



Hypoxia PET imaging beyond ^{18}F -FMISO in patients with high-grade glioma: ^{18}F -FAZA and other hypoxia radiotracers

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Abstract

Purpose High-grade gliomas are aggressive primitive brain tumors presenting aberrant vasculature, regional necrosis, and areas of hypoxia. Tumor hypoxia is associated with resistance to conventional treatment and worse prognosis. [^{18}F]-fluoromisonidazole (^{18}F -FMISO) is the most extensively investigated radiotracer for the evaluation of hypoxia. However, the use of ^{18}F -FMISO in clinical practice has been hampered mainly due to the slow clearance of the unbound tracer from normoxic tissue and its low tumor-to-background ratio (TBR). The research community has therefore investigated other radiotracers to overcome the drawbacks of ^{18}F -FMISO. This mini-review aims to present an update on the most relevant PET studies published in the last 15 years evaluating the utility of radiotracers for hypoxia imaging other than ^{18}F -FMISO in high-grade glioma (HGG) patients.

Methods A comprehensive computer literature search of studies was carried out in PubMed/MEDLINE database to identify the most relevant studies published in the last 15 years which investigated the utility of hypoxia PET tracers other than ^{18}F -FMISO in the assessment of tumor hypoxia in patients with HGG.

Results ^{18}F -flouroazomycin arabinoside (^{18}F -FAZA) has been proposed as a valid alternative to ^{18}F -FMISO for the assessment of hypoxia, due to its improved biodistribution and enhanced tumor-to-background ratio. Also 1-(2-[^{18}F]fluoro-1[hydroxymethyl]ethoxy)methyl-2-nitroimidazole(^{18}F -FRP170) seems a valuable hypoxia tracer in patients with brain tumor. The value of copper-diacetyl-bis(*N*4-methylthiosemicarbazone)(Cu-ATSM) seems controversial. Few evidences still exist regarding the utility of ^{18}F -2-(2-nitro-1*H*-imidazol-1-yl)-*N*-(2,2,3,3,3-pentafluoropropyl)-acetamide (^{18}F -EF5).

Conclusion Hypoxia PET imaging has the potential to provide useful information for the clinicians and to guide hypoxia tailored treatments. According to the present literature, the most promising hypoxic tracer seems to be ^{18}F -FAZA, but well-designed and wide trials to validate hypoxia radiotracers and evaluate their clinical utility in daily practice are still lacking.

Keywords PET · Glioma · Hypoxia · FAZA · ATSM · MRI

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Introduction

High-grade glioma (HGG) are aggressive primitive brain tumors arising from the glial tissue [1]. HGG, and particularly grade IV glioma (formerly known as glioblastoma multiforme—GBM), are characterized by aberrant vasculature (a phenomenon known as angiogenesis), regional necrosis, and viable areas of hypoxia [2]. Hypoxia is a pathophysiological status characterized by an oxygen concentration in a tissue lower than normal [3]. In brain tumors, the median pO_2 has been reported to be around 13 mmHg in contrast to the median pO_2 (35 mmHg) documented in normal brain tissue [4, 5].

A well-documented link between hypoxia, prognosis, and resistance to conventional treatments exists in hypoxic tumors [3]. The clinical relevance of mapping and measuring tumor hypoxia in HGG using non-invasive imaging techniques, such as positron emission tomography (PET) or magnetic resonance imaging (MRI), relies on the possibility of delivering hypoxia-modifying treatments, leading theoretically to an improvement of patient outcome [3, 6–8].

Quantification of hypoxia in HGG using PET has been extensively documented [9, 10]. Hypoxia PET imaging agents can be divided into nitroimidazole-based radiotracers and other compounds and further divided in fluorinated and non-fluorinated agents [11]. In the assessment of tissue hypoxia in brain tumors, further important aspects to consider for a tracer are the lipophilicity and the ability to cross the blood–brain barrier (BBB) [12].

