

Review Article

How to Modulate Tumor Hypoxia for Preclinical In Vivo Imaging Research

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Tumor hypoxia is related with tumor aggressiveness, chemo- and radiotherapy resistance, and thus a poor clinical outcome. Therefore, over the past decades, every effort has been made to develop strategies to battle the negative prognostic influence of tumor hypoxia. For appropriate patient selection and follow-up, noninvasive imaging biomarkers such as positron emission tomography (PET) radiolabeled ligands are unprecedentedly needed. Importantly, before being able to implement these new therapies and potential biomarkers into the clinical setting, preclinical in vivo validation in adequate animal models is indispensable. In this review, we provide an overview of the different attempts that have been made to create differential hypoxic in vivo cancer models with a particular focus on their applicability in PET imaging studies.

1. Background

Hypoxia, which frequently occurs in solid tumors, is related with an aggressive phenotype, chemo- and radiotherapy resistance, and thus a poor clinical outcome. To a considerable extent, hypoxia-inducible factor-1 (HIF-1), the major transcriptional regulator of the cellular response to hypoxia (Figure 1), is responsible for these observed phenomena. Encouragingly, tremendous progress with strategies to overcome the unfavorable effects of hypoxia has been made, paving the way for new personalized medicine opportunities. However, as not all patients will benefit from this new, promising radiosensitizing treatment schedules, molecular biomarkers are of utmost importance for adequate patient selection. Imaging biomarkers, such as positron emission tomography (PET) radiolabeled compounds, allow non-invasive and longitudinal assessment of molecular and functional characteristics of a tumor, thereby coping with inter- and intratumoral heterogeneity. Therefore, over the past decades, imaging biomarkers have tremendously increased in significance.

From this perspective, adequate animal models may accelerate translation of new therapies and personalized approaches, and also potential new molecular imaging biomarkers, into routine clinical practice. In hypoxia research in particular, it is often crucial to be able to distinguish between a positive and negative model for tumor hypoxia, i.e., tumors that are poorly and well oxygenated, respectively. Obviously, these models should also accurately reflect human disease. From this point of view, cell-line derived xenograft models may be inferior to genetically engineered models, orthotopic models, or patient-derived xenografts [1]. However, based on historic knowledge obtained in subcutaneous xenograft models, the fact that tumors are externally visible and easily measurable and the relatively straightforward experimental procedures that are required for their creation and follow-up, subcutaneous models are still frequently used in preclinical cancer studies, including hypoxia research.

A prerequisite to be able to differentiate between a positive and negative model for tumor hypoxia is the availability of methods to quantify tumor oxygenation. To

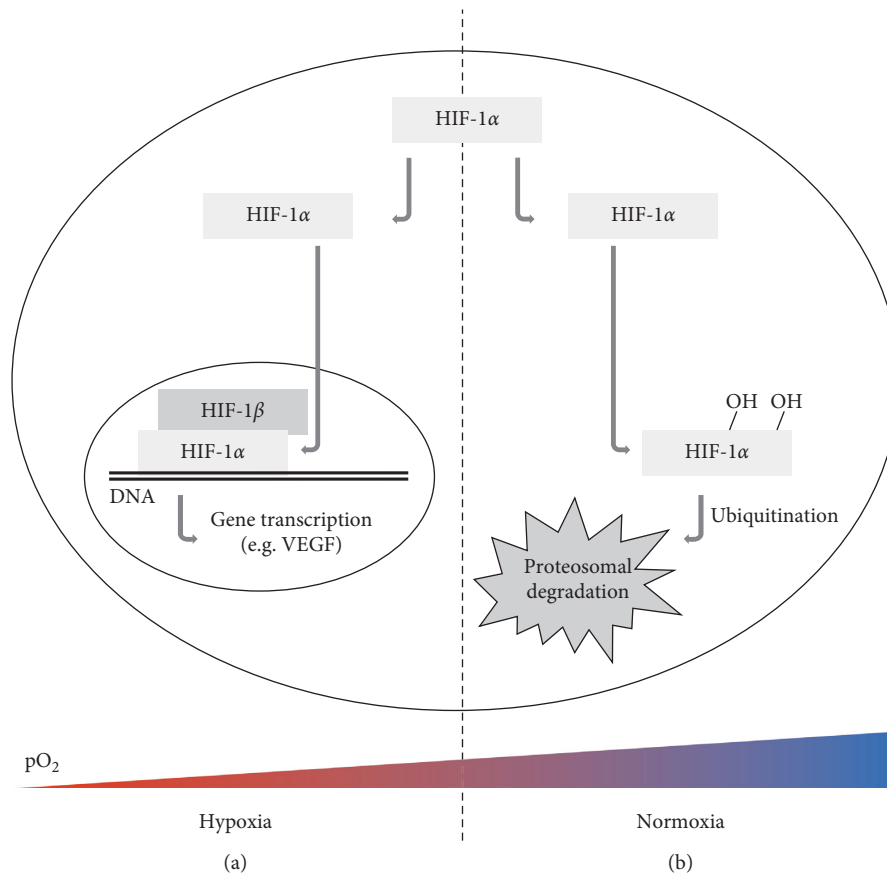


FIGURE 1: The HIF-1 pathway. (a) Under hypoxic conditions, HIF-1 α stabilizes, and its transcriptional activity is regulated by heterodimerization with the constitutively expressed HIF-1 β and binding to the hypoxia-response elements in the promoters of genes that regulate a variety of biological processes. These include genes involved in tumor survival, progression, and proliferation, such as VEGF. (b) Under normoxic conditions, the HIF-1 α subunit is rapidly degraded by the proteasome after hydroxylation and ubiquitination.

date, a variety of techniques has been used to measure tumor hypoxia, as recently reviewed by Fleming et al. [2]: oxygen (O₂) electrodes such as OxyLite; electron paramagnetic resonance (EPR); histological assessment with extrinsic biomarkers, mainly pimonidazole, and intrinsic biomarkers such as carbonic anhydrase IX (CAIX); blood O₂ level-dependent (BOLD) magnetic resonance imaging (MRI); and single photon emission tomography (SPECT) or PET with for instance hypoxia-targeting 2-nitroimidazole-based radiotracers [2, 3]. However, correlations between the different measuring techniques are often absent [4, 5] as they all provide information on different locations within the tumor, e.g., intracellular hypoxia, interstitial hypoxia, or blood oxygenation [2]. Moreover, some techniques such as OxyLite inevitably damage the tumor tissue. Therefore, the question remains if one of these techniques can be considered as the reference standard for measuring tumor hypoxia.

Over the past decades, several 2-nitroimidazole derivatives have been developed for hypoxia PET imaging (Figure 2). [¹⁸F]fluoromisonidazole ([¹⁸F]FMISO), considered as the prototype tracer, is a lipophilic compound that is highly metabolized in the liver and cleared via hepatobiliary and gastrointestinal pathways. The more hydrophilic

next-generation 2-nitroimidazole derivatives, such as [¹⁸F]flortanidazole ([¹⁸F]HX4), have better pharmacokinetic properties, resulting in chiefly renal excretion of the intact compound and only limited (<20%) hepatobiliary clearance. This gives rise to improved hypoxia-to-normoxia tissue ratios and thus images with higher contrast in comparison to [¹⁸F]FMISO [2,6–9].

Molecular imaging techniques, such as PET with 2-nitroimidazole-based probes (Figure 2), may offer considerable advantages over the other techniques because of their noninvasive nature, accuracy and reliability, and the opportunity to measure hypoxia directly, both spatially and temporally [2, 10]. From the preclinical point of view, PET imaging has the added advantages that its use is directly translatable to the clinic and that the same set of animals can be followed over time, thereby reducing the required number of animals for a single experiment and allowing individual therapy response assessments. Since animals function as their own controls over time, their intrinsic intra-animal analysis increases statistical power [1].

Furthermore, PET imaging allows absolute quantification of hypoxia. Ideally, this is achieved by performing dynamic acquisitions and kinetic modelling [11]. Accordingly, longer anesthesia regimens will be required, and

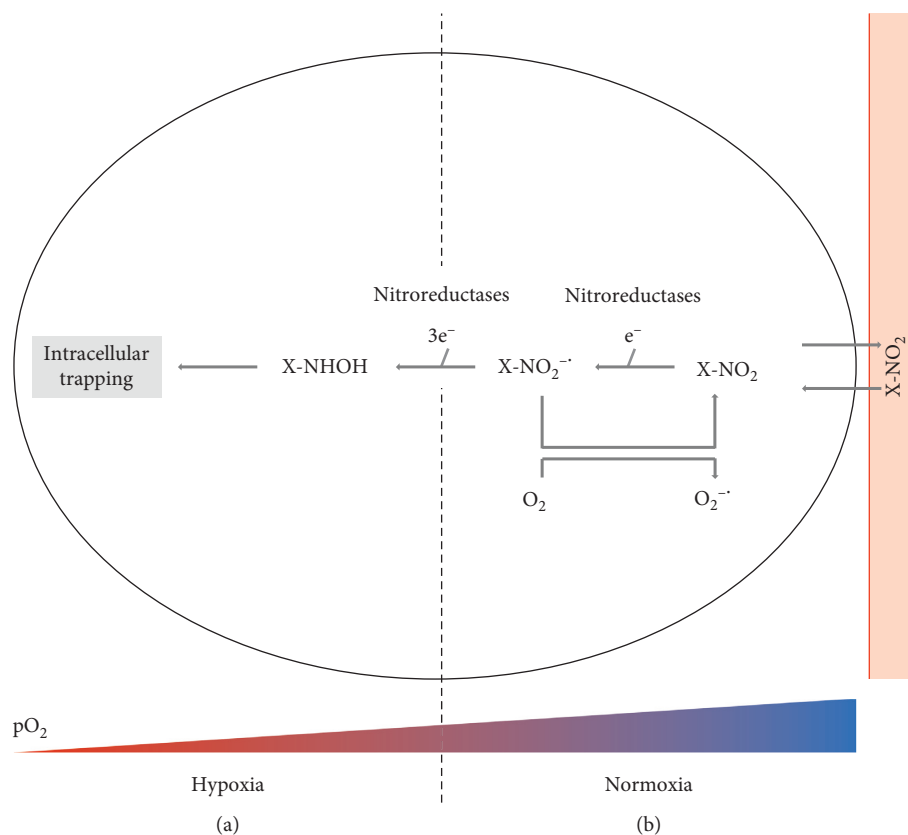


FIGURE 2: The binding mechanism of 2-nitroimidazole tracers here represented as X-NO₂. (a) Under hypoxic conditions, tracer diffuses into the cell, where the NO₂ group undergoes a series of reductions and some of the intermediate products that are formed during these reactions will bind to macromolecules within the cell. (b) Under normoxic conditions, the first reduction is reversed, giving rise to the original compound which can unhindered exit the cell. Importantly, for removal of 2-nitroimidazole background signal, an uptake time of minimal 3 hours is needed [3].

especially for hypoxia imaging, this may induce (re)oxy- genation (cf Section 2.3.1). Evidently, dynamic imaging also decreases the experimental throughput [12] which might prove cumbersome in large groups of animals ideally being scanned in identical hypoxia settings. Therefore, most studies use semiquantitative parameters derived from static images, such as standardized uptake values (SUV) or tumor-to-background ratios (TBR). Importantly, as it has been shown that these static parameters are time-dependent [3, 13, 14], it should always be taken into account that these semiquantitative approaches inherently lead to higher degrees of inaccuracy. To a certain extent, this can be overcome by respecting and maintaining an invariable tracer uptake time of at least 3 hours within an experimental set-up [3, 13]. However, for the more hydrophilic next-generation hypoxia tracers such as [¹⁸F]HX4, which are dependent on renal clearance, interanimal variability will remain substantial, due to intrinsic differences in kidney function [3]. In line with this, in therapy response evaluation studies, in which animals undergo multiple PET scans (i.e., pre- and post-therapy), drug-induced alterations of renal excretion may influence tracer clearance [3]. Unfortunately, no established standardized quantification methodologies have been developed for hypoxia PET imaging yet, particularly in the preclinical setting. Therefore, the difficulties discussed

above need to be confronted when analyzing hypoxia PET images.

