Experimental imaging

Can hypoxia-PET map hypoxic cell density heterogeneity accurately in an animal tumor model at a clinically obtainable image contrast?

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Background: PET allows non-invasive mapping of tumor hypoxia, but the combination of low resolution, slow tracer adduct-formation and slow clearance of unbound tracer remains problematic. Using a murine tumor with a hypoxic fraction within the clinical range and a tracer post-injection sampling time that results in clinically obtainable tumor-to-reference tissue activity ratios, we have analyzed to what extent inherent limitations actually compromise the validity of PET-generated hypoxia maps.

Materials and methods: Mice bearing SCCVII tumors were injected with the PET hypoxia-marker fluorooazomycin arabinoside (FAZA), and the immunologically detectable hypoxia marker, pimonidazole. Tumors and reference tissue (muscle, blood) were harvested 0.5, 2 and 4 h after FAZA administration. Tumors were analyzed for global (well counter) and regional (autoradiography) tracer distribution and compared to pimonidazole as visualized using immunofluorescence microscopy.

Results: Hypoxic fraction as measured by pimonidazole staining ranged from 0.09 to 0.32. FAZA tumor to reference tissue ratios were close to unity 0.5 h post-injection but reached values of 2 and 6 when tracer distribution time was prolonged to 2 and 4 h, respectively. A fine-scale pixel-by-pixel comparison of autoradiograms and immunofluorescence images revealed a clear spatial link between FAZA and pimonidazole-adduct signal intensities at 2 h and later. Furthermore, when using a pixel size that mimics the resolution in PET, an excellent correlation between pixel FAZA mean intensity and density of hypoxic cells was observed already at 2 h post-injection.

Conclusions: Despite inherent weaknesses, PET-hypoxia imaging is able to generate quantitative tumor maps that accurately reflect the underlying microscopic reality (i.e., hypoxic cell density) in an animal model with a clinical realistic image contrast.

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The tumor blood supply is abnormal and inefficient causing microenvironmental abnormalities, including tissue accumulation of cellular waste products (e.g., lactate, H+) and depletion of nutrients (e.g., glucose) and oxygen [1]. There is compelling evidence that tumor hypoxia, which may be transient or chronic, negatively affects the outcome as it stimulates aggressive growth and protects against radiation-induced cell injuries [2–4]. Conformal radiation techniques (e.g., intensity-modulated radiotherapy) allow tumor sub-volume dose boosting which may help to overcome hypoxia-induced resistance [5]. Furthermore, techniques that are able to reoxygenate or radiosensitize hypoxic tumors have shown promise [6,7]. However, in order to select those patients that are most likely to benefit from such treatments, knowledge on the severity of hypoxia as well as its geographical distribution is required before and possible during treatment.

Several techniques that allow assessment of tissue oxygenation have been developed including direct assessment by tissue invasive pO2 microelectrodes or indirect methods like analysis of oxygenation-responsive genes [8] or anaerobic metabolites [9]. However, there is a lack of concordance between many methods [10], and furthermore, electrodes are clinically unattractive and the usefulness and true hypoxia specificity of genetic or metabolic biomarkers have been questioned [9–13]. Nitroimidazoles, such as pimonidazole and EF5, are cell membrane-permeable agents that are reduced via the nitroreductase pathway, and transformed into highly reactive compounds in a reaction that shows a steep increase when cellular pO2 drops to below ~10 mm Hg (Km ~ 1–2 mm Hg). Highly specific antibodies that target nitroimidazole-adducts (i.e., the hypoxia-transformed pool)
have been developed and allow quantification of hypoxic cell density in tissue samples collected following systemic marker administration [14,15]. Due to tumor tissue heterogeneity accurate tumor classification may require several biopsies, which is a significant drawback. Radioactive labelling of nitroimidazoles permits PET imaging and is clinically very attractive since it allows assessment of global as well as regional tumor oxygenation which may guide specialized treatment like IMRT [16]. However, the long diffusion path from vessels to the hypoxic target and slow clearance of non-bound (hypoxia-unrelated) tracer result in substantial contaminating background signal (unlike immunohistological detection of nitroimidazole-adducts) which may compromise apparent hypoxia specificity even hours after tracer administration [17]. These inherent problems are exacerbated by the low volumetric resolution of PET (≈100 mm³ in clinical scanners) which necessitates signal averaging across large areas with viable cells experiencing an unknown distribution of oxygen partial pressures ranging from normal physiological levels to near-anoxia, often intermixed with necrotic areas with little tracer. Several nitroimidazoles have been tested, but despite large differences in partition coefficients (lipophilicity) and robustness against unwanted metabolic side reactions, no tracer stands out as being clearly superior [see for example reference [18]]. Accordingly, rather than developing new tracers with marginally better hypoxia specificity (i.e., higher intersingle and intratumor contrast), there is a need to test to what extent the intrinsic non-ideal bio-kinetics of available tracers actually compromise the accuracy of PET imaging as a means to assess global and local hypoxia. In the current study this essential question was addressed using a murine tumor with a hypoxic fraction within the range reported for patients and a tracer post-injection sampling time that results in a relatively low tumor-to-reference tissue activity ratio (and hence likely intratumoral contrast) that mimics the clinical reality.