[^{18}F]-fluoromisonidazole (^{18}F -FMISO) is the most extensively used and validated radiotracer so far for the evaluation of hypoxia in patients with brain tumors [10, 13–17]. However, other radiotracers have been investigated to overcome the main drawbacks of ^{18}F -FMISO which have limited its translation from the research setting to the clinical practice: the slow clearance of the unbound tracer from normoxic tissue, the slow plasma clearance, and the low tumor-to-background ratio (TBR) [11].

This review aimed to discuss the most relevant PET clinical studies, published in the last 15 years, evaluating the utility of PET radiopharmaceuticals for imaging tumor hypoxia, other than ^{18}F -FMISO, in patients with HGG, offering also an insight, when available, on the complementarity between PET and advanced MRI techniques in this setting.

Methods

We searched on PubMed and Scopus databases using combinations of the following search terms: ‘tumor hypoxia’, ‘brain tumor’, ‘glioma’ ‘PET’, ‘positron emission tomography’, ‘nitroimidazoles’, ‘fluoromisonidazole’, ‘FMISO’, ‘FAZA’, ‘EF5’, ‘FRP170’ ‘Cu-ATSM’, ‘DiFA’. The search results were screened for relevance and the reference lists of relevant publications were also crosschecked. Only papers published in English were considered. The final reference list was compiled by considering papers published in the last 15 years through December 2019.

^{18}F -FAZA

Several hypoxia-specific PET tracers have been proposed for tumor hypoxia imaging after ^{18}F -FMISO. One of the most promising second-generation 2-nitroimidazole radiolabeled compounds is ^{18}F -flouroazomycin arabinoside (^{18}F -FAZA) (Tables 1 and 2, Fig. 1) [11]. Compared to ^{18}F -FMISO,

^{18}F -FAZA has an improved biodistribution, related to the addition of a sugar moiety, making it less lipophilic [14]. ^{18}F -FAZA has faster diffusion and more rapid clearance from normal tissue compared to ^{18}F -FMISO, providing an enhanced tumor-to-background ratio [19].

A higher contrast with non-target tissues for ^{18}F -FAZA compared to ^{18}F -FMISO, has been reported by Souvatzoglou et al., with an average tumor-to-muscle ratio of 2.0 ± 0.3 at 2 h post-injection acquisition [20]. The same group also reported that ^{18}F -FAZA has overall superior pharmacokinetics and that the use of dynamic analysis offers further potential improvement [21]. The different pharmacokinetics of ^{18}F -FAZA can be inferred evaluating its octanol/water partition coefficient (P). This is a parameter reflecting the hydrophilic/lipophilic nature of a tracer, with lower values indicating lower lipophilicity. Whereas ^{18}F -FMISO has a P of 0.4, the corresponding value is 0.027 for ^{18}F -FAZA. The lower P, as demonstrated in preclinical experiments, results in a faster plasma clearance and lower uptake in organs (especially liver, kidneys and small intestine) 3 h post-injection compared to ^{18}F -FMISO [21, 22].

First-in-human biodistribution and dosimetry data for ^{18}F -FAZA have been recently presented by Savi et al. The authors reported an effective dose equivalent (EDE) similar to that of ^{18}F -FMISO (0.015 mSv/MBq vs. 0.013 mSv/MBq, respectively) with the highest absorbed dose measured in the urinary bladder wall [23].

Few studies investigated the application and relevance of ^{18}F -FAZA in the specific clinical setting of HGG and there is a lack of studies evaluating the utility of the combined use of ^{18}F -FAZA and MRI in the assessment of hypoxia. Postema et al. were the first group reporting clinical experience on ^{18}F -FAZA in HGG [24]. Their study included 50 oncological patients, 7/50 of whom were affected by GBM. A high tumor-to-blood ratio of the radiotracer was reported, mainly related to the lack of uptake of ^{18}F -FAZA in the normal brain tissue. Additionally, ^{18}F -FAZA provided good quality images at 2–3 h post-injection, thus avoiding later scanning. This study confirmed that ^{18}F -FAZA was highly representative of tumor hypoxia within glioblastoma. Although a disrupted BBB certainly plays a role in ^{18}F -FAZA uptake in gliomas, the radiotracer retention is strictly dependent from the reduction process, which happens only under hypoxic conditions, and therefore ^{18}F -FAZA uptake is absent in normal brain tissue.

