Role of PET Imaging in Patients with High-Grade Gliomas undergoing anti-angiogenic Therapy with Bevacizumab – Review of the Literature and Case Report

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Role of PET Imaging in Patients with High-Grade Gliomas Undergoing Anti-Angiogenic Therapy with Bevacizumab – Review of the Literature and Case Report

Irina Götz, Anca-Ligia Grosu, Timo Spehl

Abstract: Despite recent advantages in combination therapies, the prognosis of high-grade gliomas remains very poor. A new therapeutic concept in the treatment of brain tumours uses the anti-angiogenic drug bevacizumab to reduce tumour neovascularisation. However, due to a concomitant reduction of contrast agent enhancement in MRI, imaging modalities that do not depend on a disruption of the blood-brain barrier (BBB) would be desirable for therapy monitoring. Positron emission tomography (PET) imaging has emerged as a promising tool for better assessment of treatment response in patients undergoing anti-angiogenic therapy compared to MRI. The most important tracers are amino-acids like [18F]-methylion and [18F]-FDG, but also [18F]-FDG and [18F]-FLT, a biomarker of cell proliferation. In this review, we provide an overview of current knowledge concerning the value of PET in patients with brain tumours undergoing anti-angiogenic therapy and present a clinical case that illustrates the utility of PET imaging.

Key words: PET, bevacizumab, therapy monitoring, [18F]-FDG, [18F]-FLT, [18F]-FET

Introduction

High-grade gliomas (HGG) are highly aggressive brain tumours with limited treatment options. The most frequent primary brain tumour, accounting for 20 % of all intracranial tumours, is glioblastoma multiforme (WHO grade IV). Despite recent advances in combination therapy including surgery, radiation therapy, and chemotherapy, the prognosis remains very poor [1]. An important feature of tumour aggressiveness is increased tumour neovascularisation driven by the vascular endothelial growth factor (VEGF) pathway [2]. Tumour vessels are characterised by structural abnormalities that lead to an increase in the permeability of the blood-brain barrier (BBB), thus causing complications like tumour oedema and compression of adjacent structures. Under the premise of arresting tumour progression and local complications by inhibiting pro-angiogenic growth factors [3], the anti-angiogenic drug bevacizumab has been introduced into therapy of recurrent glioblastoma [4, 5] and has been approved by the FDA for this indication.

Under treatment with bevacizumab, high response rates based on morphological imaging using the conventional Macdonald criteria [6] have been reported, ranging from 30–60 % [7]. It must be borne in mind, however, that these response rates are based on a normalisation of the BBB that might not represent true tumour regression, and thus are described as “pseudo-response” [8]. There is growing evidence that bevacizumab may alter the recurrence pattern of malignant gliomas in MRI imaging by suppressing gadolinium-enhancing tumour recurrence more effectively than it suppresses non-enhancing, infiltrative tumour growth [9]. To enable a more precise response assessment, new criteria have been established for response assessment in neuro-oncology (RANO) that include T2-based fluid-attenuated inversion recovery (FLAIR) MRI sequences [10]. However, the morphological changes seen in these imaging sequences also include multiple non-specific changes like radiation-induced gliosis, peritumoural oedema, ischemia, and demyelination [11]. In this context, positron emission tomography (PET) with various tracers has been established to improve diagnostics, response assessment, and therapy planning.

We present an exemplary case of a patient with recurrent glioblastoma multiforme who was treated with bevacizumab. This case highlights the usefulness of PET imaging in response assessment compared to MRI. Furthermore, we give an overview of the current literature regarding PET imaging in patients treated with this promising new therapeutic agent.