Materials and methods

Animals

Female C3H/HeNtac mice were obtained from Taconic Laboratories (Ry, Denmark) and all experiments were performed under national and European Union approved guidelines for animal welfare.

Tumor models

SCCvII squamous carcinoma cells were a kind gift from Dr. D.W. Siemann (Department of Radiation Oncology, University of Florida, Florida, USA). Tumors were established from freshly thawed aliquots of single cell suspensions originating from enzymatically dissociated tumors by injection of ≈500 cells in both flanks (PET experiments) or the right side flank (other experiments). Tumor-bearing mice were used for experimentation 14–15 days after inoculation when tumors reached a size of 10–15 mm in the largest dimension.

Tracer preparation

The PET hypoxia tracer [18F]-FAZA was produced as previously described [19].

Ex vivo biodistribution and autoradiography

Mice were injected with 11.8 to 22.9 MBq [18F]-FAZA into the tail vein 0.5, 2 or 4 h prior to sacrifice. To obtain an immunohistologically detectable high-resolution image of the distribution of hypoxic cells, the mice also received an intraperitoneal injection with 60 mg/kg of the hypoxia marker pimonidazole 90 min prior to tissue sampling. The mice were then killed by neck dislocation, the heart was exposed and a blood sample was quickly drawn from the ventricle in a pre-weighed capillary tube and the tumor and a piece of muscle from the thigh were excised. Each tumor was cut into two at the largest diameter and one part was, together with muscle and blood, transferred to pre-weighed counting vials, weighed and analyzed for radioactivity in a Packard well counter. The other half of the tumor was quickly frozen in isopentane pre-cooled to −40°C with dry ice. The frozen tumors were cut into several 10 µm consecutive sections in a cryostat at −20°C and thaw-mounted to Superfrost/plus microscope slides. The tumor sections were then quickly dried under a flow of air and exposed overnight to a FujiFilm phosphor-imaging plate (BAS-SR2025) in an image plate cassette. Following exposure the tumor sections were removed and stored at −80°C until further processing. The spatial distribution of tracer was derived at a pixel size of 25 µm with a Bas5000 laser-excited image analyzer (Fuji Film, Tokyo, Japan) and further analyzed using the Image Gauge version 4.0 Fujifilm software (for further details see the result section).

Dynamic PET experiments

The mice were anaesthetized by inhalation of isoflurane in an anaesthesia chamber and then quickly transferred to a custom built chamber that accommodates up to three mice with tails protruding to the exterior of the chamber allowing for the insertion of tail vein catheters. The mice were kept anaesthetized throughout the catheterization procedure and for the duration of the scan by passing 2% isoflurane carried in air through the chamber. To limit respiratory-induced tumor movements, one tumor in each mouse was gently pulled away from the body by means of a surgical thread sutured to the tumor-covering skin. The other tumor served as a control to exclude that this procedure affects tumor blood flow and thus hypoxia. Prior to tracer injection, a 10-min transmission scan for attenuation and scatter correction was performed using a Concorde microPET R4. The mice were then injected with ~10 MBq of FAZA and scanned dynamically for 3 h. PET data were analyzed using the manufacturer’s software package ASIpro and self-developed software.