In the setting of treatment planning, Mapelli et al. reported the potentiality of ^{18}F -FAZA PET/CT in guiding tailored radiotherapy treatment in HGG. The group described the possibility to provide an additional boost on more hypoxic regions using ^{18}F -FAZA PET images in a simulated radiotherapy scheme. Additionally, this molecular imaging technique has been also used to assess tumor response after radiotherapy with promising implication for

Table 1 Main technical and clinical aspects of ¹⁸F-FAZA PET clinical studies in patients with glioma included in the review

First author	Study design	Grade of glioma	Number of patients	M:F	Mean age (range)	Mean injected dose (MBq)	Acquisition time (min post injection)	Image analysis	Semiquantitative parameters	MRI/other imaging modality parameters	Main findings
Postema et al. [24]	Prospective	IV	7 (extracted from a group of 50 patients with various tumors)	5:2	63.2 years (49.7–80.9)	5.2/kg	120	Visual, semi-quantitative	T/B ratio, SUVmax, relative uptake score (RUS)	N/A	Good imaging properties, acceptable T/B ratios; very promising for assessing the hypoxic fraction
Mapelli et al. [12]	Case report from clinical trial	IV	5 (1 glioma)	M	57* years	383	120	Visual	N/A	MRI: T1 with and without gadolinium contrast enhancement, T2 and FLAIR sequences, dynamic susceptibility contrast (DSC) and dynamic contrast-enhanced (DCE) perfusion	¹⁸ F-FAZA PET/CT can identify tumor areas with the highest grade, thus accurately guiding stereotactic biopsy
Mapelli et al. [22]	Case report from clinical trial	IV	1	M	77* years	396	120	Visual, semi-quantitative	T/M ratio	MRI: T1 with and without gadolinium contrast enhancement, T2 and FLAIR sequences	CT could be used for dose painting with dose escalation on the most hypoxic tumor regions

MBq megabecquerel, T/B tumor-to-blood, T/M tumor-to-muscle, N/A not available

*age of the enrolled patient

Table 2 Main technical and clinical aspects of selected ^{18}F -FRP170 PET clinical studies included in the review

First author	Study design	Grade of glioma	Num-ber of patients	M:F	Mean age (range)	Mean injected dose (MBq)	Acquisition time (min post injection)	Image analysis	Semiquantitative parameters	MRI/other imaging modality parameter	Main finding
Shibahara et al. [28]	Case series	GBM:3 AA rec:1 Oligo:2 AG: 1 DA:1	8	5:3	49 years (30–72)	370	120	Visual and semiquantitative	SUVmax	MET PET FDG PET Gd enhanced MRI Proton MR spectroscopy	High ^{18}F -FRP170 uptake is associated with remarkable hypoxia-inducible-factor (HIF-1 α) expression in case of GBM, whereas the uptake was moderate for lower grade glioma
Beppu et al. [29]	Not specified	GBM:12	12	10:2	63 years (31–81)	370	60	Visual and semiquantitative	SUVmean	MRI	High SUV uptake area corresponded to low $p\text{O}_2$ areas, measured with microelectrodes during the tumor excision
Beppu et al. [30]	Prospective	GBM:13	13	9:4	60.3 years (31–76)	370	60	Visual and semiquantitative	SUVmax, SUVmean, SUV T/N	Gd enhanced MRI	By comparison of low and high ^{18}F -FRP170 uptake areas with immunohistochemical staining for Ki-67 and hypoxia-inducible factor (HIF)-1 α within tumor specimens, a good match was found between high ^{18}F -FRP170 uptake and high HIF-1 α levels. Even high Ki-67 expression was found in high ^{18}F -FRP170 uptake areas, regardless of hypoxic levels