Molecular Mechanisms of Bevacizumab

VEGF is a vascular endothelial growth factor correlated with pathological angiogenesis and plays an important role especially in highly vascularised tumours like glioblastoma. The extent of proliferation in this tumour entity has been shown to correlate with an increased recurrence and poor survival [12]. Moreover, a direct relationship between VEGF over-expression and poor prognosis has been reported [13]. Originating from the tumour bulk, glioblastoma cells migrate along normal vascular structures into adjacent brain regions [14], thus making VEGF a promising target for therapeutic agents. Preclinical studies accordingly showed that bevacizumab inhibits tumour growth as a single-agent therapy or in combination with cytotoxic agents [15]. Bevacizumab and other anti-angiogenic agents that bind and inactivate VEGF, including cediranib (AZD2171), aflibercept (VEGF Trap), XL184, and cilengitide (EMD 121974), are thus being evaluated as a possible treatment option for use in recurrent and possibly also newly diagnosed glioblastoma.
Therapeutic Studies with Bevacizumab in Glioblastoma

The first phase-II study on bevacizumab and irinotecan (BEV/IR) in recurrent glioblastoma by Vredenburgh et al proved the feasibility of this regime and revealed an improvement in 6-months progression-free survival (PFS) of 46% compared with historical data [4]. The response rate was 57%, toxicity was moderate. Regarding side-effects, the authors reported thromboembolic complications in 4 patients and one CNS haemorrhage [4]. The response rate in the trial of Kreisl et al examining 56 patients was 35% based on the Macdonald criteria, 6-months PFS was 29% [16]. In addition to an increase in PFS, a clinical benefit was evident in terms of decreased cerebral oedema in 24 patients (50%). 15 patients were able to decrease corticosteroids and 25 patients (52%) had improved neurologic symptoms. Based on these 2 trials, the FDA granted accelerated approval of bevacizumab for the treatment of recurrent glioblastoma multiforme in May 2009.

The BRAIN study [5] confirmed that bevacizumab, alone or in combination with irinotecan, was well-tolerated and effective in recurrent glioblastoma. This was a non-comparative trial. The randomised design was intended only to prevent bias in treatment assignment. Additionally, data of the BRAIN study were analysed by Vredenburgh et al to evaluate whether bevacizumab may have corticosteroid-sparing effects [17]. The results showed sustained reduction of corticosteroids in 30% of the bevacizumab-alone group and 20% of the bevacizumab-plus-irinotecan group, respectively. However, the data has to be interpreted cautiously due to the exploratory nature of the analysis.

Other studies reported bevacizumab as an effective treatment option in radionecrosis. In a series of 6 patients with biopsy-proven cerebral radiation necrosis treated with bevacizumab, MRI follow-up demonstrated radiographic response in all patients with an average reduction of 79% for the post-gadolinium studies and 49% for the FLAIR images and was noted for a mean follow-up time of up to 5.9 months [18].

First results of 2 phase-III trials were reported at the ASCO meeting in June 2013 and were published in the New England Journal of Medicine in February 2014 [19, 20]. The RTOG study 0825 [19] reported that addition of bevacizumab to the standard treatment regime with temozolomide for newly diagnosed GBM did not improve overall survival. A small effect was seen regarding prolonged PFS, but this did not reach the significance criterion. The patient group receiving bevacizumab even showed a decline of neurocognitive status. These results discourage the use of bevacizumab for patients with the best prognosis at the outset. The analysis did not identify a group of patients who experienced benefit from first-line bevacizumab.

AVAgliao, a phase-III registration trial including >920 patients worldwide, revealed similar findings for OS. The double-blind, randomised trial evaluates the benefit of an addition of bevacizumab to the standard of care in the first-line treatment of patients with glioblastoma. Median overall survival in each arm was 17 months. Median PFS was 10.6 months in the interventional arm with bevacizumab vs 6.2 months in the control arm (p < 0.001). In contrast to the RTOG trial, this difference was statistically significant. Even more, health-related quality of life was improved for patients receiving bevacizumab in the AVAgliao trial [20] in contrast to the RTOG trial.

More encouraging results were reported for the GLARIUS study involving 182 MGMT unmethylated glioblastoma patients. Patients in the experimental arm received 4 cycles of bevacizumab over 6 weeks of radiation, then bevacizumab plus irinotecan were administered every 2 weeks until progression. At 6 months, PFS was significantly higher with BEV/IR: 9.74 vs 5.99 months (p < 0.0001). Overall survival was also significantly longer: 16.6 vs 14.8 months (p = 0.031). The experimental arm also required less corticosteroids [21].

