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ORIGINAL ARTICLE

Early treatment response evaluation using FET PET compared to MRI in glioblastoma patients at first progression treated with bevacizumab plus lomustine

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Abstract

Background The goal of this prospective study was to compare the value of both conventional MRI and O-(2-^{18}F-fluoroethyl)-L-tyrosine (FET) PET for response evaluation in glioblastoma patients treated with bevacizumab plus lomustine (BEV/LOM) at first progression.

Methods After chemoradiation with concomitant and adjuvant temozolomide, 21 IDH wild-type glioblastoma patients at first progression (age range, 33–75 years; MGMT promoter unmethylated, 81%) were treated with BEV/LOM. Contrast-enhanced MRI and FET-PET scans were performed at baseline and after 8–10 weeks. We obtained FET metabolic tumor volumes (MTV) and tumor/brain ratios. Threshold values of FET-PET parameters for treatment response were established by ROC analyses using the post-progression overall survival (OS) ≤/>9 months as the reference. MRI response assessment was based on RANO criteria. The predictive ability of FET-PET thresholds and MRI changes on early response assessment was evaluated subsequently concerning OS using uni- and multivariate survival estimates.

Results Early treatment response as assessed by RANO criteria was not predictive for an OS>9 months ($P = 0.203$), whereas relative reductions of all FET-PET parameters significantly predicted an OS>9 months ($P < 0.05$). The absolute MTV at follow-up enabled the most significant OS prediction (sensitivity, 85%; specificity, 88%; $P = 0.001$). Patients with an absolute MTV below 5 ml at follow-up survived significantly longer (12 vs. 6 months, $P < 0.001$), whereas early responders defined by RANO criteria lived only insignificantly longer (9 vs. 6 months; $P = 0.072$). The absolute MTV at follow-up remained significant in the multivariate survival analysis ($P = 0.006$).

Conclusions FET-PET appears to be useful for identifying responders to BEV/LOM early after treatment initiation.

Keywords CCNU · Amino acid PET · Glioma · Treatment-related changes · Tumour relapse

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Introduction

For decades, changes of contrast enhancement extent on MRI have been used in brain tumour patients as an indicator of therapy response or tumour progression [1, 2]. However, contrast enhancement resulting from increased blood-brain barrier permeability is nonspecific and may not always be an accurate surrogate of neoplastic tissue, tumour extent, or treatment-related changes such as pseudoprogression [3–6].

After the introduction of antiangiogenic drugs (e.g., bevacizumab) the so-called phenomenon of a "pseudoresponse" has been described, which may complicate the assessment of treatment response by evaluating changes of contrast-enhancement according to the Macdonald criteria only [2]. Within a few weeks after initiation of treatment, it has been observed that antiangiogenic drugs such as bevacizumab can markedly reduce contrast enhancement [7], indicating partial or even complete response. Some of these imaging findings on MRI may result from a quick normalization of abnormally permeable blood vessels, which is, however, not associated with the tumour's response. Thus, a decrease of contrast enhancement may not reflect actual antitumoral effects of antiangiogenic drugs [4]. Following antiangiogenic treatment, MRI frequently suggests an impressive radiological response, which can be in stark contrast to the clinical benefit caused by the antiangiogenic therapy [8].

To overcome the limitations of conventional MRI in the assessment of tumour response to antiangiogenic treatment according to the Macdonald criteria, in 2010 the Response Assessment in the Neuro-Oncology (RANO) group suggested new recommendations for evaluating the response to a therapy [1]. In particular, following antiangiogenic therapy, T2 or FLAIR signal hyperintensity were recommended as surrogate markers for nonenhancing tumour to assess tumour progression, and these alterations were included as criteria for determining tumour response or progress ("nonenhancing tumour progression") [1].

Importantly, these criteria do not provide quantitative parameters for the diagnosis of tumour progression. Furthermore, various differential diagnoses such as reactive changes after surgery, perifocal tumour oedema, radiation injury, demyelination, ischemia, and infection can lead to signal hyperintensities on T2 or FLAIR sequences, which are difficult to discern from nonenhancing tumour [4]. Consequently, alternative diagnostic methods are necessary to improve the identification of treatment response.

PET studies using the radiolabelled amino acid O-(2-[^{18}F]fluoroethyl)-L-tyrosine (FET) allow detection of the metabolically active tumour of gliomas, which is more specific than contrast enhancement or FLAIR hyperintensity on MRI [9–12]. Several studies have demonstrated the advantages of amino acid PET for treatment response evaluation of bevacizumab plus irinotecan compared to conventional MRI in patients with pretreated malignant glioma [13–15]. In particular, amino acid PET responders had a significantly longer survival than MRI responders based on RANO criteria [15, 16]. Furthermore, an amino acid PET may allow to identify non-responders earlier than conventional MRI [13, 16].