AA rec recurrent anaplastic astrocytoma, AG anaplastic ganglioglioma, DA diffuse astrocytoma, GBM glioblastoma multiforme, HIF-1 α hypoxia-inducible factor 1- α , Oligo Oligodendroglioma, $p\text{O}_2$ partial oxygen pressure, SUV standardized uptake value, SUV T/N ratio of the SUV for the tumor to the SUV for normal tissue, Gd gadolinium

clinical practice [25]. The same group reported for the first time the ability of ^{18}F -FAZA to identify the more hypoxic regions within the tumor and consequently its ability to guide stereotactic biopsy on the tumor areas with the highest aggressive potential. To achieve this goal, a co-registration of MRI and ^{18}F -FAZA PET/CT images has been performed to identify the areas showing either high perfusion MRI markers expression and high ^{18}F -FAZA uptake, therefore indicating the most representative hypoxic tumor region [26].

Other hypoxia PET tracers

Research is continuously addressing the development of new radiotracers, which can evaluate the heterogeneous nature of glial tumors, with particular regards to the extent of hypoxia within the tumor tissue, overcoming the limitations of “traditional” hypoxia PET tracers [3]. Currently, there is a lack of studies that systematically correlate these tracers with MRI in patients with HGG. Therefore, in this paragraph, we discuss the most promising hypoxic tracers considering both clinical and preclinical evidence.

2-(2-nitro-1H-imidazol-1-yl)-*N*-(2,2,3,3,3-pentafluoropropyl)-acetamide (EF5) is a lipophilic compound that binds to hypoxic tumor cells [27, 28]. Koch et al. evaluated the clinical usefulness of ^{18}F -EF5 PET by comparing ^{18}F -EF5 uptake with EF5 bindings, assessed by immunohistochemistry, in 3 patients with glioblastoma (Fig. 2). The authors found that the uptake of ^{18}F -EF5 corresponded to the regions characterized by high EF5 [29]. The same group proved that the ^{18}F -EF5 distribution is positively correlated with intertumoral variation of radiation response in twenty-two 9L gliosarcoma tumors grown in Fischer rats [30]. The main limit for the widespread use of such compound relies on the requirement for electrophilic fluorination using ^{18}F -F2, at the time of the radiolabeling process. Another limitation of ^{18}F -EF5 could be its low tumor-to-normal tissue ratio due to the slow clearance of the unbound tracer [15].

Another novel hydrophilic tracer for hypoxia assessment is 1-(2-[^{18}F]fluoro-1[hydroxymethyl]ethoxy)methyl-2-nitroimidazole (^{18}F -FRP170; Table 2). In preliminary evaluations, low distribution has been reported in normal brain tissue [31]. Shibahara et al. assessed hypoxia in glial tumors of different grades in a group of eight patients. The authors found that high ^{18}F -FRP170 uptake was associated with a remarkable hypoxia-inducible-factor (HIF-1 α) expression in case of GBM, whereas the uptake was moderate for lower-grade gliomas [32]. In four out of eight patients, an ^{18}F -FDG PET/CT scan was also performed to compare the distribution of the two radiotracers. The uptake of FDG did not match well with the FRP170 uptake, presumably due to their different pharmacodynamics. The same was true also for two of the three patients who underwent to ^{11}C methionine PET/