Immunohistochemistry

Following autoradiography one tissue section per tumor was HE stained to separate necrosis from viable tissue. The adjacent sections were analyzed for the distribution of bound pimonidazole and GLUT-1 by immunofluorescence microscopy. In short, the tumor sections were fixed for 10 min in acetone (4°C), air dried and then rehydrated for 5 min in PBS (pH 7.4) and incubated with rabbit anti GLUT-1 (Thermo Scientific) 1:100 in primary antibody diluent (PAD) (Abcam, UK), overnight at 4°C. The sections were then incubated with goat anti-rabbitCy3 (JacksonImmunoresearch) 1:600 in PBS for 30 min at 37°C. Subsequently, the sections were incubated with rabbit anti-pimonidazole diluted 1:1000 in PAD for 30 min at 37°C, followed by incubation with donkey anti-rabbit Alexa488 (Molecular Probes Inc., Oregon, USA) diluted 1:600 in PBS for 30 min at 37°C. Between each staining step the slides were rinsed with PBS. Finally, cover slips were mounted with Fluorostab (ICN Pharmaceuticals Inc., California, USA) and scanned to determine the spatial distribution of hypoxic cells (i.e., pimonidazole adducts) and GLUT-1-positive cells.

Fluorescence image acquisition and analysis

After immunohistological staining, the tissue sections were analyzed using a semiautomatic digital image system and differ-
ent filters resulting in two (hypoxia and GLUT-1) gray value images with a pixel size of 2.7 × 2.7 μm. The geographical distribution of FAZA was then compared to the exogenous (pimonidazole) and endogenous (GLUT-1) hypoxia marker using ImageGauge 4.0. In short, autoradiographic and fluorescence images were aligned and the tumor section was covered by a grid consisting of 0.16 mm² pixels, which is a compromise between two opposing constraints. A small pixel size will maximize the capture of spatial variability and the possibility that retention of the two markers is the result of the same microenvironmental stimulus. However, a pixel size smaller than the estimated accuracy of image co-registration and scatter in autoradiograms is not meaningful.

Pixel-by-pixel comparison of the two images generated scatter plots showing the correlation between the photostimulated luminescence (FAZA autoradiography) and fluorescence (a measure of pimonidazole adducts) of the same tumor section. Only pixels within the tumor section borders were included and large necrotic areas, dust particles, tissue holes and folds were excluded. Intratumoral contrast development over time was calculated by enclosing FAZA hot spots (i.e., equivalent to areas with intense pimonidazole staining) and viable tumor areas with low and homogenous FAZA signal (i.e., areas free of pimonidazole-positive cells) with circular regions of interest (ROI) with an area of 0.06 mm², and the ratio between hot spots and background was calculated. To assess to what extent discrepancies are expected between the microscopic reality and the macroscopic PET-observable averaged signal, matching autoradiograms and immunofluorescence images were divided into 5–13 (depending on the size and shape of the tumor section) pixels of 2 × 2 mm which represents a two-dimensional equivalent to PET imaging analysis. For each pixel the absolute proportion of hypoxic cells (hypoxic area/tissue area) was deduced from segmented binary pimonidazole or GLUT-1 fluorescence images as described previously [20] and compared to the mean FAZA signal intensity. To mimic the limitations that apply in real PET imaging analysis, squares with necrotic areas were not excluded from this analysis.

Statistical analysis

The spatial relationship between FAZA and pimonidazole (fluorescence intensity or hypoxic fraction) or GLUT-1 was analyzed using linear regression and Pearson’s correlation coefficients. Biodistribution data were analyzed using a t-test. P values below 0.05 were considered significant.

Results

Whole-tissue biodistribution

The retention of FAZA was quantified as tumor-to-non-hypoxic tissue ratios using two typical reference tissues. At each time point, the tracer concentration was similar in blood and muscle (Fig. 1), suggesting that classification of tumor oxygenation status based on FAZA PET images does not depend on the choice of the reference tissue. There was no significant difference between well counter based analysis of whole tissues and autoradiographic quantification based on single tissue sections (Fig. 1).

Compared to reference tissues there was no tumor accumulation of FAZA 30 min after its administration, but tumor-specific tracer retention was clearly evident at later time points, as tumor-to-reference tissue ratios (T/R) reached values of approximately 2 and 6 when tracer was allowed to circulate for 2 and 4 h, respectively.