The results of these predominantly retrospective amino acid PET studies were, however, derived from very heterogeneous patient groups, i.e., inclusion of anaplastic gliomas as well as primary and secondary glioblastomas with different molecular profiles, usually heavily pretreated by a wide range of both conventional and experimental treatment regimens, and imaged inconsistently at different time points.

More recent data from a phase 2 trial (BELOB trial) suggested that in glioblastoma patients at first progression another antiangiogenic treatment regimen—the addition of bevacizumab to the nitrosourea lomustine (BEV/LOM)—might improve OS as compared with monotherapies [17]. Besides other parameters, the rate of OS at 9 months was higher with the combination of BEV/LOM than with either agent alone. The purpose of the present study was to prospectively investigate the value of FET PET in comparison to conventional MRI for early treatment response evaluation in a more homogenous group of patients with IDH wild-type glioblastoma undergoing a newer antiangiogenic treatment regimen with BEV/LOM at first progression.

Patients and methods

Patients

From 2013 to 2015, we prospectively assessed 21 patients (age range, 33–75 years) with glioblastoma treated with BEV/LOM at first progression. All patients met the following inclusion criteria: they had a neuropathologically confirmed diagnosis of an isocitrate dehydrogenase (IDH) wild-type glioblastoma, including the evaluation of the O^6-methylguanine-DNA methyltransferase (MGMT) promoter methylation status, and had previously undergone surgical resection (if possible) or stereotactic biopsy followed by radiation therapy with concomitant and adjuvant temozolomide chemotherapy [18]. All patients had MRI-confirmed progressive disease based on RANO criteria [1] by the time BEV/LOM treatment was started. Further criteria included (i) a Karnofsky performance score ≥ 70%, (ii) adequate hematologic values (i.e., total white blood count ≥3000/μl; neutrophils ≥1.500/μl; platelets ≥100.000/μl), and (iii) sufficient hepatic (i.e., GOT and GPT 10–50 U/l) and renal function (i.e., serum creatine ≤1.2 mg/dl). Patients were excluded if they had a bleeding disorder or a recent history of intracranial bleeding or thromboembolism. The local ethics committees approved this prospective study (Denmark file number, H-1-2013-062;

Germany file number, 2438), and all patients gave written informed consent before each FET PET investigation.

Treatment and follow-up

Patients undergoing resection at the time of the first progression were eligible if surgery had confirmed the nature of the lesion, and for these patients, an immediate postoperative MRI scan within 48 h after resection was advised. Re-operated patients could not start BEV/LOM treatment until four weeks after surgery. As described previously, at progression all patients were treated with BEV/LOM (BEV dose, 10 mg/kg bodyweight i.v. every two weeks; LOM dose, 90 mg/m^2 body surface area p.o. every 6–8 weeks) [17, 19]. During treatment, all patients were evaluated for vital signs, adverse events, blood counts, and urine dipstick results every 2 weeks. The patients' clinical status was complemented by gadolinium-enhanced as well as non-enhanced MRI obtained before and at approximately 10-week intervals after starting BEV/LOM therapy. Patients were followed until death with no subjects lost during follow-up. The post-progression overall survival (OS) was defined as the time interval between first progression and death.

PET imaging

FET PET scans were performed at baseline (9 ± 11 d before BEV/LOM initiation) and follow-up after 7.7 ± 1.9 weeks. FET PET imaging was obtained at the Forschungszentrum Juelich (n = 19 patients) or the Dept. of Clinical Physiology, Nuclear Medicine & PET, Copenhagen University Hospital Rigshospitalet (n = 2 patients).

As described previously, the amino acid FET was produced via nucleophilic ^{18}F-fluorination with a radiochemical purity of greater than 98%, a specific radioactivity greater than 200 GBq/μmol and a radiochemical yield of about 60% [20]. According to the German guidelines for brain tumour imaging using labelled amino acid analogues [21], all patients fasted for at least 4 h before the PET measurements. At the Forschungszentrum Juelich, 19 patients underwent a dynamic PET scan from 0 to 50 min post-injection of 3 MBq of FET per kg of body weight at baseline and follow-up. PET imaging was performed either on an ECAT Exact HR+ PET scanner (n = 32 scans) in 3-dimensional mode (Siemens, Erlangen, Germany) (axial field of view, 15.5 cm; spatial resolution, 6 mm) or simultaneously with 3 T MR imaging using a BrainPET insert (n = 6 scans) (Siemens, Erlangen, Germany). The BrainPET is a compact cylinder that fits in the bore of the Magnetom Trio MR scanner (axial field of view, 19.2 cm; optimum spatial resolution, 3 mm) [22]. Iterative reconstruction parameters were 16 subsets, six iterations using the OSEM algorithm for ECAT HR+ PET scanner and two subsets, 32 iterations using the OP-OSEM algorithm provided by the manufacturer for the BrainPET, with correction for random, scattered coincidences, and dead time for both systems. Attenuation correction for the ECAT HR+ PET scan was based on a transmission scan, and for the BrainPET scan on a template-based approach [22]. The reconstructed dynamic data set consisted of 16 time frames (5 × 1 min; 5 × 3 min; 6 × 5 min).