CT together with the ^{18}F -FRP170 PET; the only concordant case was a recurrent anaplastic astrocytoma, possibly because cell proliferation was high enough to induce hypoxia in the tumor mass, or the vascular supply was adequate for hypoxic tumor cells to continue proliferating. Also, Beppu et al. reported interesting findings on the potential usefulness of ^{18}F -FRP170 for the detection of hypoxic areas within glial tumors. In a preliminary study involving twelve patients with GBM undergoing ^{18}F -FRP170 PET 1 h after injection, the authors reported that areas with high ^{18}F -FRP170 uptake, calculated as the SUV_{mean} within the 10 mm-ROI drawn in the areas with the highest uptake, corresponded to areas with low pO_2 , measured with microelectrodes during the tumor excision [33]. More recently, the same group compared low and high ^{18}F -FRP170 uptake areas with immunohistochemical staining for Ki-67 within tumor specimens and hypoxia-inducible factor (HIF)-1 expression in 13 patients with GBM. A good match was found between high ^{18}F -FRP170 uptake and high HIF-1 α levels and also between high Ki-67 expressions and high ^{18}F -FRP170 uptake areas [34]. According to these findings, it has therefore been postulated that ^{18}F -FRP170 uptake may be considered as a marker of proliferation rather than hypoxia.

Among novel hypoxic radiotracers, also copper-diacetyl-bis(*N*4-methylthiosemicarbazone) (Cu-ATSM) has shown some promising results (Table 3). In hypoxic cells, Cu-ATSM is trapped by the reaction with thiol groups or redox-active proteins. Conversely, Cu-ATSM is less retained in tissues with an inadequate amount of oxygen, owing to a less reducing environment, with its subsequent wash-out from the cells [16, 35, 36]. Four different Copper-isotopes were bound with ATSM compound: ^{60}Cu -, ^{61}Cu -, ^{62}Cu -, ^{64}Cu [37]. Among them, ^{64}Cu - seems to be the most promising for clinical applications, owing to its feasible half-life (12.7 h) and a more remarkable TBR, which, once assessed 1-hour post-injection, was shown to be higher than the TBR provided by 2-nitroimidazole based tracers. Moreover, it demonstrated the fastest clearance, with an easier radiolabeling procedure and synthesis, compared to other radiotracers targeting hypoxia [19]. A pioneering preclinical study evaluated the relationship between copper-diacetyl-bis(*N*4-methylthiosemicarbazone) (Cu-ATSM) uptake with pO_2 and its changes obtained by the administration of hydralazine, in a 9L gliosarcoma rat model [38] and found an inverse correlation between pO_2 and ^{61}Cu -ATSM uptake.

^{62}Cu -ATSM uptake has proved also to be an independent prognostic factor in glioma patients (in terms of PFS and OS; $p < 0.05$) and is a more promising imaging method to predict prognosis compared to ^{18}F -FDG PET/CT, as shown in a study including 56 patients with grade 2–4 glioma [39]. More recently, Tateishi et al. evaluated the feasibility of ^{62}Cu -ATSM PET to discriminate patients with different grade of glioma, even in association with ^{11}C MET PET