Taken together, there is growing evidence for the usefulness of bevacizumab in the treatment of glioblastoma with regard to PFS. However, the expectations of the community regarding overall survival were not fulfilled by the 2 phase-III trials. Additionally, it is even not clear whether bevacizumab improves or impairs the neurological condition of the patients.

Due to high costs, severe side effects, and complicated image interpretation, reliable biomarkers for therapy monitoring are required. PET has emerged as a very promising tool in this field.

Role of PET Imaging in Response Assessment to Bevacizumab Therapy

PET imaging is increasingly used in HGG. It relies on the fact that it can visualise functional changes in tumour tissue rather than morphological details. Multiple different features of brain tumours can be addressed, eg, glucose metabolism, amino-acid uptake, or proliferation activity. These can serve as valuable biomarkers for diagnostics and response assessment.

Glucose Metabolism

The most widely used PET tracer in oncology is 18F-fluorodeoxyglucose (18FDG). The use of 18FDG-PET in brain tumours was reviewed by a National Comprehensive Cancer (NCCN) panel in 2009. Based on current evidence and consensus, a role for 18FDG-PET in the management of brain tumours was proposed for diagnosis, staging/restaging, prognosis, and possibly for treatment planning and response monitoring [22]. 18FDG-PET relies on an increased glycolytic metabolism of glial tumour cells mediated by increased hexokinase activity [23] and over-expression of glucose transporters [24] (Table 1). Furthermore, 18F-FDG uptake is strongly correlated with angiogenesis markers in gliomas [25]. Thus, it could serve as a biomarker for tumour neovascularisation. There is a study that investigates 18FDG-PET for treatment monitoring of high-grade gliomas under treatment with bevacizumab and irinotecan [26]. In this study, Colavolpe et al report that 18FDG-PET was the most powerful predictor of OS and PFS in a group of 25 patients in both uni- and multivariate analysis (p < 0.001). Interestingly, in multivariate analysis, 18FDG-PET performed better in predicting survival than histological grading, steroid
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Table 1. Various tracers used for imaging of HGG undergoing bevacizumab therapy and their respective molecular properties

<table>
<thead>
<tr>
<th>Tracer</th>
<th>Radiopharmaceutical name</th>
<th>Isotope</th>
<th>Half-life</th>
<th>Molecular target (localisation)</th>
<th>Intracellular processing</th>
<th>Molecular weight</th>
<th>Penetration through intact blood-brain barrier</th>
</tr>
</thead>
<tbody>
<tr>
<td>18F-FDG</td>
<td>2-deoxy-2-[18F]-fluoro-D-glucose</td>
<td>18F</td>
<td>109 min</td>
<td>Mainly GLUT-1 (transporter), substrate for hexokinase (HK-2, enzyme)</td>
<td>Intracellular storage, “trapping”</td>
<td>181.15 g/mol</td>
<td>Yes</td>
</tr>
<tr>
<td>11C-MET</td>
<td>[11C]-methionine</td>
<td>11C</td>
<td>20 min</td>
<td>L-type amino-acid carriers (membrane)</td>
<td>Incorporated into proteins</td>
<td>149.21 g/mol</td>
<td>Yes</td>
</tr>
<tr>
<td>18F-FET</td>
<td>O-[2,18F]-fluoroethyl-L-tyrosine</td>
<td>18F</td>
<td>109 min</td>
<td>L-type amino-acid carriers (membrane)</td>
<td>Not incorporated into proteins</td>
<td>227.23 g/mol</td>
<td>Yes</td>
</tr>
<tr>
<td>18F-FLT</td>
<td>3'-deoxy-3'-[18F]-fluorothyrimidine</td>
<td>18F</td>
<td>109 min</td>
<td>Thymidin-kinase (TK-1, enzyme)</td>
<td>DNA synthesis, proportional to proliferation</td>
<td>244.22 g/mol</td>
<td>Unclear (see text)</td>
</tr>
<tr>
<td>18F-DOPA</td>
<td>3,4-dihydroxy-6-[18F]-fluoro-L-phenylalanine</td>
<td>18F</td>
<td>109 min</td>
<td>DOPA-decarboxylase (enzyme)</td>
<td>Intraneuronal storage in vesicles</td>
<td>215.18 g/mol</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Min: minutes. For details, see text.