At the Dept. of Clinical Physiology, Nuclear Medicine & PET, Copenhagen University Hospital Rigshospitalet, static and dynamic FET PET imaging (n = 4 scans in two patients) was performed on a Siemens Biograph mMR 3 T PET/MR system (Siemens, Erlangen, Germany) using a low-dose CT scan for attenuation correction [23] and a similar image reconstruction, and dynamic framing as stated above.

PET data analysis

For the evaluation of FET data, PET images over a period of 20–40 min post-injection were used. A two-dimensional auto-contouring process using a tumor-to-brain ratio (TBR) of at least 1.6 determined the mean tumoral FET uptake. This cut-off was based on a biopsy-controlled study in cerebral gliomas and differentiated best between tumoural and peritumoral tissue [9]. To exclude an influence of different scanner resolution of the HR+ scanner and the BrainPET scanner (Forschungszentrum Juelich), a circular region of interest (ROI) with a diameter of 1.6 cm was centered on maximal tumor uptake [24] for the evaluation of the maximal FET uptake. Mean and maximum TBR (TBR_{mean} and TBR_{max}) were calculated by dividing the mean and maximum standardized uptake value (SUV) of the tumor ROI by the mean SUV of a larger ROI placed at the level of the semioval centre of the contralateral unaffected hemisphere including white and grey matter [21]. The calculation of FET metabolic tumor volumes (MTV) was determined by a 3D auto-contouring process using a threshold of 1.6 with PMOD (Version 3.505, PMOD Technologies Ltd.).

MR imaging

Conventional MRI scans were performed at baseline and follow-up after 10.2 ± 2.3 weeks. All patients underwent routine MRI (1.5 T) with standard coils before and after administration of a gadolinium-based contrast agent (T1- and T2-weighted and FLAIR sequence). Early MRI-based response at 10 weeks was defined according to the RANO criteria [1]. The criteria *Stable Disease*, *Partial Response,* and *Complete Response* were considered as a response to BEV/LOM.

Statistical analysis

Descriptive statistics are provided as the mean and standard deviation or as the median and range. The Student's t-test was used to compare two groups. The Mann-Whitney rank sum test was used when variables were not normally distributed.

The distribution of dichotomized variables was determined using the Fisher exact test. A *P*-value of <0.05 was considered to be significant.

FET PET uptake threshold values (i.e., TBR_{max} and TBR_{mean}) and the MTV threshold for treatment response were established by receiver-operating characteristic (ROC) curve analysis using the OS (≤ 9 months vs. > 9 months) as the reference. This reference was derived from the results of a phase 2 trial (BELOB trial) suggesting that the proportion of patients with an OS >9 months was larger with the combination of BEV/LOM than with either agent alone [17]. Decision cut-off was considered optimal when the product of paired values for sensitivity and specificity reached its maximum. In addition, the area under the ROC curve (AUC), its standard error, and level of significance were determined as a measure of the diagnostic quality of the test. Kaplan Meier curves were subsequently generated to obtain survival estimates. Multivariate Cox proportional hazards models were constructed to test the relationship between FET metabolic responses and other predictors concerning OS. The forward stepwise selection technique was utilized to reduce the multivariate models ($P < 0.10$). Parameters that were significant in univariate analyses were included in multivariate models.

Statistical analyses were performed using SigmaStat software (SigmaPlot for Windows 11.0, Chicago, IL) and SPSS Statistics software (Release 24.0, SPSS Inc., Chicago, IL, USA).

Results

Patients

Twenty-one IDH wild-type glioblastoma patients were included (median age, 55 years; age range, 33–75 years; 8 females, 13 males; MGMT promoter unmethylated, 81%). All 21 patients completed baseline neuroimaging including FET PET and MRI as well as at follow-up. Details of patient characteristics and neuroimaging findings at baseline and follow-up are listed in Table 1.