Ex vivo autoradiography and immunohistochemistry

Hypoxic fraction (HF, fraction of viable cells with a pO₂ below ~10 mm Hg), as deduced from segmented pimonidazole fluorescence images (not shown), ranged from 0.09 to 0.32 in the SCCVII tumor model, with an average of 0.15 ± 0.03, 0.14 ± 0.02 and 0.22 ± 0.07 in the 0.5, 2 and 4 h group, respectively. There was little intratumoral heterogeneity in FAZA radioactivity 0.5 h after tracer administration, but at later time points a clear spatial correlation between pimonidazole adducts formation and FAZA concentration developed (Fig. 2). A pixel-by-pixel comparison between fluorescence signal intensity (a measure of bound pimonidazole) and photostimulated luminescence (a measure of bound and unbound FAZA) using a small pixel size of 0.4 × 0.4 mm (0.16 mm²) confirmed this visual impression (Fig. 2 and Table 1). Although there was no difference between mean regression values 2 and 4 h post-injection, biodistribution of FAZA, as determined by gamma counting in a Packard well counter or autoradiography (Fig. 1) suggests highly improved hypoxia specificity at 4 h compared to 2 h. A quantitative analysis comparing tracer accumulation in tracer hot spots and tumor areas with low and homogenous tracer distribution (as described in Materials and methods section) confirmed the improved hypoxia specificity as it resulted in ratios between hot spots and background areas of 1.5 ± 0.2, 10.8 ± 2.3 and 27.1 ± 7.1 at 0.5, 2 and 4 h, respectively (not shown).

To compare the results expected from an invasive, laborious and accurate quantification of hypoxia at a cellular resolution with what we expect to see on a crude PET image, the tumor sections were divided into pixels of 2 × 2 mm which mimics the resolution of a microPET (or a high-resolution dedicated head scanner). An index proportional to the absolute number of hypoxic cells in each pixel (pimonidazole-positive cells expressed relative to total pixel area rather than viable area: HF²) was determined, and compared to the matching mean FAZA signal intensity. This analysis revealed excellent agreement between the number of hypoxic cells and mean tracer signal in a given pixel, when tumor hypoxia was assessed at late time points (Fig. 2 and Table 1). Furthermore, despite increased intratumoral contrast, there was no apparent improvement in r-values when tracer equilibration time was prolonged from 2 to 4 h (Fig. 2 and Table 1). The spatial link between FAZA signal intensity and the endogenous putative hypoxia marker GLUT-1 was analyzed similarly.
and resulted in correlations that were weaker and highly variable (Fig. 3). Mean Pearson correlation coefficients were $-0.16$ (range: $-0.74$–$-0.35$), $0.43$ ($-0.02$–$0.81$) and $0.51$ ($0.06$–$0.84$) at 0.5, 2 and 4 h, respectively.

**Dynamic PET scans**

To monitor the real time in vivo tracer kinetics two mice, each carrying two flank tumors, were PET scanned dynamically for 3 h and tumor tracer retention was compared to an image-derived input function obtained from a volume of interest (VOI) drawn within the heart cavity, as identified from the first 5 s scan frame (Fig. 4). Kinetics and overall tracer uptake were similar in suture-fixed and non-immobilized tumors. The tumors accumulated FAZA and reached a near-plateau level after 90 min, whereas T/R continued to increase and reached values of 3–6. The apparent drop in T/R in mouse 2 at the end of the scan is likely due to accidental fluctuations in the image-derived input function caused by poor counting statistics at late time points.

To assess PET-observable intratumoral heterogeneity, the tumors were further analyzed in individual voxels of 15.6 mm$^3$.

![Fig. 2](image-url). Comparisons between total FAZA concentration (bound + unbound) and reduced (bound) pimonidazole measured by digital autoradiography and immunofluorescence microscopy, respectively. Upper row, FAZA autoradiograms in tumors collected 0.5, 2 and 4 h after tracer administration. Second row, pimonidazole stainings for the same tissue sections, covering the areas marked with red rectangles. Third row, spatial comparison between photostimulated luminescence (FAZA) and fluorescence (pimonidazole adducts) intensities using a pixel size of 0.16 mm$^2$. Lower row, spatial comparison between photostimulated luminescence and hypoxic cell density using a pixel size of 2 × 2 mm. In the scattergrams each dot represents one pixel.
which is close to the reported volumetric resolution of the micro-PET at the approximate tumor position 20 mm radial offset [21]. For clarity, and to ensure that voxels are entirely contained within the tumor volume, the time activity curves were only derived from the eight most centrally located voxels. The analysis revealed some voxel-to-voxel heterogeneity, and in mouse 2 an inverse relationship between early tracer availability (likely linked to blood perfusion) and final tracer retention was evident (Fig. 4). Such a relationship was not obvious in mouse 1, possibly due to the smaller difference between voxels with high and low uptake retention potentials (Fig. 4).