In this review, we provide an overview of the different experimental approaches and study designs that may be applicable for manipulating the tumor oxygenation state for in vivo hypoxia research (Figure 3) with a particular focus on preclinical hypoxia PET imaging, considering the inherent difficulties of this imaging technique. For each modulation approach, we will indicate its respective opportunities and pitfalls, and share our own experiences and the difficulties we run up against in our own attempts to create a differential hypoxia murine cancer or tumor model.

2. Generating Differential Hypoxia in Tumor Models

2.1. Tumor Physiological Parameters

2.1.1. Inherent Variation without Manipulations. The ability to detect differential hypoxia in a single xenograft model without underlying external manipulation is the ultimate paradigm for in vivo cancer hypoxia research. However, where inoculation of some cancer cell lines inherently leads to tumors with different degrees of tumor hypoxia regardless of the tumor volume [15], this is not the case for other cell

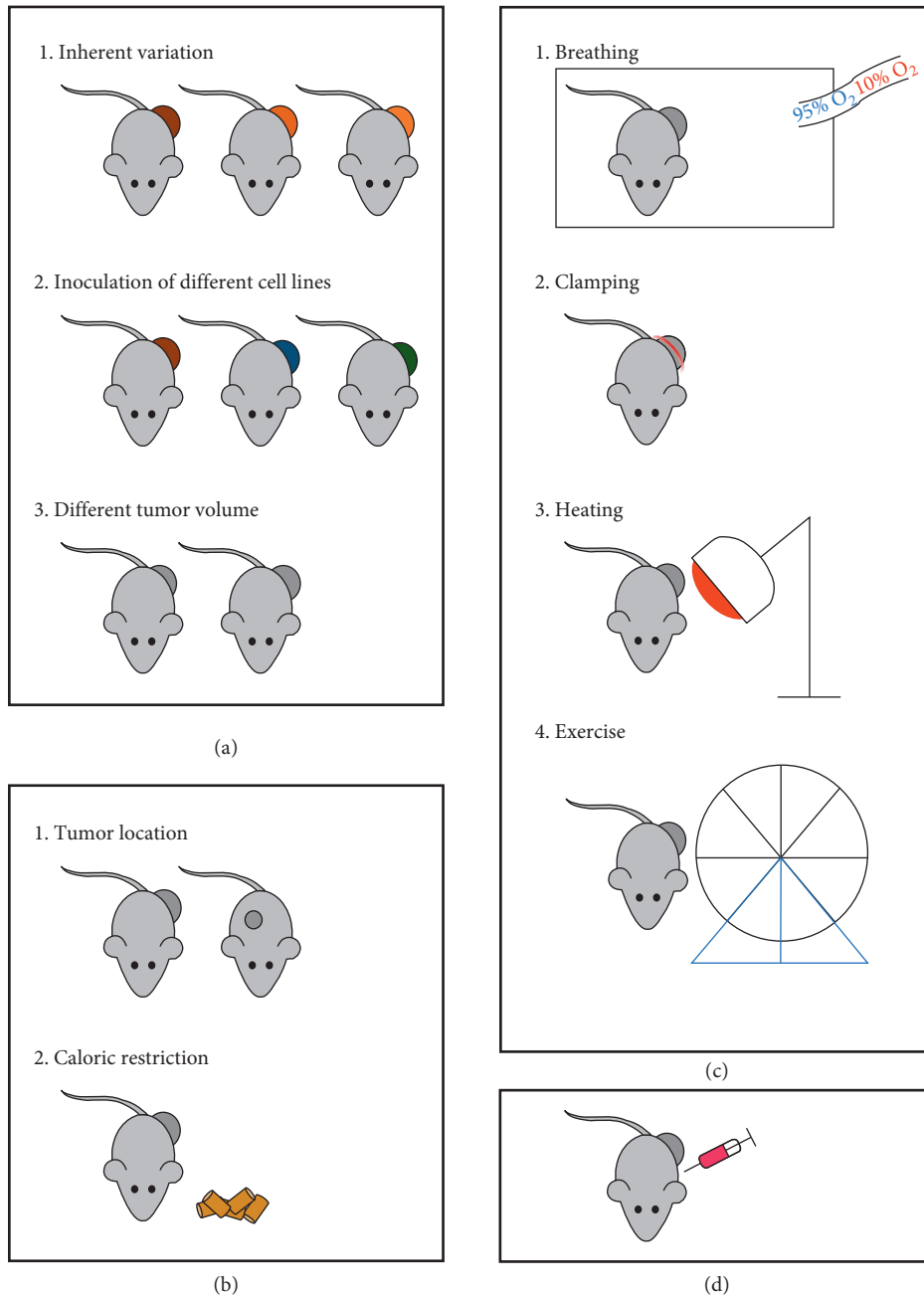


FIGURE 3: In vivo hypoxia modulation. Overview of the different experimental techniques and study designs that may be applicable for manipulating the tumor oxygenation state for hypoxia research. (a) Tumor physiological parameters. (b) Animal physiological parameters. (c) External interventions. (d) Pharmacological interventions.

lines. For instance, heterogeneous [¹⁸F]-2-nitroimidazol-pentafluoropropyl acetamide ([¹⁸F]EF5) uptake was observed in A549 non-small cell lung cancer (NSCLC) xenografts, but not in RKO and HT29 colon carcinoma xenograft models [16].

In some xenograft models, the observed intertumoral variation in tumor oxygenation allowed to show the properties of hypoxia PET as a predictive biomarker. For instance, considerable intertumoral variation in oxygenation has been observed in 9L glioma tumors, which enabled the

detection of significant correlations between [¹⁸F]EF5 uptake and response to single-dose radiotherapy [17]. In line with these results, Beck et al. performed [¹⁸F]fluoroazomycin arabinoside ([¹⁸F]FAZA) imaging on 67 EMT6 breast tumor-bearing mice and used the median tumor-to-background ratio to discriminate between hypoxic and normoxic tumors. Based on this distinction, significantly faster tumor growth of the hypoxic tumors as compared to their normoxic counterparts was observed. Moreover, it was

possible to show that administration of the radiosensitizer tirapazamine prior to radiotherapy was beneficial in hypoxic, but not in normoxic, tumors. Importantly, in this study, tumor volumes were uniformly distributed across both groups [18]. Similarly, it was found that baseline [^{18}F]FAZA TBR, which ranged from 1.17 to 5.83, was higher in radioresistant than in radiosensitive esophageal carcinoma xenograft tumors [19]. Finally, [^{18}F]FAZA TBR predicted that rhabdomyosarcoma and glioma tumors were sensitized to the effects of radiotherapy by nimorazole [20] and identified colorectal xenograft tumors that benefited from addition of the hypoxic prodrug evofosfamide to standard chemoradiotherapy regimens [21].

Despite these promising results which have been successful in validating the predictive character of hypoxia PET, this approach is not without pitfalls. For instance, in most cases, rather large cohorts of animals may be required to detect differential hypoxia, which is not preferable from an ethical point of view. Also, the relationship between tumor volume and oxygenation as such can also be an interfering factor and will therefore be discussed in a separate section (2.1.3) within this review. The most important concern may however be the lack of a clear cutoff value to discriminate between “hypoxic” and “normoxic” (i.e., “less hypoxic”) tumors in these studies. Indeed, the chosen thresholds that have been reported seem rather arbitrary [4,16,18–20,22], which renders the comparison of different studies difficult. Also, interpretation of intermediate values, i.e., values fluctuating around the threshold, may be complicated. Moreover, as these studies are predominantly semiquantitative and both SUV and TBR are extremely dependent on the pharmacokinetic properties of the used 2-nitroimidazole tracer and the PET imaging protocol [3, 14, 23], the comparison of studies with different tracers may be impossible in any case.

2.1.2. Inoculation of Different Cell Lines. The unique characteristics of different cancer cell lines give rise to different tumor phenotypes with typical features that will conceivably lead to differential tumor hypoxia. Using pimonidazole immunostaining and [^{18}F]FMISO, [^{18}F]-2-nitroimidazol-trifluoropropyl acetamide ([^{18}F]EF3) and [^{18}F]EF5 PET, differential tumor hypoxia could be observed in animal models of osteosarcoma [24], fibrosarcoma [7], glioma [25–27], head and neck cancer [28–31], lung cancer [15], melanoma [32], and prostate cancer [15, 33, 34] that were all derived from different cell lines, and in a panel of colorectal cancer (CRC) patient-derived xenograft tumors [21]. Similarly, the different stages of castration responsiveness in the Shionogi prostate cancer tumor model could be differentiated using [^{18}F]EF5 PET [35].

The investigation of different cell lines may be very relevant from a clinical point of view, as it may accurately reflect the high degree of intra- and intertumor heterogeneity observed in and between cancer patients. However, for fundamental research purposes, this approach may result in very complex analyses. When for instance therapy response is compared between different models, the different oxygenation status of the tumors may be only one of many

factors that influence therapy outcome. Indeed, the diverse genetic and phenotypic profiles arising from the different cell lines will also contribute to therapy responsiveness independently from the degree of hypoxia.

To undo the potential complexity of comparing experimental results obtained in tumors arising from different cell lines, knockdown or knockout cell lines can be used. In these cells, the expression of only one or some genes is either reduced or prevented, respectively. This allows the investigation of the functional roles of particular genes. The most obvious gene to be eliminated in regard to tumor hypoxia is HIF-1 (Figure 1). Using this approach, an inhibition in tumor growth was observed in subcutaneous HIF-1 knockout HCT116, but not RKO CRC xenograft models. Interestingly, the amount of hypoxia as determined with pimonidazole was not affected [36]. In subcutaneous lung and gastric xenograft tumors on the other hand, HIF-1 α knockdown stimulated tumor growth [37–39]. In the case of gastric cancer, knockdown additionally resulted in aggressive peritoneal dissemination via upregulation of matrix metalloproteinase-1 and in increased sensitivity to 5-FU chemotherapy through increased susceptibility to apoptosis and downregulation of drug efflux transporters [37–39]. In subcutaneous xenografts derived from HIF-1 α deficient embryonic stem cells, HIF-1 α deficiency resulted in accelerated tumor growth, decreased perfusion, and increases in tumor hypoxia as observed by pimonidazole staining [40, 41]. Genetic ablation of vascular endothelial growth factor (VEGF), a proangiogenic signal protein that is one of the downstream targets of HIF-1 (Figure 1), has been shown to give rise to increases in pimonidazole staining and thus tumor hypoxia on top of reductions in vascularity and tumor volume in a variety of tumors [36, 41–43]. Interestingly, genetic disruption of both HIF-1 and VEGF further inhibited CRC xenograft tumor growth as compared to VEGF disruption alone, but no additive effect on the hypoxic tumor compartments could be observed [36].

It should however be noted that the observed alterations in tumor vasculature rather than the degree of hypoxia itself may have influenced pimonidazole uptake (cf Section 2.4). Logically, this potential complication factor may also hold in PET imaging studies. The observed effects may also depend on the inoculation site. For instance, subcutaneous inoculation of HIF-1 α -deficient and VEGF-deficient transformed astrocytes resulted in reduced vessel density and tumor growth. Intracranial inoculation on the other hand led to accelerated growth of HIF-1 α -deficient tumor growth, whereas VEGF-deficient astrocytomas still exhibited a growth disadvantage. This suggests that the differences in the microenvironment and the vascular structure between the two inoculation sites determine the behavior and aggressiveness of the tumor [44]. The influence of the inoculation site on tumor hypoxia will be discussed more extensively in Section 2.2.1.

To wind up with, in orthotopic MDA-MB-231 breast cancer xenograft models, exposure of cells to hypoxic culturing conditions prior to inoculation not only accelerated tumor growth, but also contributed to multidrug resistance,

most importantly via increased HIF-1 α levels. Indeed, the hypoxia-driven induction of a differential protein expression makes this technique very interesting for imaging purposes. However, differences between tumors derived from hypoxic and normoxic cells tend to diminish with increases in tumor volume, starting from 100 mm³ onwards [45]. Moreover, the effects of in vitro exposure of cells to hypoxic conditions prior to inoculation may be tissue-dependent or cell line-dependent. Indeed, in recent studies, it was shown that xenografts from lung cancer cells cultured under hypoxic conditions show decelerated tumor growth but enhanced cell survival, whereas the same strategy resulted in accelerated subcutaneous tumor growth in a CRC model [46, 47]. In our own quest to create a differential hypoxic xenograft model, we investigated the effect of hypoxia-pretreatment of Colo205 cells 72 hours prior to inoculation. Fourteen days after inoculation, we observed a nonsignificant Colo205 tumor growth inhibition of 56% of the tumors arising from hypoxia-pretreated cells, but [¹⁸F]FMISO uptake was not affected by the hypoxia-pretreatment (Figure 4). This may not be surprising since tumor volume may also affect hypoxia tracer uptake, as discussed in Section 2.1.3. Therefore, seeing the unpredictable effect on tumor proliferation, we do not believe that exposure of cells to hypoxic conditions prior to inoculation is a reliable method for in vivo tumor hypoxia modulation.