Treatment delivery and side effects

At progression, 10 of 21 patients (48%) underwent neurosurgical resection before BEV/LOM initiation. Based on presence or absence of contrast-enhancing lesions in the early postoperative MRI within the first 48 h, there were six complete resections and four partial resections (Table 1). At the start of BEV/LOM therapy, six patients (29%) received dexamethasone (median dose, 4 mg; range, 4–8 mg) (Table 1). Subsequently, tapering of dexamethasone was possible in all patients. Patients received a median of three cycles of LOM (range, 1–6 cycles) and 11 cycles of BEV (range, 2–26 cycles).

There were no grade 5 events according to the Common Terminology Criteria for Adverse Events (CTCAE v4.03). CTCAE grade 4 events occurred in two patients (9%), grade 3 events in five patients (24%), and grade 1–2 events in eight patients (38%), respectively. Six patients (29%) experienced no side effects. Overall, the most common side effect was thrombocytopenia (52%).

Survival

In the whole cohort, the median time between initial diagnosis and first tumour progression was 9 months (range, 3–18 months), and the median time between initial diagnosis and death was 16 months (range, 8–31 months). The median OS, defined as the time interval between first progression and death, was 7 months (range, 3–16 months).

Optimal thresholds derived from FET PET parameters

An overview of the results of the optimal thresholds derived from FET PET is shown in Table 2. Specifically, ROC analysis revealed that at follow-up a relative reduction of TBR_{max} of more than 27% separated responders (OS >9 months) from non-responders (OS ≤9 months) with a sensitivity of 92% and a specificity of 63% (AUC, 0.78 ± 0.11; $P = 0.036$). Similarly, a relative TBR_{mean} reduction of more than 17% at follow-up revealed the same sensitivity and specificity for differentiating responders from non-responders (AUC, 0.81 ± 0.10; $P = 0.020$). A relative MTV reduction of 27% yielded a slightly higher AUC for the differentiation between responders and non-responders (AUC, 0.82 ± 0.09; $P = 0.015$).

The most significant OS prediction could be obtained by the absolute MTV at the follow-up scan (threshold, 5 ml; sensitivity 85%; specificity, 88%; AUC, 0.92 ± 0.06; $P = 0.001$) (Fig. 1). To rule out a biasing effect of a previous neurosurgical resection on the results, we excluded the completely resected patients from the analysis. Nevertheless, the results remained mainly unchanged (threshold, 5 ml; sensitivity 91%; specificity, 75%; AUC, 0.91 ± 0.08; $P = 0.019$).

Prediction of response using MRI based on RANO criteria

A *Complete Response* to BEV/LOM according to the RANO criteria could not be observed in any patient. MRI findings consistent with *Stable Disease* or *Partial Response* as assessed by RANO criteria at MRI after 10 weeks was not predictive for an OS >9 months (sensitivity 63%; specificity, 69%; $P = 0.203$; Fisher exact test) (Table 1).

Table 1 Patient characteristics, FET PET and MRI parameters, OS

Patient number	Sex / age	MGMT promoter methylation	Resection before BEV/LOM	Dex at BEV/ LOM begin	MTV baseline (ml)	MTV follow-up (ml)	MTV change (%)	TBR_{max} baseline (ml)
1	M, 53	not meth	no	8 mg	94.9	74.5	−21	4.0
2	M, 73	not meth	no	4 mg	8.3	9.5	14	2.6
3	M, 44	not meth	no	no	5.4	3.7	−31	2.3
4	M, 75	not meth	no	4 mg	20.2	21.9	8	2.9
5	F, 64	not meth	no	no	21.4	10.0	−53	2.6
6	M, 55	not meth	CR	no	82.3	0	−100	4.0
7	M, 50	not meth	no	4 mg	56.5	72.5	28	2.2
8	F, 68	not meth	CR	no	11.5	0	−100	2.0
9	M, 44	not meth	PR	no	4.7	4.7	0	2.0
10	M, 33	not meth	no	no	17.5	9.0	−49	2.6
11	M, 54	not meth	no	no	2.0	2.0	0	1.9
12	M, 45	not meth	no	no	11.4	26.3	131	2.8
13	M, 66	meth	PR	4 mg	99.3	59.0	−41	2.5
14	F, 55	not meth	PR	no	3.5	5.3	51	1.9
15	M, 51	meth	no	no	43.3	14.8	−66	3.8
16	F, 63	not meth	CR	no	0	2.0	100	0
17	F, 61	meth	CR	no	0	0	0	2.1
18	F, 55	not meth	no	8 mg	8.4	12.6	50	2.1
19	F, 51	not meth	CR	no	2.0	2.0	0	1.8
20	F, 42	not meth	PR	no	2.6	0.4	−85	2.8
21	M, 59	meth	CR	no	2.1	42.3	1914	2.6