### Discussion

The sensitivity and specificity of PET-hypoxia imaging have been questioned due to the slow tracer uptake and washout that leave a high background of unbound tracer even hours after tracer administration, which is particularly problematic when applying a low-resolution imaging technology [17]. This concern, and the often observed lack of correlation between tumor tracer retention and tissue electrode po2 measurements, has turned focus towards the development of better tracers, rather than assessing the usefulness of PET-hypoxia imaging as a means to predict outcome and guide treatment.

Most preclinical validation of hypoxia markers has been done in rodents, where tumor-to-muscle ratios (T/M), and likely hypoxia specificity, reach much higher values than is possible in clinical studies [22,23]. Rather than improved binding, this is mainly due to the short plasma tracer half-life and associated extensive washout of unbound tracer at late time points in small animals with faster metabolism. This corroborates with our data showing no global hypoxia specificity at 30 min and a large increase in T/R between 2 and 4 h (Figs. 1 and 4), although some of the increase can be explained by a 50% higher HF in the 4 h group compared to earlier time points. In patients, however, the gain in T/M is typically very modest when imaging is delayed from 2 to 4 h post-injection, which is close to the scan time limit for fluorinated tracers. The use of long-lived PET isotopes (e.g., 124I) that allows imaging as late as 48 h after tracer administration has also been disappointing [24,25], which may be linked to partly reversible tumor binding [26] or altered physicochemical properties (e.g., lipophilicity) when fluoride is substituted with another isotope [24]. Accordingly, rather than developing novel tracers it is time to move on and assess whether PET imaging, despite inherent limitations, actually can provide reliable maps depicting the number of hypoxic cells globally and locally.

By means of dynamic FAZA PET scans and tissue sampling at various time points we identified the 2 h tracer post-injection sampling point as particularly useful for further analysis, since it likely mimics clinically obtainable intertissue and intratumor image contrast based on two observations: first, the T/R ratio of ~2 in SCCVII tumors is similar to values reported in FAZA scanned head and neck cancer patients [23]; second: the hypoxic fractions of 0.09–0.32 agree well with the reported range of 0.02–0.29 in biopsy-evaluated head and neck cancer patients [27].

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### Table 1

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Tumor</th>
<th>Pimonidazole-adduct signal intensity versus FAZA signal intensity (pixel size: 0.2 × 0.2 mm)</th>
<th>HF versus FAZA signal intensity (pixel size: 2 × 2 mm)</th>
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<td>Correlation coefficients (r)</td>
<td>P-value</td>
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<td>B</td>
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<tr>
<td>0.5</td>
<td>C</td>
<td>0.35</td>
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<td>D</td>
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<tr>
<td>Mean</td>
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Discussion

The sensitivity and specificity of PET-hypoxia imaging have been questioned due to the slow tracer uptake and washout that leave a high background of unbound tracer even hours after tracer administration, which is particularly problematic when applying a low-resolution imaging technology [17]. This concern, and the often observed lack of correlation between tumor tracer retention and tissue electrode po2 measurements, has turned focus towards the development of better tracers, rather than assessing the usefulness of PET-hypoxia imaging as a means to predict outcome and guide treatment.

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To assess the time-dependent development of FAZA hypoxia specificity we compared FAZA and pimonidazole-related signal intensities pixel-wise (pixel size: 0.16 mm²) in individual tumor sections, which is more conclusive than a section-to-section comparison of whole tissue section-derived mean values. First, it generates multiple data points for each section. Second, the pimonidazole-related fluorescence signal strength depends on the input function, the exact staining protocol, including primary and secondary antibodies, microscope settings, illumination stability and other factors, which may cause slight variations between sections from the same tumor or different tumors. The pixel-by-pixel analysis (Fig. 2 and Table 1) confirmed our earlier findings that FAZA accumulates in hypoxic cells [19]. Despite a T/R ratio of ~1 at 30 min post-injection, the pixel-by-pixel analysis revealed a weak (low r-value) yet significant spatial link between FAZA and pimonidazole. The correlation increased substantially at later time points, but unexpectedly, there was no increase in r-values at 4 h compared to 2 h, despite greatly increased intertissue (Fig. 1) and intratumoral (Fig. 2 and hot spot analysis; Result section) contrast over time. This may relate to some inherent weaknesses in this kind of analysis. First, any inaccuracy in image co-registration, will reduce the spatial link between FAZA and pimonidazole, and the significance of the problem will depend on the chosen pixel size and the pattern of hypoxia with the largest influence in tumors (or sections) with numerous small isolated islets of hypoxia (patchy appearance) compared to tumors with larger confluent areas of hypoxia. Second, the inherent scatter in autoradiography will lower any spatial link and again to a greater extent in tumors displaying the patchy type of hypoxia compared to tumors with
larger confluent areas of hypoxia. Such analysis may therefore underestimate the true hypoxia specificity of a tracer and, furthermore, may suffer from a possible tumor-type dependent bias. More importantly, co-localisation analysis will also not per se be able to reveal whether PET-hypoxia imaging can map overall and local tumor hypoxia appropriately (e.g., proportionality between FAZA signal and absolute number of hypoxic cells).