2.1.3. Tumor Volume. In rat rhabdomyosarcoma tumors, it was observed that the hypoxic volume assessed with both pimonidazole staining and [¹⁸F]FMISO autoradiography increased with increases in tumor volume [48]. In murine sarcoma models on the other hand, inverse correlations between autoradiographically determined [¹⁸F]fluoroerythronitroimidazole ([¹⁸F]FETNIM) and tumor volume were found [49], whereas in other models, [¹⁸F]FETNIM, [¹⁸F]FMISO, or [¹⁸F]FAZA uptake did not correlate with tumor volume [50–52]. These conflicting observations may be due to the presence of tumor necrosis [49–51], since, 2-nitroimidazole PET tracers will be not retained by necrotic cancer cells. As necrosis may be more wide-spread in larger tumors [16], whether or not areas of necrosis are included within the determined volume of interest (VOI) can potentially lead to underestimation or slight overestimation of the amount of tumor hypoxia, respectively, dependent on the quantification method. These important observations should be considered particularly when exclusively focusing on tumor volume.

The use of autoradiographs allows an easy and accurate quantification of the amount of tumor necrosis, although only in ex vivo tissue samples. Corrections for the influence of necrosis on in vivo hypoxia are more complicated. For instance, in a CH3 mammary carcinoma model, [¹⁸F]FMISO TBR did not correlate with tumor volume, despite the existing correlation between tumor volume and pO₂ electrode measurements [5]. Again, this apparent discrepancy between these two measuring techniques has been attributed to the presence of tumor necrosis [54]. Indeed, the automatic thresholding technique which was used in this study to

delineate tumors on PET images [5] may exclude macroscopically necrotic areas without tracer uptake. The polarographic method on the other hand cannot distinguish between viable and necrotic tumor fractions. Interestingly however, by applying necrosis correction in murine rhabdomyosarcoma tumors, it was observed that polarographically-determined pO₂ values did not really change as tumors grew larger and did not correlate with the degree of necrosis, unless when tumors reached a weight of more than 2 grams [55].

Depending on the tumor delineation method (manual or automatic) on static PET images, areas of macroscopic necrosis will or will be not included within the VOI. Importantly, in studies that investigated the relationship between tumor volume and hypoxia PET tracer uptake [16,23,26,27,31,33,56–58], it is not always clear if areas of necrosis were included within the VOI, which renders the interpretation and comparability difficult. Moreover, the resolution of preclinical PET may in any case be too low to discriminate areas of microscopic necrosis. The use of parametric images based on different tracer uptake patterns over time between normoxic, hypoxic, and necrotic regions also looked promising to overcome these shortcomings [11], but the need for dynamic imaging may preclude general use in rodent models for tumor hypoxia.

To what extent tumor volume influences the degree of hypoxia remains an intriguing question. Therefore, we do not recommend the use of different volumes as a model for tumor hypoxia for preclinical PET investigations. If opting for this methodology regardless, the exclusion of macroscopic areas of necrosis during hypoxia PET tracer quantification is advisable.

Taken together, to be able to make a more well-defined distinction between “hypoxic” and “normoxic” tumors, and to broaden the intertumoral oxygenation range, experimental manipulation may be required. In what follows we will discuss the different procedures that have been adopted for this purpose.

2.2. Animal Physiological Parameters

2.2.1. Tumor Location. In a study by Graves et al., the uptake of the glucose analogue 2-deoxy-2-[¹⁸F]fluoro-D-glucose ([¹⁸F]FDG), [¹⁸F]FAZA, and pimonidazole was compared between genetically induced lung tumors in situ, subcutaneous, and orthotopic A549 xenograft models. [¹⁸F]FDG uptake was comparable between all models, whereas [¹⁸F]FAZA and pimonidazole were only trapped in subcutaneous tumors, but not in lesions growing within the lung. Those observations were confirmed by administering the hypoxic prodrug PR-104 to all models, as therapy response was only observed in subcutaneous tumors [59]. Taken together, these data show that the presence of hypoxia in lung cancer may depend on the inoculation site and thus the present microenvironment. It has been hypothesized that this may be either a result of the tumor vasculature being more functional in orthotopic than in subcutaneous tumors, or the accessibility of O₂ via the alveoli in orthotopic

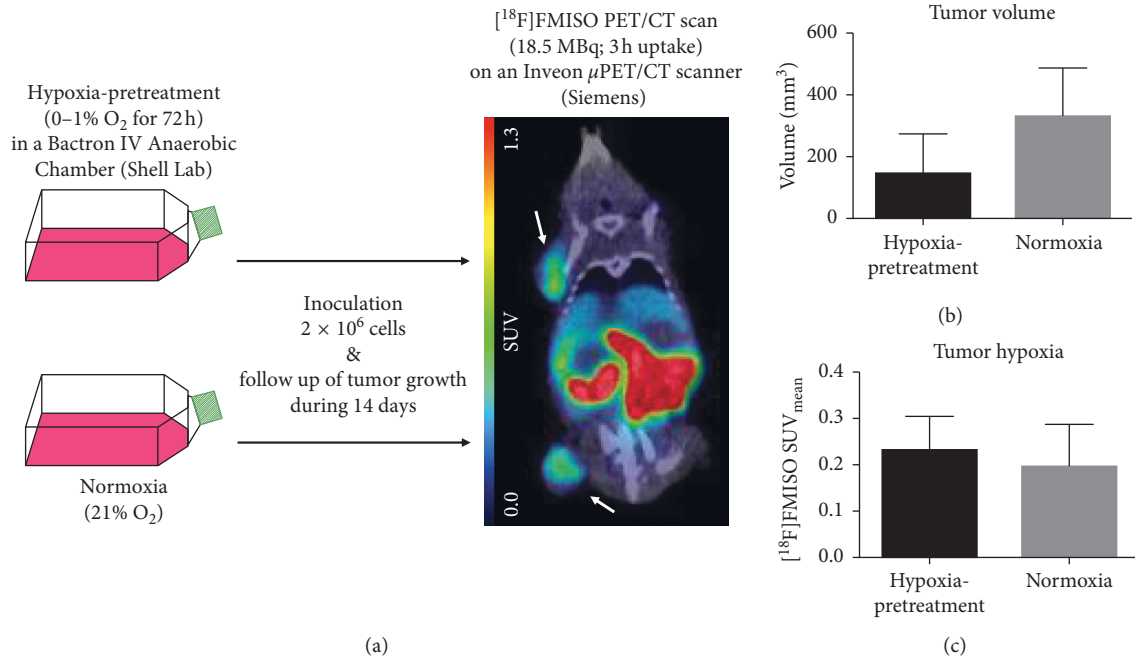


FIGURE 4: Hypoxia-pretreatment experiment, approved by the Antwerp University Ethical Committee (2012-69). All applicable institutional and European guidelines for animal care and use were followed. (a) Colo205 cells (Perkin Elmer) were exposed to hypoxia (0-1% O₂) for 72 h in a Bactron IV Anaerobic Chamber (Shell Lab) or to normoxia (21% O₂) in a common incubator. CD-1 nude mice (Charles River, $n = 5$) were inoculated with 2×10^6 “hypoxic” cells in the shoulder and with 2×10^6 “normoxic” cells in the hind leg. Fourteen days later, animals underwent a [¹⁸F]FMISO PET/CT scan as previously described [53]. Images were analyzed as previously described [53]. One representative mouse is shown. Arrows indicate tumors. As [¹⁸F]FMISO clearance mainly occurs via the hepatobiliary pathway and the gastrointestinal tract [8], tracer uptake can be observed in liver and intestines. (b) “Hypoxic” tumors were numerically, but not significantly, smaller than “normoxic” tumors ($144 \pm 129 \text{ mm}^3$ vs $328 \pm 158 \text{ mm}^3$ [mean \pm SEM], respectively; $p = 0.19$). (c) [¹⁸F]FMISO SUV did not differ between “hypoxic” and “normoxic” tumors (0.228 ± 0.074 vs 0.195 ± 0.090 , respectively; $p = 0.71$).

but not in subcutaneous tumors, thereby suggesting that this inoculation site-specific differential hypoxia is seen exclusively in lung cancer [60]. Nevertheless, our research group recently observed significantly lower tumor hypoxia, as assessed with CAIX immunohistochemistry, in an orthotopic CRC xenograft model as compared to subcutaneous tumors [61].

As already mentioned earlier in Section 2.1.2, the growth and differentiation of HIF-1 α -deficient and VEGF-deficient astrocytomas is also dependent on the inoculation site and its corresponding microenvironment [44]; however, the degree of hypoxia was not quantified in the cited study. Similarly, it has been observed that orthotopically and subcutaneously grown gliomas derived from the same cell line exhibit different gene expression profiles [62]. Thus, it is clear that the inoculation site influences tumor characteristics. Again, a major drawback of such technique is the different growth rate of subcutaneously and orthotopically growing tumors. Moreover, the different growth patterns [59] will inevitably lead to different tumor volumes. Both factors may complicate the interpretation of data on tumor hypoxia (cf Section 2.1.3).

2.2.2. Caloric Restriction. The Warburg effect, i.e., the upregulation of glycolysis in cancer cells regardless of the partial O₂ pressure, accounts for tumors’ high dependency

on the supply of nutrients, especially glucose. Therefore, caloric restriction (CR), which is the reduction in energy uptake without malnutrition as compared to ad libitum feeding, has been considered as a promising synergistic treatment option. CR can be achieved via different ways: via intermittent fasting, via short-term fasting, or via chronic daily energy restriction whereby animals are only fed a certain percentage of normal intake [63]. As tumor hypoxia is inextricably bound with the Warburg effect, CR may provide another obvious way to experimentally influence tumor oxygenation. Indeed, in A549 lung cancer xenograft models, it was shown with EF5 immunostaining that daily food restriction caused significant decreases in tumor hypoxia, on top of significant inhibition of tumor growth. In line with this, HIF-1 α expression, VEGF expression, and microvessel density (MVD) were all reduced [64]. Similar observations were made in rat, mouse, and human brain tumor models, where decreases in HIF-1 α , VEGF, and MVD could be observed [65, 66]. In line with this, it was shown in orthotopic allograft breast cancer models that CR and radiotherapy worked synergistically [67]. Within the context of tumor hypoxia, VEGF inhibition by CR was suggested to account for its observed radiosensitizing capacities, as this may result in vascular normalization and thus increases in the O₂ tension [63].

Taken together, these data are promising for the use of CR to create a differential hypoxic tumor model. However, it has been reported that some cell lines may be resistant for

CR [68]. Moreover, some considerations should be taken into account when using CR in combination with PET imaging. First, CR lowers blood glucose and body weight [63–67] and these disturbances should be taken into account during PET image quantification. Second, the dietary status may determine the intratumoral tracer distribution pattern. In A549 xenograft models, [^{18}F]FDG accumulated predominantly in the hypoxic cancer cells of fasted animals, whereas in fed animals, radiotracer accumulated in the noncancerous stroma [69]. Third, CR also influences tumor volume [64–67], which potentially could alter tumor hypoxia as discussed in Section 2.1.3.

2.3. External Interventions

2.3.1. Breathing. Mortensen et al. separated a hypoxic and a normoxic mammary carcinoma group based on the median [^{18}F]FAZA TBR, although in this study, intertumoral oxygenation heterogeneity was increased by exposing some of the animals to carbogen breathing prior to [^{18}F]FAZA scanning and radiotherapy [4]. The exposure of tumor-bearing animals acutely or chronically to a gas mixture containing a reduced O_2 concentration (e.g., 5–10% O_2) or, exactly the opposite, to an increased O_2 concentration (most commonly carbogen, i.e., 95% O_2), whether or not under hyperbaric pressure conditions, is indeed the most applied technique to modulate tumor oxygenation in vivo. In this way, O_2 diffusion distances decrease or increase, respectively, thereby deteriorating or improving the degree of chronic hypoxia within the tumor. In the study of Mortensen et al., significantly different tumor control probabilities were observed in both tumor groups. Interestingly, when the carbogen-breathing animals were excluded from the analysis, significance of the radiotherapy efficacy was lost. The authors therefore correctly suggested that in general, intertumoral variability in tumor hypoxia within one model may be too low without any external manipulations [4].

