Early treatment response assessment using ^{18}F-FET PET compared to contrast-enhanced MRI in glioma patients following adjuvant temozolomide chemotherapy

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ABSTRACT

Background: The goal of this study was to compare the value of contrast-enhanced MRI and O-(2-[^{18}F]fluoroethyl)-L-tyrosine (^{18}F-FET) PET for response assessment in glioma patients following adjuvant temozolomide chemotherapy (TMZ).

Methods: After biopsy or resection and completion of radiotherapy with concomitant TMZ, 41 newly diagnosed and histomolecularly characterized glioma patients (glioblastoma, 90%; age range, 20-79 years) were subsequently treated with adjuvant TMZ. MR and ^{18}F-FET PET imaging were performed at baseline and after the second cycle of adjuvant TMZ. We obtained ^{18}F-FET metabolic tumor volumes (MTV) as well as mean and maximum tumor-to-brain ratios (TBR_{mean}, TBR_{max}). Threshold values of ^{18}F-FET PET parameters to predict outcome were established by ROC analyses using a median progression-free survival (PFS) of ≥9 months and overall survival (OS) of ≥15 months as reference. MRI response assessment was based on the Response Assessment in Neuro-Oncology (RANO) working group criteria. The predictive value of changes of ^{18}F-FET PET and MRI parameters on survival was evaluated subsequently using univariate and multivariate survival estimates.

Results: After two cycles of adjuvant TMZ chemotherapy, a treatment-induced reduction of MTV and TBR_{max} predicted a significantly longer PFS and OS (both $P \leq 0.03$; univariate survival analyses) while RANO criteria were not significant ($P > 0.05$). Multivariate survival analysis revealed that TBR_{max} changes predicted a prolonged PFS ($P = 0.012$) and changes of MTV a prolonged OS ($P = 0.005$) independent of O^6-methylguanine-DNA-methyltransferase promoter methylation and other strong prognostic factors.

Conclusions: Changes of ^{18}F-FET PET parameters appear to be helpful for identifying responders to adjuvant TMZ early after treatment initiation.

KEYWORDS

Amino acid PET; treatment monitoring; treatment-related changes; pseudoprogression; metabolic tumor volume

INTRODUCTION

The prognosis of patients with glioblastoma is still relatively poor, with median overall survival rates ranging between 15 and 20 months (*1-3*). Since 2005, first-line treatment consists of cytoreductive surgery, followed by radiotherapy with concomitant and adjuvant temozolomide (TMZ) chemotherapy, according to the EORTC-NCIC 22981/26981 protocol (*1*). More recently, in glioblastoma patients, further survival benefit has been achieved by adding tumor-treating fields concurrent to adjuvant TMZ chemotherapy (*4,5*), or by lomustine/TMZ combination chemotherapy in glioblastoma patients with O^6-methylguanine-DNA-methyltransferase (MGMT) promoter methylation (*6*). Nevertheless, in many centers, radiotherapy with concomitant and adjuvant TMZ is still the standard of care.

For decades, the method of choice for treatment response assessment in brain tumor patients is contrast-enhanced anatomical MRI. Predominantly, changes of contrast enhancement are used as a surrogate of treatment response or tumor progression (*7,8*). However, contrast enhancement resulting from increased blood-brain barrier permeability is nonspecific and may not always be an accurate indicator of neoplastic tissue, tumor extent, or treatment effect (*9-11*). Importantly, since the introduction of chemoradiation with TMZ, there has been an increasing awareness of progressive enhancing lesions on MRI, which are related to the treatment. These findings eventually either remain stable or may ultimately even regress, as observed during follow-up MR imaging without any change of treatment. Accordingly, this phenomenon was termed pseudoprogression (*12-14*). Typically, this phenomenon occurs within the first 12 weeks after chemoradiation completion (*7*) and may also occur beyond the 12-week time window (*15,16*). Similarly, radiation necrosis, which usually manifests several months later than pseudoprogression, may also lead to

contrast enhancement on MRI (*17*). Additionally, nonspecific contrast enhancement may result from postoperative inflammation, ischemia, and seizures (*18,19*). Consequently, alternative diagnostic methods are needed to improve the evaluation of treatment response.

In the recent past, numerous studies have shown that PET using the radiolabeled amino acid O-(2-[^{18}F]fluoroethyl)-L-tyrosine (^{18}F-FET) provides valuable additional diagnostic information for various indications in neurooncology, including the assessment of treatment response (*20,21*). Moreover, the Response Assessment in Neuro-Oncology (RANO) working group has emphasized that for gliomas and brain metastases, the additional clinical value of amino acid PET compared to standard MRI is excellent as it provides valuable diagnostic information for treatment response assessment (*22,23*).

However, studies evaluating the value of ^{18}F-FET PET for treatment response assessment in glioma patients (*24-27*) are predominantly based on mostly heterogeneous patient groups (i.e., usually heavily pretreated glioma patients with different histomolecular diagnoses and/or inconsistent imaging time points). Additionally, very few studies have addressed the value of ^{18}F-FET PET only for the assessment of response to chemoradiation with concurrent TMZ in newly diagnosed glioblastoma patients treated according to the EORTC/NCIC 22981/26981 trial (*28-30*).

To evaluate the response to adjuvant TMZ chemotherapy using ^{18}F-FET PET and contrast-enhanced MRI, we performed a study in newly diagnosed glioma patients. We aimed to identify which ^{18}F-FET PET parameter in comparison to MRI is

suited best for predicting a significantly longer survival early after adjuvant TMZ treatment initiation.