Despite the usefulness of 18FDG-PET in general oncology, it is limited as a tracer in brain neoplasms due to its physiological uptake of grey matter, where small tumours can be masked and low-grade tumours may not be discernible from white matter due to lower uptake. Therefore, multiple other biomarkers have been tested that have low uptake in normal brain tissue and thus allow for better contrast in PET imaging. Among the most promising PET tracers are radio-labelled amino-acids (AA), such as 18F-fluoroethyl-L-tyrosine (FET), whose half-life of 109 minutes makes it more readily available than the previously widely used 11C-methionine (MET). Uptake of radio-labelled AA is fairly low in normal brain tissue [28, 29]. Increased AA uptake in gliomas is related to an over-expression of L-type amino-acid transporters in the cell membrane [30, 31] (Table 1). These carriers are particularly up-regulated in HGG and it has been suggested in a rat model that tumours can stimulate transporter expression, especially in their vasculature [32]. 18F-FET-PET is increasingly used as a diagnostic agent for detection of tumour recurrence and to exclude radiation necrosis, where MRI is of low specificity [28, 29, 33]. Moreover, it facilitates radiation therapy planning and thus could lead to better overall survival. In the first study on this issue with 44 patients with recurrent high-grade gliomas, fractioned stereotactic radiotherapy was performed after definition of the tumour volume using MET-PET/MRI/CT or merely MRI/CT. Patients from the first group had, in an univariate analysis, a significantly longer survival compared to those patients whose treatment was based on MRI/CT only [34]. Ongoing studies like the multicentre, randomised, phase-II German GLIAA study (clinicaltrials.gov: NCT01252459) will examine if 18F-FET-PET-based radiotherapy planning can truly increase overall survival.

In addition to radiation therapy planning, 18F-FET PET could play a major role in the assessment of response in patients undergoing therapy with bevacizumab. Case reports hint that 18F-FET-PET may indicate therapy failure earlier than MRI [35, 36]. There are 2 studies that suggest that 18F-FET-PET could serve as a reliable biomarker to predict treatment failure and is superior to MRI based on RANO criteria for the detection of tumour progression [37, 38]. In the study of Galldiks et al [37], FET-PET predicted a significantly longer PFS and OS than RANO criteria-based MRI reading. Furthermore, in 40 % of the patients, FET-PET was discordant with MRI and revealed treatment failure earlier than MRI (median time benefit 10.5 weeks). Similar results have been reported by Hutterer et al [38], where in 36.4 % of all cases, FET-PET and MRI were discordant and PET was able to detect treatment failure earlier than MRI. FET-PET was also a very good predictor of therapy response (for details see Table 2). Both authors used identical criteria for therapy response (45-% reduction of tumour volume, as defined by Hutterer et al [38]) and similarly conclude that 18F-FET-PET could serve as a valuable biomarker to detect treatment failure earlier than conventional imaging methods. In addition, a recent study by Heinzl et al [39] has demonstrated that using 18F-FET-PET to detect therapy failure in HGG treated with bevacizumab is actually cost-effective, reducing both costs and therapy-associated side effects where there is no benefit to be expected. In this study, the number needed to diagnose was as low as 2.4 [39]. As a footnote, 18F-FET-PET has also been reported to be of good value in therapy monitoring of patients with rare indications like progressive brain-stem gliomas [40].
In a pilot study with 21 HGG patients, Chen et al. reported on the treatment efficacy and monitoring of brain tumours. In this study, 18F-FLT, a thymidine analogue that visualises tumour cell proliferation, was used for early treatment response. In another study, 18F-FLT was shown to be superior to 18FDG regarding evaluation of overall survival as early as 6 weeks after treatment initiation [49]. Response was defined as a > 25-% reduction of FLT uptake in the tumour mass compared to pre-treatment scans. In 2011, another study confirmed these results [50]. Using the same criteria, Schwarzenberg et al. reported that changes in tumour FLT uptake were highly predictive of PFS and OS in patients with recurrent malignant glioma undergoing bevacizumab therapy and FLT-PET was more predictive than MRI [50]. In another study, 18F-FLT kinetics were analysed in recurrent HGG in 15 patients, and it reported that a persistently decreased 18F-FLT uptake (by > 45 % of tumour volume) was a predictor for longer survival [51].