In a variety of cancer xenograft models, the breathing technique has proven to be very useful to detect different degrees of hypoxia with numerous hypoxia PET tracers, including [^{18}F]FMISO [5, 6, 14, 29, 50, 53, 70–72], [^{18}F]EF3 [6, 7, 73], [^{18}F]FETNIM [50], [^{18}F]FAZA [4, 14, 22, 71, 74, 75], [^{18}F]fluoroetanidazole ([^{18}F]FETA) [76], and [^{18}F]HX4 [8, 14, 77]. An important issue of this breathing technique is that tracer metabolism and trace uptake of background regions may also be affected [14], which may hamper image quantification using background normalization. It has been observed that carbogen or hypoxic gas breathing significantly altered [^{18}F]FMISO uptake in fat tissue [50] and [^{18}F]FETNIM and [^{18}F]FAZA uptake in muscle [14, 50, 71]. Similar observations were made by Cairns et al., who found drops in pO_2 in normal muscle tissue when animals breathed 5–7% O_2 and rises in pO_2 when ambient air was reintroduced [78]. Taken together, exposure to an altered breathing atmosphere obviously alters hypoxia tracer uptake of noncancerous tissue too. Therefore, it may not be appropriate to calculate TBRs, without thorough understanding of the magnitude and

dynamics of these changes. By contrast, it has also been argued that calculating TBRs is a way to anticipate the possible confounding effects on background tracer uptake [75].

Carbogen breathing is often combined with the administration of nicotinamide, a vitamin B3 derivate that induces vasodilation and thus improves tumor perfusion. In this way, not only chronic but also acute hypoxia can be counterbalanced [79]. Indeed, regional short-lived changes in blood flow could possibly influence tracer circulation and cause considerable day-to-day variations in hypoxia tracer uptake. Nevertheless, given the relatively low temporal resolution of (micro)PET (i.e., 10 seconds dynamic frames) and the long uptake period required for 2-nitroimidazole-based tracers, acute hypoxia may not be detectable with PET [80]. Moreover, in different models, it was shown that the combination of carbogen and nicotinamide gave rise to similar or even less pronounced decreases in the hypoxic fraction compared to carbogen alone [81, 82]. These two facts question the additional value of nicotinamide. In spite of this, it has been observed that depending on the amount of carbon dioxide in the gas mixture, breathing of a high- O_2 gas mixture may cause vasoconstriction [83], thereby deteriorating tumor oxygenation state and also tracer delivery. In this particular case, administering nicotinamide may compensate for that [75, 81, 84], but it should be noted that changes in tumor vascularity are not necessarily accompanied by a reduced accessibility of radionuclide tracers to tumor tissue [85, 86]. The eventual positive or negative influence of breathing an altered atmosphere on the oxygenation state and on tumor progression appears to be tissue-dependent and cell line-dependent [46, 83, 87–89].

The effectiveness of breathing models has also been shown in numerous experiments in which hypoxia-evoked therapy resistance was investigated. The most straightforward, yet clinically less relevant approach is the investigation of the influence of breathing an altered O_2 atmosphere on the effect of a single therapy dose. For instance, in fibrosarcoma, colon carcinoma, and hepatoma models, tumor growth decreased significantly when a single dose of 5-fluorouracil (5-FU) was administered during short-term carbogen breathing [90–93]. In line with these results, our study demonstrated a reduced 5-FU chemotherapy effect in colorectal carcinoma xenografts exposed to short-term hypoxic breathing conditions during administration of a single dose of 5-FU, as predicted on a baseline [^{18}F]FMISO scan [53]. In yet another study, rhabdomyosarcoma-bearing rats and lung tumor-bearing mice were exposed to modified O_2 concentrations 4 hours per day during 5 consecutive days. Using this approach, the investigators were able to show that treatment efficacy of the hypoxic prodrug TH-302 was dependent on tumor oxygenation: daily short-term exposure of the xenograft models to carbogen abolished the effect of TH-302, whereas 7% O_2 breathing increased the therapeutic potential [77]. Accordingly, the hypoxic prodrug CEN-209 was induced more effectively in subcutaneous HCT116 tumors of mice exposed to a hypoxic atmosphere, whereas hyperbaric O_2 breathing suppressed CEN-209 activation when the drug was administered after irradiation [94]. The

use of breathing an altered atmosphere for improving radiotherapy response is also being studied extensively. For instance, accelerated radiotherapy with carbogen and nicotinamide (ARCON) enhanced therapeutic response in a variety of tumor models, including mammary adenocarcinoma and sarcoma models [88, 95, 96].

Nevertheless, the breathing approach may become technically challenging for longitudinal therapy schedules. In particular, it has been shown in murine and rat tumors that the effect of breathing an adapted atmosphere can be reversed immediately when the intervention stops [82, 97, 98], is time-dependent [84], and may even be lost after prolonged exposure times [22, 99]. Things may even become more complex when considering that anesthesia potentially influences the experimental outcome. Indeed, to avoid motion-related artefacts during preclinical in vivo imaging, the use of anesthetics is inevitable, and these anesthetics or their carrier gasses (in case of volatile drugs) can have a substantial influence on tumor oxygenation. For instance, drops in pO_2 in tumor and muscle tissue of ketamine/xylazine-anesthetized animals when compared to isoflurane-anesthetized mice have been observed [100]. In CT26 colorectal carcinoma-bearing mice, [^{18}F]FAZA PET uptake was increased in both tumor and muscle tissue in ketamine/xylazine-anesthetized animals as compared to isoflurane-anesthetized animals. Yet these changes were less pronounced in muscle, resulting in higher [^{18}F]FAZA TBR in ketamine/xylazine-anesthetized animals [101]. In a murine adenocarcinoma model on the other hand, no difference in [^{18}F]FMISO TBR between the two anesthesia regimens was found, despite the observation of higher tumoral uptake in ketamine/xylazine-anesthetized animals compared to their isoflurane counterparts [102]. Surprisingly however, in both imaging studies, it was shown that [^{18}F]FAZA and [^{18}F]FMISO TBR, respectively, did not differ between animals breathing room air or animals breathing O_2 during isoflurane anesthesia [101, 102].

2.3.2. Clamping. The O_2 state of tumors can be temporarily reduced by physically occluding the blood supply to the tumor. This clamping technique gives rise to severe hypoxia. In general, animals are subcutaneously inoculated in the hind leg. When tumors have reached the desired volume, animals are anesthetized whereupon the proximal part of the leg is clamped with a rubber band or a metal clamp for a limited time period. In this way, transient acute tumor hypoxia is mimicked. For instance, it was observed that clamping induces expression of HIF-1 α [103]. Deteriorated radiotherapy response after clamping has also been observed [104]. Similarly, this clamping technique has been successfully applied in an orthotopic liver tumor model, where severe hypoxia could be induced via hepatic artery ligation [105].

On top of being painful and stressful [106], clamping has some other major limitations. First, by occluding the blood supply to the tumor, the delivery of therapy or radiotracer will also be extremely hampered [104]. It was hypothesized that this could be prevented by administering [^{18}F]FMISO

prior to initiation of the clamping procedure. Indeed, in two different glioblastoma models, this approach resulted in significantly increased tracer and pimonidazole uptake. On the opposite, it was emphasized that clamping after PET tracer administration may just cause trapping of unbound radiotracer in the tumor [107]. A second drawback of the clamping technique is that ischemia can occur instead of hypoxia, leading to irreversible damage and cell death. Third, the occluded artery may clot during the clamping, hampering reperfusion [105].

2.3.3. Temperature Modification. As excellently reviewed by Song et al. [108], ample evidence in a variety of xenograft models shows that tumor oxygenation improves during and after heating at 39 to 42°C. This observation is probably the result of a combination of an increase in tumor blood perfusion and a decrease in the O_2 consumption rate. Moreover, mild hyperthermia oxygenates both chronic and acute hypoxic cells, and this effect may last for up to 48 hours after heating [108]. A second proposed mechanism of action of hyperthermia is that it may kill hypoxic cells directly [109, 110]. In order to avoid this so-called hyperthermic cytotoxicity, which is beneficial from the therapeutic point of view, but may complicate therapy response evaluation experiments focusing on tumor hypoxia, the applicable temperature range is rather narrow.

Practically, the tumor-bearing leg of restrained or anesthetized animals is immersed into heated water. In a subcutaneous CRC model, this resulted in an immediate decrease in the hypoxic fraction, assessed with the extrinsic hypoxia markers pimonidazole and EF5, but the effect disappeared 24 hours after heating [111]. In a mammary carcinoma model, HIF-1 α increased immediately after 1 hour of hyperthermia treatment and was restored 48 hours later. VEGF however was elevated up to 48 hours after treatment, which on its turn was responsible for the induction of angiogenesis, increased tumor perfusion, and consequent decreases in tumor hypoxia [112].

Another way to achieve hyperthermia is whole-body heating. By placing mice in a heated environmental chamber, body temperature and thus tumor temperature can be raised, leading to improved tumor perfusion [113]. After 6 hours of whole-body heating, pimonidazole staining showed an initial decrease in hypoxia in a CRC xenograft model, possibly by an increase in vascular volume, but slightly increased again after heating [113].

Although it was suggested that temporal increases in HIF-1 α may lead to tumor reoxygenation and may therefore be beneficial in particular cases [112], the role of HIF-1 in therapy resistance is irrefutable and its upregulation via heating may most likely be detrimental. Second, raising the temperature of deep-seated orthotopic tumors may be technically challenging or even impossible using external heating. Whole-body heating may overcome this short-coming but can cause systemic stress. Third, it has been argued that anesthetics, which are indispensable in imaging research, may limit the thermoregulatory response as they cause vasoconstriction and thus may hamper the

efficacy of hyperthermia [113]. Fourth, seeing its transient nature, long-term use of hyperthermia may be complicated. Thus, tumor heating may not be a straightforward approach for the creation of a differential hypoxic tumor model.

2.3.4. Exercise. In two studies by Jones et al., the effects of long-term voluntary wheel running on tumor perfusion, hypoxia, and angiogenesis were investigated in two different orthotopic cancer models. In both models, significantly higher hypoxia, epitomized by an increase in HIF-1 α expression, and also improved tumoral blood perfusion were observed in the exercise group as compared to the sedentary group. As speculated by the authors, exercise may cause an increase in O₂ delivery towards muscle and heart tissue, thereby redirecting the O₂ supply from tumors to these metabolically active tissues. This in turn will activate the HIF-1 cascade in the tumor in order to preserve the local homeostasis, which possibly leads towards angiogenesis [114, 115]. Similarly, increased orthotopic breast tumor perfusion without affecting tumor oxygenation has been observed after forced daily treadmill running [116]. In order to investigate the influence of this altered microenvironment on chemotherapy efficacy, the group of Jones et al. conducted another study in two different murine orthotopic breast cancer models using the same study protocol. As opposed to the authors' previous data, tumor hypoxia, now assessed with EF5 immunohistochemistry, was significantly lower in the exercise groups as compared to controls. MVD and vessel maturity were significantly higher in the exercised animals, which led to increases in tumor perfusion as observed before [117]. Importantly, these results were in line with other preclinical data obtained in a rat orthotopic prostate cancer model [118].

Voluntary wheel running as used in the mouse studies described above [114, 115, 117], may be an animal-friendly, stressless way to influence tumor oxygenation. Moreover, as it is sufficient to provide running wheels in the animals' cages, this method is relatively cheap and uncomplicated since it does not require sophisticated technical equipment. Another major advantage is that low intensity exercise does not influence tumor growth [114, 115, 117]. Nevertheless, results may seem contradictory. In the studies where increases in HIF-1 were observed, hypoxia as such was not quantified [114, 115]. Importantly, the upregulation of HIF-1 α can also be the result of other mechanisms than a hypoxic microenvironment. The observed changes in perfusion [114, 115] on the other hand are most probably acute phenomena, whereas the hypoxia marker EF5 [117] primarily stains chronic hypoxia. Also, the site of inoculation should always be taken into consideration. As already discussed in Section 2.2.1, the subcutaneous space and its adjacent tissues are poorly perfused, which may explain at least partially why subcutaneously implanted tumors experience acute hypoxia during exercise [119]. More cell lines and more models should be studied in order to fully explore the high potential of exercise to create a differential hypoxic tumor model.