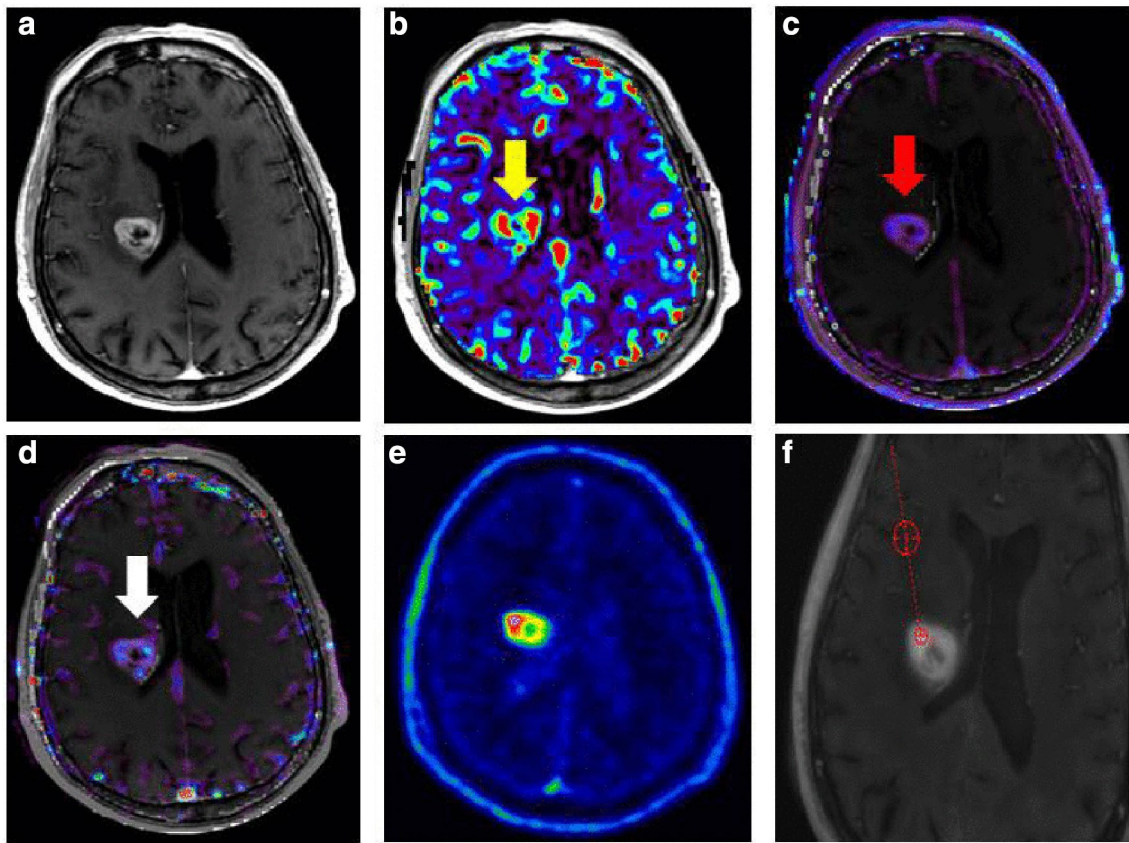


Fig. 1 (Adapted with the permission of Mapelli et al. [26]. A 77-year-old patient with a suspected brain tumor. **a** MRI gadolinium-enhanced T1 image showing an enhancing, oedematous lesion in the right basal ganglia and "corona radiata" with mass effect on the right ventricle. The lesion also showed high values of relative cerebral blood volume, **b** (rCBV; yellow arrow), transfer constant (K^{trans}), **c** red arrow and fractional plasma volume (Vp)(**d**)—white arrow. **e**

^{18}F -FAZA PET image showed uptake in correspondence of the brain lesion with a central photopenic area possibly due to necrotic tissue. **f** MRI and ^{18}F -FAZA PET/CT images were co-registered and fused and an area of both high perfusion MRI markers and high ^{18}F -FAZA uptake was selected for biopsy. The histological analysis confirmed the presence of a grade IV glioblastoma