Patient number	TBR_{max} follow-up (ml)	TBR_{max} change (%)	TBR_{mean} baseline (ml)	TBR_{mean} follow-up (ml)	TBR_{mean} change (%)	MRI response	OS (months)
1	4.3	8	2.5	2.7	8	PR	9
2	2.0	−23	2.2	2.0	−9	PD	3
3	2.0	−13	2.1	2.0	−5	SD	16
4	2.7	−7	2.4	2.3	−4	PD	6
5	2.0	−23	2.1	1.8	−14	PR	7
6	0	−100	2.6	0	−100	PD	14
7	3.2	45	2.0	2.2	10	PD	6
8	0	−100	2.0	0	−100	SD	16
9	1.9	−5	2.0	1.9	−5	PD	11
10	1.8	−31	2.2	1.8	−18	SD	11
11	1.7	−11	1.9	1.7	−11	SD	6
12	2.5	−11	2.3	2.1	−9	PD	4
13	1.9	−24	2.1	1.9	−10	PD	5
14	2.0	5	1.9	2.0	5	PD	7
15	2.3	−39	2.6	2.1	−19	PR	6
16	2.8	100	0	2.1	100	PD	7
17	0	−100	1.7	0	−100	SD	12
18	3.1	48	1.7	2.0	18	PD	9
19	2.0	11	1.7	1.7	0	PD	11
20	1.7	−39	2.0	1.6	−20	SD	12
21	2.8	8	1.9	2.0	5	PD	5

BEV/LOM = bevacizumab plus lomustine; **CR** = complete resection; **Dex** = dexamethasone; **F** = female; **M** = male; **meth, not meth** = MGMT promoter methylated / not methylated; **MGMT** = O^6-methylguanine–DNA methyltransferase; **MTV** = metabolic tumor volume; **OS** = post-progression overall survival, defined as the time interval between first progression and death; **PD** = progressive disease based on RANO criteria based on RANO criteria; **PR** = partial resection / partial response based on RANO criteria; **SD** = stable disease based on RANO criteria; **TBR_{max}, TBR_{mean}** = maximum and mean tumor-to-brain ratio

Table 2 Differentiation between responders (OS >9 months) and non-responders (OS ≤9 months) by imaging parameters

Parameter	Relative reduction of TBR_{max}	Relative reduction of TBR_{mean}	Relative reduction of MTV	Absolute MTV at follow-up	MRI response
Best threshold	27%	16%	27%	5 ml	PR or SD
Sensitivity (95% CI)	92% (64–100%)	92% (64–100%)	77% (46–95%)	85% (55–98%)	63% (31–86%)
Specificity (95% CI)	63% (24–91%)	63% (24–91%)	63% (24–91%)	88% (47–100%)	69% (42–87%)
AUC ± standard error	0.78 ± 0.11	0.81 ± 0.10	0.82 ± 0.09	0.92 ± 0.06	n.a.
P-value	0.036	0.020	0.015	0.001	0.203

95% CI = 95% confidence interval; **AUC** = area under the curve; **MTV** = metabolic tumor volume; **n.a.** = not available, **PR** = partial response based on RANO criteria; **SD** = stable disease based on RANO criteria; **TBR_{max}, TBR_{mean}** = maximum and mean tumor-to-brain ratio.

Univariate survival analysis

When comparing relative FET MTV changes from baseline to follow-up using the ROC-derived threshold value of ≥27% reduction, eight responders (OS >9 months) and 13 non-responders (OS ≤9 months) were identified with a median FET MTV reduction of −60% for responders and 15% for non-responders, respectively ($P < 0.001$). The median OS was 11 months for responders vs. 7 months for nonresponders ($P = 0.028$) (Table 3). Using the absolute FET MTV threshold of 5 ml at follow-up, nine responders and 12 non-responders were identified with a median FET MTV of 2.0 ml for responders and 18.4 ml for non-responders at follow-up, respectively ($P < 0.001$). The median OS was 12 months for responders vs. 6 months for nonresponders ($P < 0.001$) (Fig. 2). Similarly, this prolonged effect on OS has also been observed in patients with biopsy and partial resection only (threshold, 5 ml; 11 vs. 6 months; $P = 0.002$) (Table 3).

In contrast, MRI responders based on RANO criteria at 10 weeks showed only a trend towards longer OS (9 vs. 6 months; $P = 0.072$). Patients' age, neurosurgical resection before BEV/LOM initiation, and a methylated MGMT promoter did not predict a prolonged OS. An overview of the results of univariate survival analyses is presented in Table 3.