Accordingly, using a clinically relevant pixel size for non-invasive imaging, we next calculated the spatial correlations between the FAZA signal and the number of hypoxic cells per pixel as deduced from segmented pimonidazole images (HF*). Hypoxic fraction, a measure of viable cells below a certain pO\textsubscript{2} value (~10 mm Hg), is considered a robust and reliable analytical technique, largely independent from minor protocol deviations, for the assessment of hypoxia at a cellular resolution. Our pixel-by-pixel comparison of mean FAZA signal intensity and pimonidazole-defined hypoxic cell density revealed an excellent agreement (Fig. 2, Table 1). Again there was no difference between 2 and 4 h post-injection sampling time points, suggesting that the image contrast at 2 h is sufficient for accurate imaging of tumor heterogeneity. Since the FAZA signal (like the pimonidazole-linked fluorescence signal) will be much more intense in near anoxic cells than in cells only slightly below 10 mm Hg, some discordance is expected when comparing intensities (FAZA) with threshold-identified hypoxic cell densities, making regression coefficients close to 0.9 even more remarkable.

Despite these encouraging results, applying a kinetic model for analysis of tumor and blood flow activity curves (TACs) may further improve the accuracy of hypoxia maps. In particular, such analysis may allow identification of voxels, clusters of voxels or whole tumors overlooked by threshold identification, that contains a mixture of severely hypoxic cells intermixed with necrosis with no tracer signal [28,29]. For such tumor entities, TACs with small perfusion peaks and subsequent slow tracer accumulation (cumulative TAC type) are expected, which makes them separable from well-perfused, non-tracer accumulating voxels (washout TAC type) at early time points. In accordance, in the present study we observed an inverse relationship between initial tracer availability and final tracer retention, at least in voxels with widely differing tracer retention potential (Fig. 4). Whether such an analysis will actually improve the validity of hypoxia maps is unclear, since the link between blood flow and hypoxia is non-trivial and may be tumor-type dependent. Accordingly, the tumor vasculature is poorly organized which may uncouple oxygen delivery capacity and blood flow, and inherent differences in metabolic rates (e.g., oxygen consumption rate) may cause hypoxia in highly perfused tumors. Regardless of the prognostic value added by prolonged dynamic scans, they are problematic in clinical settings as they are costly, labour intensive and may cause unacceptable inconvenience to the patients.

The use of endogenous hypoxia markers has been extensively investigated [30–33]. Besides high availability and low cost the main advantage of biopsy-based immunohistochemical analysis over PET-hypoxia imaging is that several genes that are relevant for tumor progression and treatment resistance, but not necessarily linked to hypoxia, can be included. In the present study the expression of the hypoxia-regulated glucose transporter GLUT-1 was compared to the retention pattern of FAZA. GLUT-1 and FAZA showed substantial spatial overlap but also areas of mismatch (Fig. 3) which may relate to regional blood flow changes, a hallmark of solid tumors [1]. Acute perfusion changes may cause FAZA retention in previously well-oxygenated areas (still no accumulation of endogenous hypoxia markers) or lack of tracer retention in recently reoxygenated areas (hypoxia marker still present) [34]. However, discordance may also imply a true lack of hypoxia specificity since GLUT-1 is induced under conditions of low glucose [35] and suppressed at low pH values [12,13], which is typical of the tumor microenvironment [1]. This issue remains highly controversial with many conflicting results and further studies are warranted in order to decide whether endogenous and exogenous markers provide the same information.

In conclusion, despite obvious inherent limitations, PET-hypoxia imaging is able to generate accurate quantitative maps that reflect the underlying microscopic reality (hypoxic cell density) in a tumor model that mimics the clinical challenges. Future studies should focus on determining the prognostic value of PET-hypoxia imaging and its usefulness in image-guided therapy.
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References

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