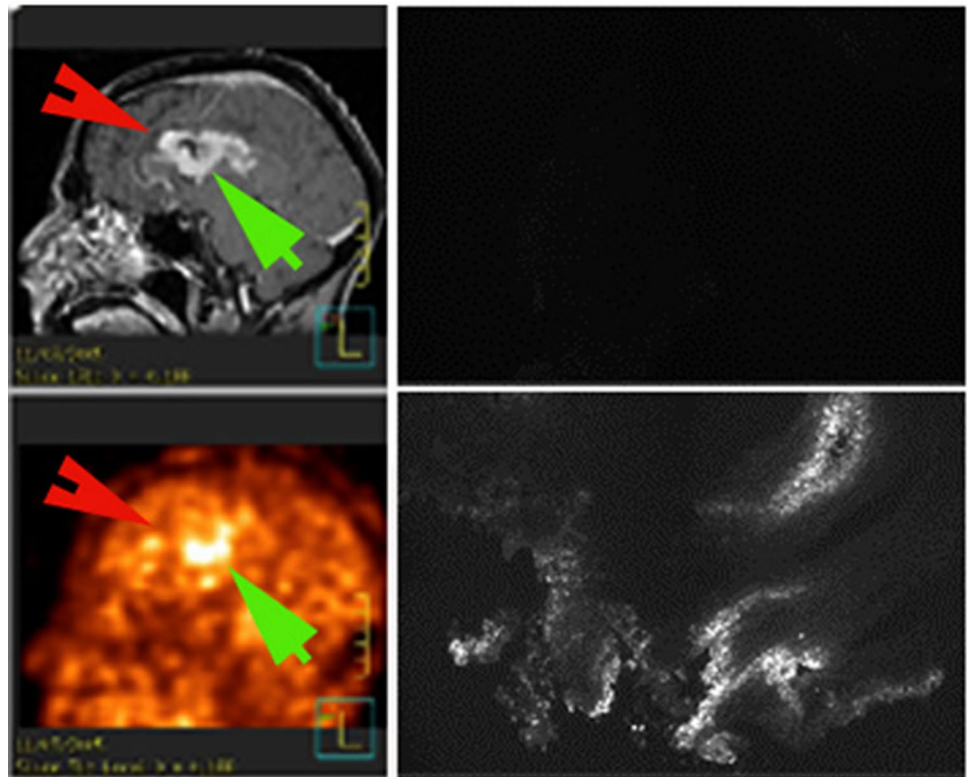
and/or ^{18}F -FDG PET. The authors found higher SUV_{\max} and TBR in both ^{62}Cu -ATSM PET and ^{11}C MET PET scans in patients with GBM than in patients with grade II or III gliomas. By establishing a TBR cut-off of 1.9, the authors confirmed that ^{62}Cu -ATSM PET could be a useful tool for distinguishing patients with GBM from patients with lower-grade glioma [40].

Intratumoral distribution of Cu-ATSM has also been compared with the distribution of ^{18}F -FMISO and ^{18}F -FDG [41–43]. The rationale of this comparison relies on the fact that ^{18}F -FMISO and ^{64}Cu -ATSM have different retention mechanisms and different half-lives, $t_{1/2} = 110$ min and 12.7 h, respectively. Furthermore, in a 9L gliosarcoma rat model, Dence and coworkers found a strong positive correlation between intra-tumoral ^{18}F -FMISO uptake, assessed 2 h post injections, with ^{64}Cu -ATSM uptake either at 10 min and 24 h post-injection [44].

However, further evidence warns to consider Cu-ATSM as a marker for hypoxia *tout court* since the validity of ^{64}Cu -ATSM uptake as a marker of hypoxia is dependent on tumor type [45]. Indeed, it is still a matter of debate whether Cu-ATSM could be able to detect a pure hypoxic phenomenon rather than cell-redox potential and vascular delivery [46, 47].

More recently, another PET tracer, 1-(2,2-dihydroxyethyl-3-[^{18}F]-fluoropropyl)-2-nitroimidazole (^{18}F -DiFA) has been proposed for measuring hypoxia [48]. This tracer accumulates in hypoxic tissue via a glutathione conjugation reaction and displays a more hydrophilic nature than ^{18}F -FMISO [8]. In vitro experiments in Fadu head and neck cancer cells, preincubated under hypoxia ($\text{O}_2 = 1\%$), documented higher radioactivity uptake at 2 h after incubation with for ^{18}F -DiFA compared to ^{18}F -FMISO (0.53 ± 0.02 vs. 0.34 ± 0.07 ; $p < 0.01$) [49]. A subsequent preclinical study,

Fig. 2 Adapted with the permission from Koch et al. [29]. CE MRI image of tumor (upper left) and the correspondent PET image (lower left). The anterior area of the tumor (red arrow) displays little ^{18}F -EF5 uptake and tissue obtained from this region has no EF5 binding, determined by IHC (upper right). The inferior area (green arrow) is characterized by high ^{18}F -EF5 uptake and tissue obtained from this area has high EF5 binding, determined by IHC (lower right)



conducted in EMT6 mammary carcinoma cell-bearing mice, showed higher accumulation in tumor than ^{18}F -FMISO at 1 h and 2 h post-injection [50]. Preliminary clinical data in seven patients with different types of tumors undergoing both ^{18}F -FMISO and ^{18}F -DiFA PET scan showed at a visual analysis similar diagnostic ability for tumor hypoxia for ^{18}F -DiFA (evaluated at 1 h and 2 h) compared to ^{18}F -FMISO (assessed 4 h post-injection). Unfortunately, no data are still available for brain tumors [48].