Proliferation Activity
In addition to radio-labelled amino-acids, 3’-deoxy-3’-18F-fluorothymidine PET (18F-FLT) is increasingly used for therapy monitoring in brain tumours. 18F-FLT is a thymidine analogue that visualises tumour cell proliferation [41]. Uptake of FLT correlates with the activity of thymidine-1-kinase which is expressed during the DNA synthesis phase of tumour cells [42]. After phosphorylation, FLT is trapped inside the cell [43]. FLT uptake correlates with the Ki-67 proliferation index and FLT uptake has been shown to correlate with tumour grading and cell proliferation [44] (Table 1). It has been successfully investigated in brain malignancies [45, 46] and has been demonstrated to predict overall survival in HGG patients [47].

18F-FLT has also been investigated for its utility in treatment response assessment in HGG undergoing therapy with bevacizumab: in preclinical studies, 18F-FLT has been shown to be a sensitive marker of treatment efficacy in a rat model [48]. In this study, it was superior to 18FDG regarding evaluation of treatment efficacy. First clinical data were reported as early as 2007: in a pilot study with 21 HGG patients, Chen et al. were able to demonstrate that 18F-FLT-PET is highly predictive of overall survival as early as 6 weeks after treatment initiation [49]. Response was defined as a > 25-% reduction of FLT uptake in the tumour mass compared to pre-treatment scans. In 2011, another study confirmed these results [50]. Using the same criteria, Schwarzenberg et al. reported that changes in tumour 18F-FLT uptake were highly predictive of PFS and OS in patients with recurrent malignant glioma undergoing bevacizumab therapy and FLT-PET was more predictive than MRI for early treatment response [50]. In another study, 18F-FLT kinetics were analysed in recurrent HGG in 15 patients, and it reported that a persistently decreased 18F-FLT uptake (by means of measurements after 2 and 6 weeks) in the tumour was a predictor for longer survival [51].

There is one study that combines the amino-acid 3,4-dihydroxy-6-[18F]-fluoro-L-phenylalanine (18F-DOPA) with 18F-FLT-PET [52]. Here, voxel-wise changes in tracer uptake were analysed in 24 patients with HGG. Harris et al. reported that voxel-wise increase in PET uptake in areas of pre-treatment contrast enhancement defined by MRI stratified 3-month progression-free survival and 6-month overall survival (OS)
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[52]. Log rank analyses, however, revealed that only the volume fraction of increased $^{18}$F-DOPA uptake between 2 post-treatment time points stratified long- and short-term OS, while $^{18}$F-FLT uptake did not. The authors state that $^{18}$F-DOPA might be slightly superior to $^{18}$F-FLT.

However, there is growing uncertainty about the mechanisms of transport of $^{18}$F-FLT into brain tumours. There is data suggesting that the uptake of $^{18}$F-FLT is highly dependent on BBB breakdown and much less on phosphorylation itself [53]. This hypothesis was confirmed in another study where uptake of $^{18}$F-FLT was largely related to leakage into extracellular space via a disrupted BBB, whereas the effect of nucleoside transporters was regarded to be much lower in comparison [54]. Taken together, these facts might limit the general use of $^{18}$F-FLT in patients undergoing therapy with bevacizumab. In addition, the presence of benign lesions showing BBB disruption cannot be distinguished from malignant tumours [55] in $^{18}$F-FLT-PET. Thus, $^{18}$F-FLT-PET needs to be carefully evaluated. Further investigations in this field will be necessary before $^{18}$F-FLT can be recommended for general use in brain tumours.