Multivariate survival analysis

The absolute FET MTV at follow-up remained significant in the multivariate survival analysis ($P = 0.006$; HR, 0.158; 95% CI, 0.042–0.595), indicating an independent predictor for OS. In contrast, relative changes of TBR_{max} and TBR_{mean}, as well as MTV, were not significant (Table 4).

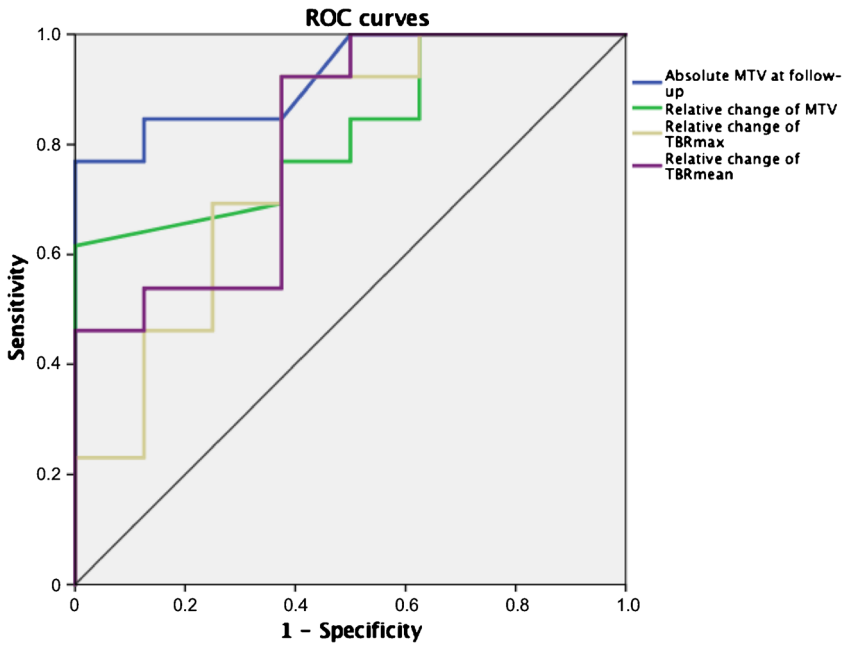

Fig. 1 ROC curves for the absolute MTV at follow-up and relative changes of MTV, TBR_{max}, and TBR_{mean}

Table 3 Results of univariate survival analyses

Parameter	Threshold / Criterion	OS (months) Responders / Criterion present	OS (months) Non-Responders / Criterion not present	*P*-value
Relative reduction of TBR_{max}	27%	11	7	0.038
Relative reduction of TBR_{mean}	16%	11	7	0.038
Relative reduction of MTV	27%	11	7	0.028
Absolute MTV at follow-up	5 ml	12	6	< 0.001
MRI response based on RANO criteria	PR or SD	9	6	0.072
Neurosurgical resection before BEV/LOM	resection obtained	11	6	0.196
MGMT promoter methylation	meth	5	9	0.257
Age	55 years	9	6	0.532

BEV/LOM = bevacizumab plus lomustine; **MTV** = metabolic tumor volume;; **OS** = post-progression overall survival, defined as the time interval between first progression and death $\mathbf{TBR_{max}}$, $\mathbf{TBR_{mean}}$ = maximum and mean tumor-to-brain ratio.

Comparison of early MRI and FET PET findings during BEV/LOM

In a subset of patients ($n = 15$; without additional tumor resection before BEV/LOM initiation or with partial resection only), baseline MRI findings were compared to that at follow-up. It could be observed that MR imaging findings were almost unchanged ($n = 4$; patients#3, #10, #11, #20 were assigned to *Stable Disease* based on RANO criteria). In contrast, in three of these four patients, FET MTV decreased considerably (median MTV decrease, −49%; range, −31% to −85%). Figure 3 presents an example (patient #3). The OS of that patient was 16 months. Moreover, in another non-responding patient (patient #1), MRI at follow-up was consistent with *Partial Response*. Conversely, in that patient, the OS was not >9 months.