2.3.5. Other Approaches. Other, less-studied external interventions to decrease tumor hypoxia include electrical stimulation [120] and the addition of Matrigel during tumor inoculations [121]. By studying the effect of electrical stimulation of the sciatic nerve in intramuscularly implanted liver and rhabdomyosarcoma tumors, significant increases in tumor pO₂ and tumor blood flow were observed in both models, accompanied by decreases in the O₂ consumption rate [120]. However, seeing its invasiveness and technical complexity, this technique may be less applicable, especially in longitudinal therapy response evaluation studies. In line with this, the use of Matrigel is also not supported, as no differences in hypoxia could be observed between tumors originating from FaDu cells in medium with or without Matrigel, respectively [121].

2.4. Pharmacological Interventions. Last decades, the search for radiosensitizers and hypoxia-targeting therapies has led to the development of a variety of promising pharmacological interventions to overcome tumor hypoxia. For an excellent recent review of this matter, we refer to Horsman and Overgaard [110]. Some examples of these therapeutics with potential usefulness for the creation of differential hypoxia are described below.

In order to increase O₂ delivery to tumors, administration of erythropoietin (EPO), a substance naturally produced by the body, seems evident. For instance, in glioblastoma and carboplatin-treated NSCLC xenograft tumors, EPO administration resulted in significantly lower hypoxia as assessed polarographically or with HIF-1 α immunofluorescence, respectively [122, 123]. However, EPO also improved muscle oxygenation [122], which potentially complicates hypoxia tracer TBR calculations (cf Section 2.3.1). Since EPO also acts as a stimulatory growth factor, it may have detrimental tumor effects via processes that are not hypoxia-related [110].

A more promising drug may be metformin, a relatively safe antidiabetic with a favorable pharmacokinetic profile. It could be argued that the administration of metformin to laboratory animals may mimic the clinical situation to a certain extent, as the drug is widely prescribed to diabetes patients. However, it should be emphasized that in most preclinical experiments, the drug is administered to non-diabetic mice, often using doses exceeding those considered safe in humans which may impair direct translation. Among different anticancer effects attributed to this drug, metformin is supposed to improve tumor oxygenation via its inhibitory effect on complex I of the mitochondrial electron transport chain [124]. Zannella et al. showed in HCT116 CRC models that administration of a single dose of metformin indeed decreased tumor hypoxia and consequently improved radiotherapy response using [¹⁸F]FAZA PET [125], results that we were able to confirm in a comparable set-up using [¹⁸F]HX4 PET in A549 NSCLC xenografts [126]. These observations are supported by similar observations obtained in xenograft models of prostate cancer and lung carcinoma using another inhibitor of the

mitochondrial complex I, BAY 87-2243; [^{18}F]FAZA uptake was significantly reduced after administration of this novel drug [86]. In FaDu xenografts, no effect of a single dose of metformin on EF5 staining was observed, but treatment for seven consecutive days caused a nonsignificant decrease in tumor hypoxia [127]. Despite these observations, the way in which metformin influences radiation response is not fully understood and may not be fully attributable to hypoxia-related phenomena [128]. Moreover, in highly hypoxic pediatric sarcoma models, it was shown that metformin had no additive value to chemotherapy response, as opposed to tumors with a better oxygenation state [129].

Another way to influence tumor oxygenation is the use of vascular-targeting agents. A well-studied example of such drugs is hydralazine, a vasodilator that relaxes arterial smooth muscle. At high doses, hydralazine decreases tumor perfusion through the “steal” phenomenon and thus increases tumor hypoxia [79]. For instance, uptake of the hypoxia tracer PrognostixTM ([$^{99\text{m}}\text{Tc}$]HL91) was increased after hydralazine administration in NSCLC and CRC xenograft models when compared to controls [130, 131]. Similarly, drops in pO_2 were observed after hydralazine administration in CRC models [132]. However, the use of hydralazine to create a hypoxic model for investigating the cytotoxicity of the hypoxic prodrug AQ4N produced conflicting results [133, 134]. It was therefore suggested that in some tumor models, the effect of hydralazine may be too limited and too short-lived [134]. Other vascular-targeting agents include vascular disrupting agents [110], such as the novel combretastatin analogue OXi-4503, a tubulin-binding agent that selectively targets endothelial cells and damages existing tumor vessels [135, 136]. However, administration of such vascular-targeting drugs may lead to severe reductions in blood flow and thus ischemic cell death. This may in turn lead to difficulties in interpreting longitudinal PET imaging results as radiotracer delivery may be severely hampered. Another category includes the angiogenesis inhibiting agents [110]. It is generally believed that administration of angiogenesis inhibitors in a correct dose leads to vascular normalization and consequent decreases in tumor hypoxia [137], as shown with [^{18}F]FMISO PET in two patient-derived pancreas xenograft models after dovitinib treatment [138]. However, also increases in [^{18}F]FMISO after antiangiogenesis treatment have been reported [139]. Moreover, for all vascular targeting agents, timing of administration in combination with other therapies, mainly radiotherapy, is still controversial. It is supposed that vascular disrupting agents should be administered shortly after irradiation in order to be additive [110], which makes them unsuitable for the creation of differential hypoxic models.

Yet another drug-based approach is the use of agents that specifically target hypoxic cells. A first class includes O_2 mimetics such as nimorazole. In Denmark, this drug is incorporated in routine radiotherapy treatment of head and neck cancer [119]. A second class includes hypoxic prodrugs such as TH-302 [110]. For instance, it was shown that TH-302 significantly decreased the hypoxic fraction, as assessed with [^{18}F]HX4 PET, in rhabdomyosarcoma and NSCLC xenograft tumors. However, administration of TH-302 for

five consecutive days also resulted in a significant growth delay as compared to control tumors [77], which limits its potential as an exclusive hypoxia modulator.

Despite the promising potential of some of the therapies discussed above as a radiosensitizer, pharmacological intervention may not be the optimal choice for the creation of a differential hypoxic model. Indeed, drugs may disturb the physiological state of the tumor or alter the clearance properties of the tracer [3] and may moreover give rise to complex drug interactions when performing therapy response evaluation studies.

3. Conclusions

In an ideal world, one would be able to detect inherently differential hypoxia within a single cohort of tumor-bearing laboratory animals. However, external manipulations may be indispensable, especially for the investigation and validation of novel hypoxia-targeting therapies. In this article, we reviewed a substantial number of promising techniques with the potential to alter tumor oxygenation in a preclinical *in vivo* setting. Obviously, none of these models will accurately mimic the complexity of human disease. Indeed, each individual discussed technique entails specific practical or ethical drawbacks and is subject to the influence of the other parameters. For instance, when creating a differential hypoxia model using the breathing approach, one should also take into consideration that the studied cell line, the range of tumor volumes, the food type, and fasting periods, body temperature, or anesthesia during imaging studies may all have a substantial influence on the degree of tumor hypoxia, or hypoxia PET tracer uptake, independently from the administered breathing gasses. Therefore, in theory, all of these factors should be monitored very strictly in order to prevent experimental disturbances. Tumor hypoxia is such a transient, complex, and very sensitive process, as a result of which it is extremely susceptible to a lot of internal and external influencing factors.

Indeed, the smallest disturbance in one of the discussed variables mentioned above may individually or in combination lead to unpredictable, unexpected, unreliable, or unreplicable experimental outcomes. Nevertheless, application in a clinical setting is not free of influence from external factors. In this respect, the use of mouse models offers important advantages over clinical research, such as the ease of biopsy specimen collection, the nondependence on laborious patient recruitment and not running the risk of failing to reach target goals, and the curtailment of patient heterogeneity, among others.

Mouse models have actually proven their usefulness, as, for instance, [^{18}F]FMISO has successfully been implemented in patients after extensive preclinical *in vivo* validation. Validation of other hypoxia tracers is however still evolving. In this regard, this review provides a comprehensive overview and a better understanding of the applicable *in vivo* hypoxia modulation methods, but it also reveals that experimental modulation of tumor oxygenation remains a challenge. All of the reviewed methods may serve for

specific experimental designs or hypotheses and in this way, they undoubtedly all contribute to the ongoing search for new biomarkers and cures for cancer.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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References

- [1] M. de Jong, J. Essers, and W. M. Van Weerden, "Imaging preclinical tumour models: improving translational power," *Nature Reviews Cancer*, vol. 14, no. 7, pp. 481–493, 2014.
- [2] I. N. Fleming, R. Manavaki, P. J. Blower et al., "Imaging tumour hypoxia with positron emission tomography," *British Journal of Cancer*, vol. 112, no. 2, pp. 238–250, 2014.
- [3] L. J. Wack, D. Mönnich, W. Van Elmpt et al., "Comparison of [¹⁸F]-FMISO, [¹⁸F]-FAZA and [¹⁸F]-HX4 for PET imaging of hypoxia – a simulation study," *Acta Oncologica*, vol. 54, no. 9, pp. 1370–1377, 2015.
- [4] L. S. Mortensen, M. Busk, M. Nordmark et al., "Assessing radiation response using hypoxia PET imaging and oxygen sensitive electrodes: a preclinical study," *Radiotherapy and Oncology*, vol. 99, no. 3, pp. 418–423, 2011.
- [5] L. Bentzen, S. Keiding, M. R. Horsman et al., "Assessment of hypoxia in experimental mice tumours by [¹⁸F]fluoromisonidazole PET and pO₂ electrode measurements," *Acta Oncologica*, vol. 41, no. 3, pp. 304–312, 2002.
- [6] P. Mahy, M. De Bast, T. De Groot et al., "Comparative pharmacokinetics, biodistribution, metabolism and hypoxia-dependent uptake of [¹⁸F]-EF3 and [¹⁸F]-MISO in rodent tumor models," *Radiotherapy and Oncology*, vol. 89, no. 3, pp. 353–360, 2008.
- [7] P. Mahy, M. De Bast, P. H. Leveque et al., "Preclinical validation of the hypoxia tracer 2-(2-nitroimidazol-1-yl)-N-(3,3,3-[¹⁸F]trifluoropropyl)acetamide, [¹⁸F]EF3," *European Journal of Nuclear Medicine and Molecular Imaging*, vol. 31, no. 9, pp. 1–10, 2004.
- [8] L. J. Dubois, N. G. Lieuwes, M. H. M. Janssen et al., "Pre-clinical evaluation and validation of [¹⁸F]HX4, a promising hypoxia marker for PET imaging," *Proceedings of the National Academy of Sciences*, vol. 108, no. 35, pp. 14620–14625, 2011.
- [9] M. Doss, J. J. Zhang, M.-J. Bélanger et al., "Biodistribution and radiation dosimetry of the hypoxia marker ¹⁸F-HX4 in monkeys and humans determined by using whole-body PET/CT," *Nuclear Medicine Communications*, vol. 31, pp. 1016–1024, 2010.
- [10] M. R. Horsman, L. S. Mortensen, J. B. Petersen et al., "Imaging hypoxia to improve radiotherapy outcome," *Nature Reviews Clinical Oncology*, vol. 9, no. 12, pp. 674–687, 2012.
- [11] D. Thorwarth, S. M. Eschmann, F. Paulsen, and M. Alber, "A kinetic model for dynamic [¹⁸F]-Fmiso PET data to analyse tumour hypoxia," *Physics in Medicine and Biology*, vol. 50, no. 10, pp. 2209–2224, 2005.
- [12] W. A. Weber and F. Kiessling, "Imaging in oncology research," in *Small Animal Imaging*, F. Kiessling, B. J. Pichler, and P. Hauff, Eds., pp. 793–820, Springer International Publishing, Cham, Switzerland, 2nd edition, 2017.
- [13] S. Carlin, H. Zhang, M. Reese et al., "A comparison of the imaging characteristics and microregional distribution of 4 hypoxia PET tracers," *Journal of Nuclear Medicine*, vol. 55, no. 3, pp. 515–521, 2014.
- [14] S. G. J. A. Peeters, C. M. L. Zegers, N. G. Lieuwes et al., "A comparative study of the hypoxia PET tracers [¹⁸F]HX4, [¹⁸F]FAZA, and [¹⁸F]FMISO in a preclinical tumor model," *International Journal of Radiation Oncology Biology Physics*, vol. 91, no. 2, pp. 351–359, 2015.
- [15] M. T. Wyss, M. Honer, P. A. Schubiger, and S. M. Ametamey, "NanoPET imaging of [¹⁸F]fluoromisonidazole uptake in experimental mouse tumours," *European Journal of Nuclear Medicine and Molecular Imaging*, vol. 33, no. 3, pp. 311–318, 2005.
- [16] R. Ali, S. Apte, M. Vilalta et al., "¹⁸F-EF5 PET is predictive of response to fractionated radiotherapy in preclinical tumor models," *PLoS One*, vol. 10, no. 10, Article ID e0139425, 2015.
- [17] C. J. Koch, A. L. Shuman, W. T. Jenkins et al., "The radiation response of cells from 9L gliosarcoma tumours is correlated with [F18]-EF5 uptake," *International Journal of Radiation Biology*, vol. 85, no. 12, pp. 1137–1147, 2010.
- [18] R. Beck, B. Roper, J. M. Carlsen et al., "Pretreatment ¹⁸F-FAZA PET predicts success of hypoxia-directed radiochemotherapy using tirapazamine," *Journal of Nuclear Medicine*, vol. 48, no. 6, pp. 973–980, 2007.
- [19] E. Melsens, E. De Vlieghere, B. Descamps et al., "Hypoxia imaging with ¹⁸F-FAZA PET/CT predicts radiotherapy response in esophageal adenocarcinoma xenografts," *Radiotherapy and Oncology*, vol. 13, no. 1, p. 39, 2018.
- [20] L.-B.-A. Tran, A. Bol, D. Labar et al., "Predictive value of ¹⁸F-FAZA PET imaging for guiding the association of radiotherapy with nimorazole: a preclinical study," *Radiotherapy and Oncology*, vol. 114, no. 2, pp. 189–194, 2015.
- [21] J. Haynes, T. D. McKee, A. Haller et al., "Administration of hypoxia-activated prodrug evofosfamide after conventional adjuvant therapy enhances therapeutic outcome and targets cancer-initiating cells in preclinical models of colorectal cancer," *Clinical Cancer Research*, vol. 24, no. 9, pp. 2116–2127, 2018.
- [22] L.-B.-A. Tran, A. Bol, D. Labar et al., "Hypoxia imaging with the nitroimidazole ¹⁸F-FAZA PET tracer: a comparison with OxyLite, EPR oximetry and ¹⁹F-MRI relaxometry," *Radiotherapy and Oncology*, vol. 105, no. 1, pp. 29–35, 2012.
- [23] L. Dubois, W. Landuyt, L. Cloetens et al., "[¹⁸F]EF3 is not superior to [¹⁸F]FMISO for PET-based hypoxia evaluation as measured in a rat rhabdomyosarcoma tumour model," *European Journal of Nuclear Medicine and Molecular Imaging*, vol. 36, no. 2, pp. 209–218, 2008.
- [24] C. Campanile, M. J. E. Arlt, S. D. Kramer et al., "Characterization of different osteosarcoma phenotypes by PET