Conclusion

Well-designed trials are required to validate hypoxia radiotracers and evaluate their clinical utility in daily clinical practice. Hypoxia PET imaging has the potential to provide useful information for clinicians to guide hypoxia-tailored treatments. Based on current literature, the most promising second-generation hypoxic tracer seems to be ^{18}F -FAZA, although additional research trials should be performed before introducing this tracer into the clinical scenario. The

ideal tracer for quantifying hypoxia has not yet been found in brain tumor imaging. Although hydrophilic compounds present undeniable advantages compared to lipophilic tracers in terms of pharmacokinetics, there is still an open debate between supporters of lipophilic tracers and supporters of hydrophilic tracers. Despite the undeniable advantages of hydrophilic profile in terms of pharmacokinetics, the impact of BBB disruption on tracer delivery should be further investigated.

Another problem to consider is that hypoxia PET imaging requires fully equipped facilities with trained staff for tracer production and accurate image analysis. A consensus in the quantification methodology of hypoxia in PET imaging is still missing. Another point that needs to be addressed is the comparison of the hypoxic volume (defined in PET imaging) and the volume of the radio-resistant portion of the tumor. Last but not least, trials evaluating the utility of hypoxia PET imaging in the assessment of response to hypoxia-targeted treatments are warranted to define the potential impact in patient outcome.

Table 3 Main technical and clinical aspects of selected Cu-ATSM clinical studies included in the review

First author	Study design	Grade of glioma	Num-ber of patient	M:F	Mean age (range)	Mean injected dose (MBq)	Acquisition time (min post injection)	Image analysis	Semiquantitative parameters	MRI/other imaging modality parameter	Main findings
Tateishi et al. [36]	Prospective	IV: 10 III (AOD): 4 III (AOA): 1 II (OA): 3 II (DA): 3 II (OD): 2	23	10:13	54.2 years (19–81)	740	Dynamic data acquisition for 30–40 min	Visual, semi-quantitative	SUV _{max} SUV _{T/B}	MET PET/CT FDG PET/CT T1 weighted MRI with MPRAGE sequences	Higher ⁶² Cu-ATSM uptake and higher tumor-to-background ratio (TBR) in patients with glioblastoma multiforme, than in patients with grade II or grade III glioma. A TBR cut-off of 1.9 could discriminate among glioblastoma and lower grade glioma patients
Torihara et al. [35]	prospective	IV: 29 III: 14 II: 13	56	28:28	56 years (N/A)	482	Dynamic data acquisition for 30 min	Semiquantitative	SUV _{max} SUV _{T/B}	FDG PET/CT	SUV _{max} was proven as an independent predictor of both progression-free survival and overall survival

AOA anaplastic oligoastrocytoma, AOD anaplastic oligodendrogloma, DA diffuse astrocytoma, OA oligoastrocytoma, OD oligodendrogloma, SUV_{max} maximum standardized uptake value, TBR tumor to background ratio, N/A not available, FDG fluorodeoxyglucose, MET ¹¹Cmethionine, MPRAGE magnetization-prepared rapid acquisition with gradient echo, MRI magnetic resonance imaging, MBq megabecquerel, SUV_{T/B} ratio between tumor SUV_{max} and background SUV_{max}

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Compliance with ethical standards

Conflict of interest Quartuccio N, Laudicella R, Mapelli P, Guglielmo P, Pizzuto DA, Boero M, Arnone G, Picchio M declare no conflict of interest related to this work.

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