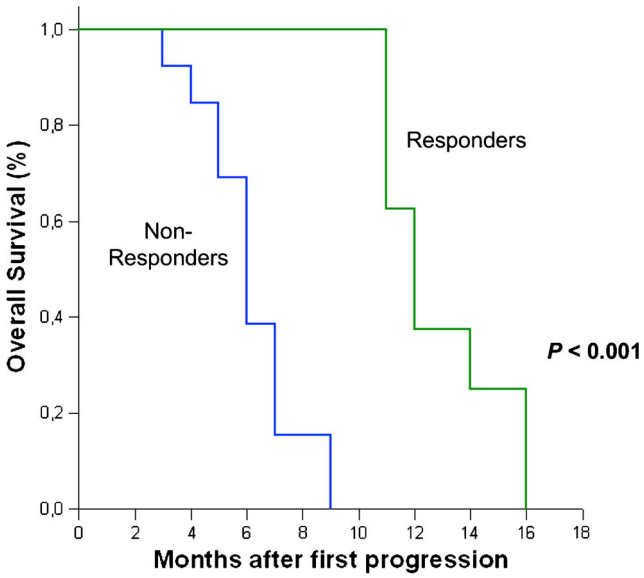

Fig. 2 Kaplan-Meier overall survival curve separated by the absolute FET PET MTV (threshold, 5 ml) at follow-up

Discussion

The main finding of the present study is that early FET PET-derived imaging parameters such as TBR and MTV provide valuable clinical information on tumor response in GBM patients at first progression treated with BEV/LOM, which cannot be derived from MRI response assessment based upon RANO criteria at the initial posttreatment evaluation. In particular, in contrast to MRI responders, FET PET parameters significantly predicted a favourable OS (> 9 months). Furthermore, in a subset of patients, a clear metabolic response as assessed by FET PET could be observed, whereas MRI remained unchanged as indicated *Stable Disease* based on RANO criteria (Fig. 3).

It should be noted that the MRI response assessment based upon RANO criteria at later time points in patients with progressive GBM responding to BEV/LOM prognosticates a prolonged OS [25]. Our data, however, suggests that FET PET is more sensitive in early response assessment, which could be useful in patient management, e.g., re-evaluation of other treatment options.

Our results are in line with a previous prospective study by Schwarzenberg and colleagues. Progressive high-grade glioma patients ($n = 30$) were treated with BEV and irinotecan and examined using standard MRI and 3,4-dihydroxy-6-[^{18}F]-fluoro-L-phenylalanine (FDOPA) PET at baseline, as well as two weeks and 6 weeks after starting the treatment [15]. In that study, the majority of patients were heavily pretreated (e.g., six patients had undergone 6–9 treatment regimens), but, in accordance with our study, the absolute FDOPA MTV at both follow-up time points was the most potent predictor for a significantly prolonged OS. Similar to FET, FDOPA belongs to the group of radiolabeled amino acids, and regarding tumor delineation, both tracers provide equivalent information [26]. In contrast to our study, the latter

Table 4 Results of multivariate survival analyses

Parameter	Threshold	Hazard ratio	95%-confidence interval	*P*-value
Relative reduction of TBR_{max} and TBR_{mean}	27% / 16%	0.644	0.189–2.199	0.492
Relative reduction of MTV	27%	0.395	0.114–1.372	0.144
Absolute MTV at follow-up	5 ml	0.158	0.042–0.595	0.006

MTV = metabolic tumor volume; **TBR_{max}, TBR_{mean}** = maximum and mean tumor-to-brain ratio

study identified a higher threshold tumor volume at follow-up for outcome prediction (18 ml compared to 5 ml in the present study), most probably related to a different methodology of MTV estimation.

Furthermore, other previous studies used FET PET for the evaluation of treatment effects of BEV and irinotecan in heavily pretreated patients with high-grade gliomas and suggested that the relative change of FET MTV between baseline and follow-up imaging was also predictive for an improved outcome (threshold of relative change, 45%) [13, 14]. However, in these studies, the predictive value of the absolute MTV at follow-up was not evaluated. Similarly, in our study, relative MTV changes were also predictive for a prolonged OS. Again, compared to our study the MTV threshold value was different (27%), most probably related to a larger and more homogenous group of patients with distinctly less pretreatment in the present data set. Note that in previous amino acid PET studies the treatment regimen BEV in combination with irinotecan has been evaluated regarding the treatment response. Therefore, the present results on response assessment to BEV/LOM using FET PET are not entirely comparable.

The previously published amino acid PET studies in this field have also evaluated the post progression-free survival (PFS) after the start of bevacizumab plus irinotecan based on RANO criteria [13–15]. For various reasons, the diagnosis of progression is difficult. In particular, pseudoresponse (not infrequently observed in clear contrast to the clinical benefit caused by BEV therapy effects) [8], the lack of quantitative values of T2 or FLAIR signal change for the diagnosis of non-enhancing progression [1, 4], and different MRI patterns of tumour progression during BEV therapy [27–29] may complicate the evaluation of PFS. For these reasons, this study focused on OS only and we did not calculate the PFS after BEV/LOM initiation.

