea, vomiting and fatigue as the most frequently occurring toxicities. Other trials successfully combined TH-302 with doxorubicin in patients with advanced soft tissue sarcoma [27] or with gemcitabine in patients with advanced pancreatic cancer [28]. A phase 3 double-blind, placebo-controlled trial has been initiated in which patients with advanced pancreatic cancer were randomized to gemcitabine combined with TH-302 or placebo [29]. Recently our group has evaluated the efficacy of TH-302 in a rat rhabdomyosarcoma and a human H460 xenograft model, using growth delay as endpoint. TH-302 in both models significantly inhibited tumour growth and markedly sensitized tumours to radiation. Furthermore, the therapeutic effect of TH-302 was dependent on the tumour oxygenation status prior to local radiotherapy that was modified by either carbogen (to improve oxygenation) or low oxygen containing gas (to increase hypoxia) breathing [30].

Increasing tumour oxygenation has shown potential for improving radiotherapy efficacy in several randomized clinical trials [31,32]. In spite of positive results, these strategies using hyperbaric oxygen or carbogen combined with vasodilating agents have not gained clinical traction due to practical limitations, toxicity and relatively modest clinical benefit [33]. An alternative strategy to achieve improved tumour oxygenation is to decrease cellular oxygen consumption using for example metformin, an inhibitor of the mitochondrial NADH dehydrogenase, also known as complex 1, activity in the mitochondrial electron transport respiration chain [34]. Recently, it has been demonstrated that metformin increases tumour response to radiotherapy, through a reduction in oxygen consumption and improved tumour oxygenation [35]. For future personalized cancer medicine, evaluation of hypoxia biomarkers and patient stratification will be essential to apply hypoxia targeting treatments to change radiotherapy response.

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