- imaging in preclinical animal models,” *Journal of Nuclear Medicine*, vol. 54, no. 8, pp. 1362–1368, 2013.
- [25] A. Corroyer-Dulmont, E. A. Pérès, E. Petit et al., “Non-invasive assessment of hypoxia with 3-[¹⁸F]-fluoro-1-(2-nitro-1-imidazolyl)-2-propanol ([¹⁸F]-FMISO): a PET study in two experimental models of human glioma,” *Biological Chemistry*, vol. 394, no. 4, pp. 529–539, 2013.
- [26] S. Valable, E. Petit, S. Roussel et al., “Complementary information from magnetic resonance imaging and ¹⁸F-fluoromisonidazole positron emission tomography in the assessment of the response to an antiangiogenic treatment in a rat brain tumor model,” *Nuclear Medicine and Biology*, vol. 38, pp. 781–793, 2011.
- [27] A. M. Stokes, C. P. Hart, and C. C. Quarles, “Hypoxia imaging with PET correlates with antitumor activity of the hypoxia-activated prodrug evofosfamide (TH-302) in rodent glioma models,” *Tomography*, vol. 2, no. 3, pp. 229–237, 2016.
- [28] E. G. C. Troost, J. Bussink, J. H. A. M. Kaanders et al., “Comparison of different methods of CAIX quantification in relation to hypoxia in three human head and neck tumor lines,” *Radiotherapy and Oncology*, vol. 76, no. 2, pp. 194–199, 2005.
- [29] E. G. C. Troost, P. Laverman, M. E. P. Philippens et al., “Correlation of [¹⁸F]FMISO autoradiography and pimonidazole immunohistochemistry in human head and neck carcinoma xenografts,” *European Journal of Nuclear Medicine and Molecular Imaging*, vol. 35, no. 10, pp. 1803–1811, 2008.
- [30] J. Wobb, S. A. Krueger, J. L. Kane et al., “The effects of pulsed radiation therapy on tumor oxygenation in 2 murine models of head and neck squamous cell carcinoma,” *International Journal of Radiation Oncology Biology Physics*, vol. 92, no. 4, pp. 820–828, 2015.
- [31] A. Silvoniemi, J. Silén, S. Forsback et al., “Evaluation of repeated [¹⁸F]EF5 PET/CT scans and tumor growth rate in experimental head and neck carcinomas,” *EJNMMI Research*, vol. 4, no. 1, p. 393, 2014.
- [32] J.-V. Gaustad, T. G. Simonsen, L. M. K. Andersen, and E. K. Rofstad, “Vascular abnormalities and development of hypoxia in microscopic melanoma xenografts,” *Journal of Translational Medicine*, vol. 15, no. 1, p. 241, 2017.
- [33] P. Mena-Romano, C. Chang, C. Glowa et al., “Measurement of hypoxia-related parameters in three sublines of a rat prostate carcinoma using dynamic ¹⁸F-FMISO-PET-CT and quantitative histology,” *American Journal of Nuclear Medicine and Molecular Imaging*, vol. 5, pp. 348–362, 2015.
- [34] J. A. O’Donoghue, P. Zanzonico, A. Pugachev et al., “Assessment of regional tumor hypoxia using ¹⁸F-fluoromisonidazole and ⁶⁴Cu(II)-diacetyl-bis(N4-methylthiosemicarbazone) positron emission tomography: comparative study featuring microPET imaging, pO₂ probe measurement, autoradiography, and fluorescent microscopy in the R3327-AT and FaDu rat tumor models,” *International Journal of Radiation Oncology Biology Physics*, vol. 61, no. 5, pp. 1493–1502, 2005.
- [35] D. T. T. Yapp, J. Woo, A. Kartono et al., “Non-invasive evaluation of tumour hypoxia in the Shionogi tumour model for prostate cancer with ¹⁸F-EF5 and positron emission tomography,” *BJU International*, vol. 99, no. 5, pp. 1154–1160, 2007.
- [36] D. T. Dang, F. Chen, L. B. Gardner et al., “Hypoxia-inducible factor-1 promotes nonhypoxia-mediated proliferation in colon cancer cells and xenografts,” *Cancer Research*, vol. 66, no. 3, pp. 1684–1936, 2006.
- [37] W. Li, Y.-Q. Chen, Y.-B. Shen et al., “HIF-1 α knockdown by miRNA decreases survivin expression and inhibits A549 cell growth in vitro and in vivo,” *International Journal of Molecular Medicine*, vol. 32, no. 2, pp. 271–280, 2013.
- [38] J. Nakamura, Y. Kitajima, K. Kai et al., “HIF-1 α is an unfavorable determinant of relapse in gastric cancer patients who underwent curative surgery followed by adjuvant 5-FU chemotherapy,” *International Journal of Cancer*, vol. 127, no. 5, pp. 1158–1171, 2009.
- [39] M. Hiraki, Y. Kitajima, K. Kai et al., “Knockdown of hypoxia-inducible factor-1 α accelerates peritoneal dissemination via the upregulation of MMP-1 expression in gastric cancer cell lines,” *Experimental and Therapeutic Medicine*, vol. 4, no. 3, pp. 355–362, 2012.
- [40] P. Carmeliet, Y. Dor, J.-M. Herbert et al., “Role of HIF-1 α in hypoxia-mediated apoptosis, cell proliferation and tumour angiogenesis,” *Nature*, vol. 394, no. 6692, pp. 485–490, 1998.
- [41] Y. Tsuzuki, D. Fukumura, B. Oosthuysen et al., “Vascular endothelial growth factor (VEGF) modulation by targeting hypoxia-inducible factor-1 α \rightarrow hypoxia response element \rightarrow VEGF cascade differentially regulates vascular response and growth rate in tumors,” *Cancer Research*, vol. 60, pp. 6248–6252, 2000.
- [42] T. Takeda, H. Okuyama, Y. Nishizawa et al., “Hypoxia inducible factor-1 α is necessary for invasive phenotype in *Vegf*-deleted islet cell tumors,” *Scientific Reports*, vol. 2, no. 1, p. 494, 2012.
- [43] M. Inoue, J. H. Hager, N. Ferrara et al., “VEGF-A has a critical, nonredundant role in angiogenic switching and pancreatic beta cell carcinogenesis,” *Cancer Cell*, vol. 1, no. 2, pp. 193–202, 2002.
- [44] B. Blouw, H. Song, T. Tihan et al., “The hypoxic response of tumors is dependent on their microenvironment,” *Cancer Cell*, vol. 4, no. 2, pp. 133–146, 2003.
- [45] L. Milane, Z. Duan, and M. Amiji, “Role of hypoxia and glycolysis in the development of multi-drug resistance in human tumor cells and the establishment of an orthotopic multi-drug resistant tumor model in nude mice using hypoxic pre-conditioning,” *Cancer Cell International*, vol. 11, no. 1, p. 3, 2011.
- [46] L. Yu and C. A. Hales, “Long-term exposure to hypoxia inhibits tumor progression of lung cancer in rats and mice,” *BMC Cancer*, vol. 11, no. 1, p. 331, 2011.
- [47] L. Zhang and R. P. Hill, “Hypoxia enhances metastatic efficiency in HT1080 fibrosarcoma cells by increasing cell survival in lungs, not cell adhesion and invasion,” *Cancer Research*, vol. 67, no. 16, pp. 7789–7797, 2007.
- [48] L. Dubois, W. Landuyt, K. Haustermans et al., “Evaluation of hypoxia in an experimental rat tumour model by [¹⁸F]fluoromisonidazole PET and immunohistochemistry,” *British Journal of Cancer*, vol. 91, no. 11, pp. 1947–1954, 2004.
- [49] J.-K. Chung, Y. S. Chang, Y. J. Lee et al., “The effect of tumor size on F-18-labeled fluorodeoxyglucose and fluoroerythronitroimidazole uptake in a murine sarcoma model,” *Annals of Nuclear Medicine*, vol. 13, no. 5, pp. 303–308, 1999.
- [50] T. Grönroos, L. Bentzen, P. Marjamäki et al., “Comparison of the biodistribution of two hypoxia markers [¹⁸F]FETNIM and [¹⁸F]FMISO in an experimental mammary carcinoma,” *European Journal of Nuclear Medicine and Molecular Imaging*, vol. 31, no. 4, pp. 513–520, 2004.
- [51] H. J. Tochon-Danguy, J. I. Sachinidis, F. Chan et al., “Imaging and quantitation of the hypoxic cell fraction of viable tumor in an animal model of intracerebral high grade glioma using