Regarding the efficacy of BEV/LOM therapy, data from a phase 2 trial (BELOB trial) suggested that in glioblastoma patients at first progression the addition of BEV to LOM might improve OS as compared with monotherapies [17]. In particular, the rate of OS at 9 and 12 months was higher with the combination of BEV/LOM than with either agent alone. Based on these results of the BELOB trial, the EORTC (European Organization for Research and Treatment of

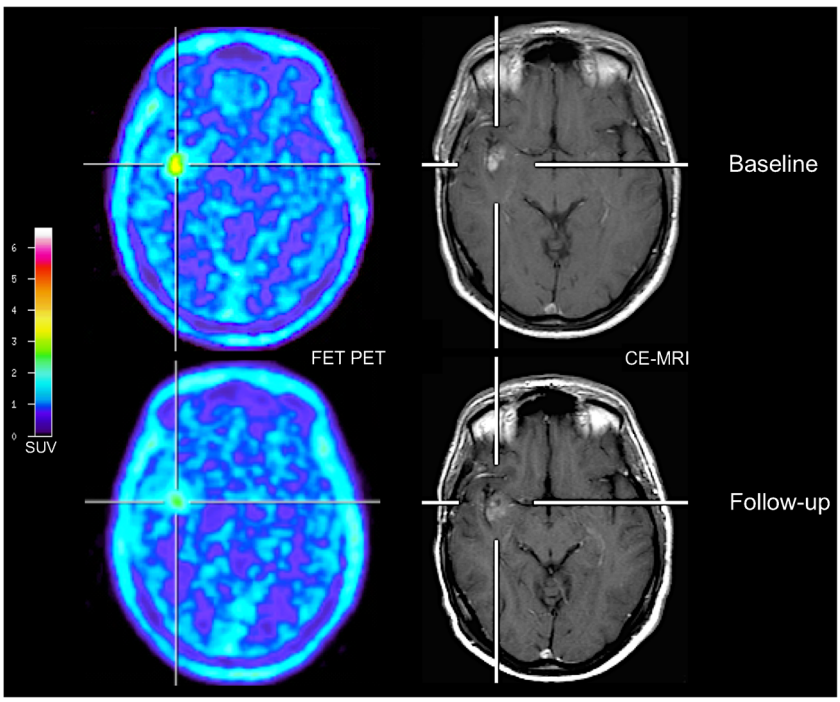

Fig. 3 Comparison of FET PET and contrast-enhanced MR images at baseline (*top row*) and follow-up (*bottom row*). At follow-up (patient #3), a clear decrease of both the metabolically active tumor volume (−31%) as well as maximum and mean tumor/brain ratios (−13% and −5%, respectively) is observed whereas the MRI shows no significant change of contrast enhancement (*Stable Disease* according to RANO criteria)

Cancer) 26101 phase 2 trial has been modified into a phase 3 trial [19]. However, that EORTC 26101 phase 3 trial was unable to confirm the conclusion of the phase 2 BELOB trial that the addition of BEV to LOM improves OS in glioblastoma patients at first progression [19]. In that study, the OS of patients treated with BEV/LOM was 9.1 months, insignificantly prolonged compared to 8.6 months for LOM monotherapy. In our study, the OS was shorter (7.0 months), most problaby due to the high fraction of patients with an unmethylated MGMT promoter (81%). Despite the lack of efficacy regarding the OS prolongation in that phase 3 trial, we observed in our study a BEV/LOM treatment effect, with a significantly longer OS in a subgroup of patients. However, the reasons for these positive treatment effects on OS are unclear. Nevertheless, data from our study suggest that FET MTV might be an early predictive biomarker, which identifies a patient subgroup potentially responding to BEV/LOM. The use of this approach would facilitate accrual to clinical trials evaluating treatment effects of newer therapies in patients with progressive glioblastoma.

The present study has a limitation. Note that our patient sample was small and our results should therefore be considered with caution. On the other hand, the patient cohort is fairly homogenous, i.e., only IDH wild-type glioblastoma patients at first progression with the same pretreatment were included, and in 81% of the patients, the MGMT promoter methylation status was the same. Furthermore, the most significant predictor for OS, the absolute FET MTV at follow-up, was scrutinized by multivariate survival analyses and remained significant.

In summary, FET PET-derived imaging parameters seem to predict response to BEV/LOM treatment and, moreover, may provide significant information for the patients' OS. Importantly, this information was derived over and above that which could be gained by assessing early treatment response using MRI based on RANO criteria. Thus, in clinical practice, this important information in response assessment could be useful for decision-making, e.g., re-evaluation of treatment options. A clinical trial with a larger patient cohort should be performed to assess further the diagnostic potential of FET PET in determining the effects of neurooncological treatment options.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed written consent was obtained from all individual participants included in the study.

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