Outlook: Imaging of Angiogenesis and Hypoxia in Brain Tumours

In malignant gliomas, αβ3 integrin plays a key role in tumour angiogenesis and invasion [56]. To date, there is a single study reporting the use of $^{18}$F-labelled glycosylated Arg-Gly-Asp peptide ($^{18}$F-Galacto-RGD) to successfully visualise αβ3 expression in patients with glioblastoma [57]. This tracer may thus be a promising tool not only for planning and monitoring integrin-targeted therapies but possibly also for bevacizumab therapy planning.

Another interesting biomarker in HGG undergoing therapy with bevacizumab might be hypoxia. $^{18}$F-misonidazole ($^{18}$F-MISO) is the most intensively studied PET tracer for hypoxia detection, and baseline $^{18}$F-MISO uptake has been shown to correlate with tumour aggressiveness in glioblastoma [58]. Furthermore, regional hypoxia measured by $^{18}$F-MISO correlated with time to progression and survival [59]. In solid tumours, it has been proposed that drugs that induce vascular normalisation could alleviate hypoxia and increase the efficacy of conventional therapies if both are carefully scheduled. Thus, $^{18}$F-MISO was proposed as a potential tool for tracking the normalisation window in patients undergoing anti-angiogenic therapy, which is considered as the period of radiation and chemotherapy response enhancement due to improvement of oxygenation [60], but this has not been researched so far. In how far $^{18}$F-MISO is useful to evaluate anti-angiogenic therapy in glioblastoma is not clear, but currently under investigation, eg, in the HYPONCO study (clinicaltrials.gov: NCT01200134).

To draw a reliable conclusion regarding the best imaging method under anti-angiogenic therapy we need more validation trials comparing MRI and PET, ideally performed on the same day. Contrast enhancement on MRI might not be accurate enough after anti-angiogenic-agent therapy like bevacizumab and leads to pseudo-response. Amino-acid PET has a high sensitivity and specificity in detecting tumour tissue and it is not influenced by the blood-brain barrier. These are good arguments for PET in such situations.

It is worthwhile to correlate both imaging techniques with histology. However, it is often hardly possible. So we have to decide about pseudo-progression or tumour progression from the clinical course and repeated imaging scans.

Conclusion

PET imaging is increasingly used in brain tumour imaging. Many different functional aspects of tumour growth and metabolism can be investigated and used for diagnosis, treatment planning, and response assessment. Use of novel therapeutic agents like bevacizumab that severely alter tumour appearance in conventional imaging requires reliable biomarkers for therapy monitoring, as demonstrated by the case presented in this paper. PET is a promising, possibly cost-efficient method to reliably assess tumour response to bevacizumab therapy and may thus be included in the clinical management of recurrent high-grade gliomas treated with anti-angiogenic drugs.

Conflict of Interest

The authors have no conflict of interest to disclose.

References:
In December 2009, a 51-year-old woman was diagnosed with glioblastoma multiforme in the left thalamus, putamen, and insular cortex. Histology was acquired by stereotactic biopsy. In July 2010, the gadolinium-enhanced MRI and treatment with 16 mg dexamethasone and 533 mg iodo-alpha-methyl-L-tyrosine in a human glioma xenograft model were performed in the region of the left thalamus and further proliferation in brain tumors. J Nucl Med 2009; 60: 1948–58.


After radiation (Figure 1D), $^{18}$F-FET-PET presented a small decrease of uptake but still a visible tumour mass. In addition, progressive infiltration of the mesencephalon was seen, as compared to a small, focal enhancing lesion on MRI (Figure 2). In contrast, MRI showed only minimal contrast enhancement without relevant changes to the MRI prior to re-irradiation. The patient died 16 months after primary diagnosis. This case illustrates the often discordant results between contrast enhancement in MRI and amino-acid uptake in PET and demonstrates the clinical utility of PET for response assessment and therapy management.

We assume that the decrease of contrast enhancement was due to a reduction of the brain-blood barrier damage by bevacizumab and not due to a real tumour reduction. However, it was not possible to decide whether the MRI or the PET scan showed the real tumour dimension. An autopsy was not performed.