- [18F]fluoromisonidazole (FMISO),” *Nuclear Medicine and Biology*, vol. 29, no. 2, pp. 191–197, 2002.
- [52] B. Solomon, D. Binns, P. Roselt et al., “Modulation of intratumoral hypoxia by the epidermal growth factor receptor inhibitor gefitinib detected using small animal PET imaging,” *Molecular Cancer Therapeutics*, vol. 4, no. 9, pp. 1417–1422, 2005.
- [53] S. De Bruycker, C. Vangestel, T. Van Den Wyngaert et al., “Baseline [¹⁸F]FMISO μPET as a predictive biomarker for response to HIF-1α inhibition combined with 5-FU chemotherapy in a human colorectal cancer xenograft model,” *Molecular Imaging and Biology*, vol. 18, no. 4, pp. 606–616, 2016.
- [54] A. A. Khalil, M. R. Horsman, and J. Overgaard, “The importance of determining necrotic fraction when studying the effect of tumour volume on tissue oxygenation,” *Acta Oncologica*, vol. 34, no. 3, pp. 297–300, 1995.
- [55] K. De Jaeger, F. M. Merlo, M.-C. Kavanagh et al., “Heterogeneity of tumor oxygenation: relationship to tumor necrosis, tumor size, and metastasis,” *International Journal of Radiation Oncology Biology Physics*, vol. 42, no. 4, pp. 717–721, 1998.
- [56] P. Zanzonico, J. O’Donoghue, J. D. Chapman et al., “Iodine-124-labeled iodo-azomycin-galactoside imaging of tumor hypoxia in mice with serial microPET scanning,” *European Journal of Nuclear Medicine and Molecular Imaging*, vol. 31, no. 1, pp. 117–128, 2003.
- [57] D. Zhao, A. Constantinescu, E. W. Hahn, and R. P. Mason, “Tumor oxygen dynamics with respect to growth and respiratory challenge: investigation of the Dunning prostate R3327-HI tumor,” *Radiation Research*, vol. 156, no. 5, pp. 510–520, 2001.
- [58] J.-I. Saitoh, H. Sakurai, Y. Suzuki et al., “Correlations between in vivo tumor weight, oxygen pressure, ³¹P NMR spectroscopy, hypoxic microenvironment marking by β-D-iodinated azomycin galactopyranoside (β-D-IAZGP), and radiation sensitivity,” *International Journal of Radiation Oncology Biology Physics*, vol. 54, no. 3, pp. 903–909, 2002.
- [59] E. E. Graves, M. Vilalta, I. K. Cecic et al., “Hypoxia in models of lung cancer: implications for targeted therapeutics,” *Clinical Cancer Research*, vol. 16, no. 19, pp. 4843–4852, 2010.
- [60] A. Maity and C. Koumenis, “Location, location, location - makes all the difference for hypoxia in lung tumors,” *Clinical Cancer Research*, vol. 16, no. 19, pp. 4685–4687, 2010.
- [61] S. Rapic, C. Vangestel, J. Verhaeghe et al., “Characterization of an orthotopic colorectal cancer mouse model and its feasibility for accurate quantification in positron emission tomography,” *Molecular Imaging and Biology*, vol. 19, no. 5, pp. 762–771, 2017.
- [62] K. Camphausen, B. Purow, M. Sproull et al., “Orthotopic growth of human glioma cells quantitatively and qualitatively influences radiation-induced changes in gene expression,” *Cancer Research*, vol. 65, no. 22, pp. 10389–10393, 2005.
- [63] R. J. Klement and C. E. Champ, “Calories, carbohydrates, and cancer therapy with radiation: exploiting the five R’s through dietary manipulation,” *Cancer and Metastasis Reviews*, vol. 33, no. 1, pp. 217–229, 2014.
- [64] B.-Q. Lin, Z.-Y. Zeng, S.-S. Yang, and C.-W. Zhuang, “Dietary restriction suppresses tumor growth, reduces angiogenesis, and improves tumor microenvironment in human non-small-cell lung cancer xenografts,” *Lung Cancer*, vol. 79, no. 2, pp. 111–117, 2013.
- [65] M. A. Mahlke, L. A. Cortez, M. A. Ortiz et al., “The anti-tumor effects of calorie restriction are correlated with reduced oxidative stress in ENU-induced gliomas,” *Pathobiology of Aging & Age-related Diseases*, vol. 1, no. 1, p. 7189, 2011.
- [66] P. Mukherjee, “Antiangiogenic and proapoptotic effects of dietary restriction on experimental mouse and human brain tumors,” *Clinical Cancer Research*, vol. 10, no. 16, pp. 5622–5629, 2004.
- [67] A. Saleh, B. Simone, J. Palazzo et al., “Caloric restriction augments radiation efficacy in breast cancer,” *Cell Cycle*, vol. 12, no. 12, pp. 1955–1963, 2014.
- [68] N. Y. Kalaany and D. M. Sabatini, “Tumours with PI3K activation are resistant to dietary restriction,” *Nature*, vol. 458, no. 7239, pp. 725–731, 2009.
- [69] G. Zhang, J. Li, X. Wang et al., “The reverse Warburg effect and ¹⁸F-FDG uptake in non-small cell lung cancer A549 in mice: a pilot study,” *Journal of Nuclear Medicine*, vol. 56, no. 4, pp. 607–612, 2015.
- [70] L. Bentzen, S. Keiding, M. R. Horsman et al., “Feasibility of detecting hypoxia in experimental mouse tumours with ¹⁸F-fluorinated tracers and positron emission tomography: a study evaluating [¹⁸F]fluoromisonidazole and [¹⁸F]fluoro-2-deoxy-D-glucose,” *Acta Oncologica*, vol. 39, no. 5, pp. 629–637, 2000.
- [71] G. Reischl, D. S. Dorow, C. Cullinane et al., “Imaging of tumor hypoxia with [¹²⁴I]IAZA in comparison with [¹⁸F]FMISO and [¹⁸F]FAZA - first small animal PET results,” *Journal of Pharmacy & Pharmaceutical Sciences*, vol. 10, pp. 203–211, 2007.
- [72] K.-I. Matsumoto, L. Szajek, M. Krishna et al., “The influence of tumor oxygenation on hypoxia imaging in murine squamous cell carcinoma using [⁶⁴Cu]Cu-ATSM or [¹⁸F]fluoromisonidazole positron emission tomography,” *International Journal of Oncology*, vol. 30, pp. 873–881, 2007.
- [73] P. Mahy, M. De Bast, J. Gillart et al., “Detection of tumour hypoxia: comparison between EF5 adducts and [¹⁸F]EF3 uptake on an individual mouse tumour basis,” *European Journal of Nuclear Medicine and Molecular Imaging*, vol. 33, no. 5, pp. 553–556, 2006.
- [74] F. C. Maier, M. Kneilling, G. Reischl et al., “Significant impact of different oxygen breathing conditions on non-invasive in vivo tumor-hypoxia imaging using [¹⁸F]-fluoroazomycin-arabino-furanoside ([¹⁸F]FAZA),” *Radiation Oncology*, vol. 6, no. 1, p. 165, 2011.
- [75] M. Piert, H.-J. Machulla, M. Picchio et al., “Hypoxia-specific tumor imaging with ¹⁸F-fluoroazomycin arabinoside,” *Journal of Nuclear Medicine*, vol. 46, pp. 106–113, 2005.
- [76] H. Barthel, H. Wilson, D. R. Collingridge et al., “In vivo evaluation of [¹⁸F]fluoroetomidazole as a new marker for imaging tumour hypoxia with positron emission tomography,” *British Journal of Cancer*, vol. 90, no. 11, pp. 2232–2242, 2004.
- [77] S. G. J. A. Peeters, C. M. L. Zegers, R. Biemans et al., “TH-302 in combination with radiotherapy enhances the therapeutic outcome and is associated with pretreatment [¹⁸F]HX4 hypoxia PET imaging,” *Clinical Cancer Research*, vol. 21, no. 13, pp. 2984–2992, 2015.
- [78] R. A. Cairns, T. Kalliomaki, and R. P. Hill, “Acute (cyclic) hypoxia enhances spontaneous metastasis of KHT murine tumors,” *Cancer Research*, vol. 61, pp. 8903–8908, 2001.
- [79] P. Sonveaux, “Provascular strategy: targeting functional adaptations of mature blood vessels in tumors to selectively influence the tumor vascular reactivity and improve cancer treatment,” *Radiotherapy and Oncology*, vol. 86, no. 3, pp. 300–313, 2008.

- [80] M. Busk, M. R. Horsman, P. E. G. Kristjansen et al., "Aerobic glycolysis in cancers: implications for the usability of oxygen-responsive genes and fluorodeoxyglucose-PET as markers of tissue hypoxia," *International Journal of Cancer*, vol. 122, no. 12, pp. 2726–2734, 2008.
- [81] J. Bussink, J. H. Kaanders, P. F. Rijken et al., "Vascular architecture and microenvironmental parameters in human squamous cell carcinoma xenografts: effects of carbogen and nicotinamide," *Radiotherapy and Oncology*, vol. 50, no. 2, pp. 173–184, 1999.
- [82] S. P. Robinson, F. A. Howe, M. Stubbs, and J. R. Griffiths, "Effects of nicotinamide and carbogen on tumour oxygenation, blood flow, energetics and blood glucose levels," *British Journal of Cancer*, vol. 82, pp. 2007–2014, 2000.
- [83] T. J. Dunn, R. D. Braun, W. E. Rhemus et al., "The effects of hyperoxic and hypercarbic gases on tumour blood flow," *British Journal of Cancer*, vol. 80, no. 1-2, pp. 117–126, 1999.
- [84] S. A. Hill, D. R. Collingridge, B. Vojnovic, and D. J. Chaplin, "Tumour radiosensitization by high-oxygen-content gases: influence of the carbon dioxide content of the inspired gas on pO₂, microcirculatory function and radiosensitivity," *International Journal of Radiation Oncology Biology Physics*, vol. 40, no. 4, pp. 943–951, 1998.
- [85] B. Thézé, N. Bernards, A. Beynel et al., "Monitoring therapeutic efficacy of sunitinib using [¹⁸F]FDG and [¹⁸F]FMISO PET in an immunocompetent model of luminal B (HER2-positive)-type mammary carcinoma," *BMC Cancer*, vol. 15, no. 1, p. 534, 2015.
- [86] E. Chang, H. Liu, K. Unterschemmann et al., "¹⁸F-FAZA PET imaging response tracks the reoxygenation of tumors in mice upon treatment with the mitochondrial complex I inhibitor BAY 87-2243," *Clinical Cancer Research*, vol. 21, no. 2, pp. 335–346, 2015.
- [87] N. Khan, H. Li, H. Hou et al., "Tissue pO₂ of orthotopic 9L and C6 gliomas and tumor-specific response to radiotherapy and hyperoxygenation," *International Journal of Radiation Oncology Biology Physics*, vol. 73, no. 3, pp. 878–885, 2009.
- [88] G. Stüben, M. Stuschke, K. Knühmann et al., "The effect of combined nicotinamide and carbogen treatments in human tumour xenografts: oxygenation and tumour control studies," *Radiotherapy and Oncology*, vol. 48, no. 2, pp. 143–148, 1998.
- [89] H. W. M. Van Laarhoven, J. Bussink, J. Lok et al., "Effects of nicotinamide and carbogen in different murine colon carcinomas: immunohistochemical analysis of vascular architecture and microenvironmental parameters," *International Journal of Radiation Oncology Biology Physics*, vol. 60, no. 1, pp. 310–321, 2004.
- [90] P. M. McSheehy, S. P. Robinson, A. S. Ojugo et al., "Carbogen breathing increases 5-fluorouracil uptake and cytotoxicity in hypoxic murine RIF-1 tumors: a magnetic resonance study in vivo," *Cancer Research*, vol. 58, pp. 1185–1194, 1998.
- [91] P. M. J. McSheehy, R. E. Port, L. M. Rodrigues et al., "Investigations in vivo of the effects of carbogen breathing on 5-fluorouracil pharmacokinetics and physiology of solid rodent tumours," *Cancer Chemotherapy and Pharmacology*, vol. 55, no. 2, pp. 117–128, 2004.
- [92] H. W. M. Van Laarhoven, G. Gambarota, J. Lok et al., "Carbogen breathing differentially enhances blood plasma volume and 5-fluorouracil uptake in two murine colon tumor models with a distinct vascular structure," *Neoplasia*, vol. 8, no. 6, pp. 477–487, 2006.
- [93] Y. J. L. Kamm, G. J. Peters, W. E. Hull et al., "Correlation between 5-fluorouracil metabolism and treatment response in two variants of C26 murine colon carcinoma," *British Journal of Cancer*, vol. 89, no. 4, pp. 754–762, 2003.
- [94] J. Wang, A. Foehrenbacher, J. Su et al., "The 2-nitroimidazole EF5 is a biomarker for oxidoreductases that activate the bioreductive prodrug CEN-209 under hypoxia," *Clinical Cancer Research*, vol. 18, no. 6, pp. 1684–1695, 2012.
- [95] A. Rojas, V. K. Hirst, A. S. Calvert, and H. Johns, "Carbogen and nicotinamide as radiosensitizers in a murine mammary carcinoma using conventional and accelerated radiotherapy," *International Journal of Radiation Oncology Biology Physics*, vol. 34, no. 2, pp. 357–365, 1996.
- [96] M. R. Horsman and J. Overgaard, "Preclinical studies on how to deal with patient intolerance to nicotinamide and carbogen," *Radiotherapy and Oncology*, vol. 70, no. 3, pp. 301–309, 2004.
- [97] O. Thews and P. Vaupel, "Zeitliche Veränderungen der Tumoroxygenierung und -durchblutung während normo- und hyperbarer inspiratorischer Hyperoxie," *Strahlentherapie und Onkologie*, vol. 192, no. 3, pp. 174–181, 2015.
- [98] K.-I. Matsumoto, M. Bernardo, S. Subramanian et al., "MR assessment of changes of tumor in response to hyperbaric oxygen treatment," *Magnetic Resonance in Medicine*, vol. 56, no. 2, pp. 240–246, 2006.
- [99] J. H. Kaanders, J. Bussink, and A. J. Van Der Kogel, "ARCON: a novel biology-based approach in radiotherapy," *The Lancet Oncology*, vol. 3, no. 12, pp. 728–737, 2002.
- [100] C. Baudalet and B. Gallez, "Effect of anesthesia on the signal intensity in tumors using BOLD-MRI: comparison with flow measurements by laser Doppler flowmetry and oxygen measurements by luminescence-based probes," *Magnetic Resonance Imaging*, vol. 22, no. 7, pp. 905–912, 2004.
- [101] M. Mahling, K. Fuchs, W. M. Thaiss et al., "A comparative pO₂ probe and [¹⁸F]-fluoro-azomycin-arabino-furanoside ([¹⁸F]FAZA) PET study reveals anesthesia-induced impairment of oxygenation and perfusion in tumor and muscle," *PLoS One*, vol. 10, no. 4, Article ID e0124665, 2015.
- [102] V. Kersemans, B. Cornelissen, R. Hueting et al., "Hypoxia imaging using PET and SPECT: the effects of anesthetic and carrier gas on [⁶⁴Cu]-ATSM, [^{99m}Tc]-HL91 and [¹⁸F]-FMISO tumor hypoxia accumulation," *PLoS One*, vol. 6, no. 11, Article ID e25911, 2011.
- [103] A. Ferrario, K. F. Tiehl Von, N. Rucker et al., "Antiangiogenic treatment enhances photodynamic therapy responsiveness in a mouse mammary carcinoma," *Cancer Research*, vol. 60, pp. 4066–4069, 2000.
- [104] K. Kubota, M. Tada, S. Yamada et al., "Comparison of the distribution of fluorine-18 fluoromisonidazole, deoxyglucose and methionine in tumour tissue," *European Journal of Nuclear Medicine and Molecular Imaging*, vol. 26, no. 7, pp. 750–757, 1999.
- [105] P. Brader, C. C. Riedl, Y. Woo et al., "Imaging of hypoxia-driven gene expression in an orthotopic liver tumor model," *Molecular Cancer Therapeutics*, vol. 6, no. 11, pp. 2900–2908, 2007.
- [106] S. Rockwell, J. E. Moulder, and D. F. Martin, "Effectiveness and biological effects of techniques used to induce hypoxia in solid tumors," *Radiotherapy and Oncology*, vol. 5, no. 4, pp. 311–319, 1986.
- [107] E. G. C. Troost, P. Laverman, J. H. A. M. Kaanders et al., "Imaging hypoxia after oxygenation-modification: comparing [¹⁸F]FMISO autoradiography with pimonidazole

- immunohistochemistry in human xenograft tumors,” *Radiation Therapy and Oncology*, vol. 80, no. 2, pp. 157–164, 2006.
- [108] C. W. Song, H. Park, and R. J. Griffin, “Improvement of tumor oxygenation by mild hyperthermia,” *Radiation Research*, vol. 155, no. 4, pp. 515–528, 2001.
- [109] M. R. Horsman and J. Overgaard, “Hyperthermia: a potent enhancer of radiotherapy,” *Clinical Oncology*, vol. 19, no. 6, pp. 418–426, 2007.
- [110] M. R. Horsman and J. Overgaard, “The impact of hypoxia and its modification of the outcome of radiotherapy,” *Journal of Radiation Research*, vol. 57, no. S1, pp. i90–i98, 2016.
- [111] X. Sun, X.-F. Li, J. Russell et al., “Changes in tumor hypoxia induced by mild temperature hyperthermia as assessed by dual-tracer immunohistochemistry,” *Radiation Therapy and Oncology*, vol. 88, no. 2, pp. 269–276, 2008.
- [112] E. J. Moon, P. Sonveaux, P. E. Porporato et al., “NADPH oxidase-mediated reactive oxygen species production activates hypoxia-inducible factor-1 (HIF-1) via the ERK pathway after hyperthermia treatment,” *Proceedings of the National Academy of Sciences*, vol. 107, no. 47, pp. 20477–20482, 2010.
- [113] A. Sen, M. L. Capitano, J. A. Spornyak et al., “Mild elevation of body temperature reduces tumor interstitial fluid pressure and hypoxia and enhances efficacy of radiotherapy in murine tumor models,” *Cancer Research*, vol. 71, no. 11, pp. 3872–3880, 2011.
- [114] L. W. Jones, B. L. Viglianti, J. A. Tashjian et al., “Effect of aerobic exercise on tumor physiology in an animal model of human breast cancer,” *Journal of Applied Physiology*, vol. 108, no. 2, pp. 343–348, 2010.
- [115] L. W. Jones, J. Antonelli, E. M. Masko et al., “Exercise modulation of the host-tumor interaction in an orthotopic model of murine prostate cancer,” *Journal of Applied Physiology*, vol. 113, no. 2, pp. 263–272, 2012.
- [116] J. M. Wiggins, L. Rice, S. Lepler et al., “The impact of daily exercise on tumor perfusion,” in *Proceedings of the 110th Annual Meeting of the American Association for Cancer Research (AACR)*, Abstract nr 5913, AACR, Philadelphia, PA, USA, April 2017.
- [117] A. S. Betof, C. D. Lascola, D. Weitzel et al., “Modulation of murine breast tumor vascularity, hypoxia and chemotherapeutic response by exercise,” *Journal of the National Cancer Institute*, vol. 107, no. 5, article djv040, 2015.
- [118] D. J. McCullough, J. N. Stabley, D. W. Siemann, and B. J. Behnke, “Modulation of blood flow, hypoxia, and vascular function in orthotopic prostate tumors during exercise,” *Journal of the National Cancer Institute*, vol. 106, no. 4, article dju036, 2014.
- [119] D. W. Siemann and M. R. Horsman, “Modulation of the tumor vasculature and oxygenation to improve therapy,” *Pharmacology & Therapeutics*, vol. 153, pp. 107–124, 2015.
- [120] B. F. Jordan, P. Sonveaux, O. Feron et al., “Nitric oxide-mediated increase in tumor blood flow and oxygenation of tumors implanted in muscles stimulated by electric pulses,” *International Journal of Radiation Oncology Biology Physics*, vol. 55, no. 4, pp. 1066–1073, 2003.
- [121] F. P. Fliedner, A. E. Hansen, J. T. Jørgensen, and A. Kjær, “The use of matrigel has no influence on tumor development or PET imaging in FaDu human head and neck cancer xenografts,” *BMC Medical Imaging*, vol. 16, no. 1, p. 5, 2016.
- [122] S. Scigliano, S. Pinel, S. Poussier et al., “Measurement of hypoxia using invasive oxygen-sensitive electrode, pimonidazole binding and ¹⁸F-FDG uptake in anaemic or erythropoietin-treated mice bearing human glioma xenografts,” *International Journal of Oncology*, vol. 32, pp. 69–77, 2008.
- [123] D. Doleschel, A. Rix, S. Arns et al., “Erythropoietin improves the accumulation and therapeutic effects of carboplatin by enhancing tumor vascularization and perfusion,” *Theranostics*, vol. 5, no. 8, pp. 905–918, 2015.
- [124] W. W. Wheaton, S. E. Weinberg, R. B. Hamanaka et al., “Metformin inhibits mitochondrial complex I of cancer cells to reduce tumorigenesis,” *eLife*, vol. 3, article e02242, 2014.
- [125] V. E. Zannella, A. Dal Pra, H. Muaddi et al., “Reprogramming metabolism with metformin improves tumor oxygenation and radiotherapy response,” *Clinical Cancer Research*, vol. 19, no. 24, pp. 6741–6750, 2013.
- [126] S. De Bruycker, C. Vangestel, T. Van Den Wyngaert et al., “¹⁸F-flortanidazole hypoxia PET holds promise as a prognostic and predictive imaging biomarker in a lung cancer xenograft model treated with metformin and radiotherapy,” *Journal of Nuclear Medicine*, 2018, In press.
- [127] T. M. Ashton, E. Fokas, L. A. Kunz-Schughart et al., “The anti-malarial atovaquone increases radiosensitivity by alleviating tumour hypoxia,” *Nature Communications*, vol. 7, p. 12308, 2016.
- [128] M. Koritzinsky, “Metformin: a novel biological modifier of tumor response to radiation therapy,” *International Journal of Radiation Oncology Biology Physics*, vol. 93, no. 2, pp. 454–464, 2015.
- [129] C. Garofalo, M. Capristo, M. C. Manara et al., “Metformin as an adjuvant drug against pediatric sarcomas: hypoxia limits therapeutic effects of the drug,” *PLoS One*, vol. 8, no. 12, Article ID e83832, 2013.
- [130] B.-F. Lee, N.-T. Chiu, C.-C. Hsia, and L.-H. Shen, “Accumulation of Tc-99m HL91 in tumor hypoxia: in vitro cell culture and in vivo tumor model,” *Kaohsiung Journal of Medical Sciences*, vol. 24, no. 9, pp. 461–472, 2008.
- [131] S. Kinuya, K. Yokoyama, X.-F. Li et al., “Hypoxia-induced alteration of tracer accumulation in cultured cancer cells and xenografts in mice: implications for pre-therapeutic prediction of treatment outcomes with ^{99m}Tc-sestamibi, ²⁰¹Tl chloride and ^{99m}Tc-HL91,” *European Journal of Nuclear Medicine and Molecular Imaging*, vol. 29, no. 8, pp. 1006–1011, 2002.
- [132] G. Mees, R. Dierckx, C. Vangestel et al., “Pharmacologic activation of tumor hypoxia: a means to increase tumor 2-deoxy-2-[¹⁸F]fluoro-D-glucose uptake?,” *Molecular Imaging*, vol. 12, no. 1, pp. 49–58, 2013.
- [133] L. H. Patterson and S. R. McKeown, “AQ4N: a new approach to hypoxia-activated cancer chemotherapy,” *British Journal of Cancer*, vol. 83, no. 12, pp. 1589–1593, 2000.
- [134] E. Manley and D. J. Waxman, “Impact of tumor blood flow modulation on tumor sensitivity to the bioreductive drug banoxantrone,” *Journal of Pharmacology and Experimental Therapeutics*, vol. 344, no. 2, pp. 368–377, 2013.
- [135] A. B. Iversen, M. Busk, and M. R. Horsman, “Induction of hypoxia by vascular disrupting agents and the significance for their combination with radiation therapy,” *Acta Oncologica*, vol. 52, no. 7, pp. 1320–1326, 2013.
- [136] T. R. Wittenborn and M. R. Horsman, “Targeting tumour hypoxia to improve outcome of stereotactic radiotherapy,” *Acta Oncologica*, vol. 54, no. 9, pp. 1385–1392, 2015.
- [137] R. K. Jain, “Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy,” *Science*, vol. 307, no. 5706, pp. 58–62, 2005.
- [138] E. Hernández-Agudo, T. Mondejar, M. L. Soto-Montenegro et al., “Monitoring vascular normalization induced by

- antiangiogenic treatment with ^{18}F -fluoromisonidazole-PET,” *Molecular Oncology*, vol. 10, no. 5, pp. 704–718, 2016.
- [139] M. Murakami, S. Zhao, Y. Zhao et al., “Evaluation of changes in the tumor microenvironment after sorafenib therapy by sequential histology and ^{18}F -fluoromisonidazole hypoxia imaging in renal cell carcinoma,” *International Journal of Oncology*, vol. 41, no. 5, pp. 1593–1600, 2012.



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