PATIENTS AND METHODS

Patients

From 2015 - 2019, we examined 41 consecutive adult patients (mean age, 52 ± 13 years; age range, 20 - 79 years; 19 females) with a Karnofsky performance status ≥ 70% and newly diagnosed glioma (predominantly glioblastoma, 90%) using MR and ^{18}F-FET PET imaging. All patients underwent resection or stereotactic biopsy and had histomolecularly confirmed gliomas, and completed radiotherapy with concomitant TMZ chemotherapy according to the EORTC/NCIC 22981/26981 trial (*1*). Neuroimaging was performed at baseline (within 7 days before adjuvant TMZ initiation) and after the second cycle of adjuvant TMZ. Further details on the patients' characteristics are listed in Table 1.

Treatment and Follow-Up

Following resection or biopsy, all patients were treated with radiotherapy (60 Gy) and concomitant and adjuvant TMZ chemotherapy over 6 cycles according to the EORTC/NCIC 22981/26981 trial (*1*). Contrast-enhanced conventional MRI was performed within the first 48 h after resection and every 8-12 weeks. Patients were assessed by neurological examination and the Karnofsky Performance Score at baseline and every 8-12 weeks during the treatment and after treatment completion. The patients' outcome was prospectively followed. The progression-free survival (PFS) was defined as the time interval between histomolecularly confirmed glioma diagnosis and tumor progression according to RANO criteria (*7*). The overall survival (OS) was defined as the time interval between histomolecularly confirmed glioma diagnosis and death.

Conventional MR Imaging

In accordance with the International Standardized Brain Tumor Imaging Protocol (BTIP) (*31*), MR imaging was performed using a 1.5 T or 3.0 T MRI scanner with a standard head coil before and after administration of a gadolinium-based contrast agent (0.1 mmol/kg body weight). The sequence protocol comprised 3D isovoxel T1-weighted, 2D T2-weighted, and 2D fluid-attenuated inversion recovery-weighted sequences. MRI changes at first follow-up compared to the baseline scan were assigned according to the RANO criteria (*7*). The criteria for *Stable Disease*, *Partial Response*, and *Complete Response* were considered for assessing the response to treatment.

^{18}F-FET PET Imaging

As described previously, the amino acid ^{18}F-FET was produced via nucleophilic ^{18}F-fluorination with a radiochemical purity of greater than 98%, molar radioactivity greater than 200 GBq/μmol, and a radiochemical yield of about 60% (*32*). According to international guidelines for brain tumor imaging using labeled amino acid analogues (*33*), patients fasted for at least 4 h before the PET measurements. All patients underwent a dynamic PET scan from 0 to 50 minutes post-injection of 3 MBq of ^{18}F-FET per kg of body weight at baseline (within 7 days before starting of adjuvant TMZ) and after the second cycle of adjuvant TMZ. PET imaging was performed either on an ECAT Exact HR+ PET scanner in 3-dimensional mode (n = 64 scans; Siemens, Erlangen, Germany; axial field-of-view, 15.5 cm) or simultaneously with 3T MR imaging using a BrainPET insert (n = 15 scans; Siemens, Erlangen, Germany; axial field of view, 19.2 cm). The BrainPET is a compact cylinder that fits into the bore of the Magnetom Trio MR scanner (*34*).

Iterative reconstruction parameters were 16 subsets, 6 iterations using the OSEM algorithm for the ECAT HR+ PET scanner and two subsets, 32 iterations using the OP-OSEM algorithm for the BrainPET. Data were corrected for random, scattered coincidences, dead time, and motion, 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 (*34*). The reconstructed dynamic data sets consisted of 16 time frames (5 x 1 min; 5 x 3 min; 6 x 5 min) for both scanners.

To optimize the comparability of the results related to the influence of the two different PET scanners, reconstruction parameters, and post-processing steps, a 2.5 mm 3D Gaussian filter was applied to the BrainPET data before further processing. In phantom experiments using spheres of different sizes to simulate lesions, this filter kernel demonstrated the best comparability between PET data obtained from the ECAT HR+ PET and the BrainPET scanner (*35*).

^{18}F-FET PET Data Analysis

For the evaluation of ^{18}F-FET data, summed PET images over 20-40 minutes post-injection were used. Mean tumoral ^{18}F-FET uptake was determined by a two-dimensional auto-contouring process using a tumor-to-brain ratio (TBR) of at least 1.6. This cut-off was based on a biopsy-controlled study in glioma patients and differentiated best between tumoral and peritumoral tissue (*36*). A circular region-of-interest (ROI) with a diameter of 1.6 cm was centered on the maximal tumor uptake for the evaluation of the maximal ^{18}F-FET uptake, as previously reported (*37*). Mean and maximum TBRs (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 in the semioval center of the contralateral unaffected hemisphere

including white and grey matter (*33*). The calculation of ^{18}F-FET metabolic tumor volumes (MTV) was determined by a three-dimensional auto-contouring process using a threshold of 1.6 using the software PMOD (Version 3.505, PMOD Technologies Ltd.).

Neuropathological Tumor Classification and Analysis of Molecular Markers

All tumors were histomolecularly classified according to the World Health Organization (WHO) Classification of Tumors of the Central Nervous System of 2016 (*38*). For molecular biomarker analysis, tumor DNA was extracted from formalin-fixed and paraffin-embedded tissue samples with a histologically estimated tumor cell content of 80% or more. For assessment of the isocitrate dehydrogenase (IDH) mutation status, the presence of an IDH1-R132H mutation was evaluated by immunohistochemistry using a mutation-specific antibody in a standard immunohistochemical staining procedure as reported (*39,40*). If immunostaining for IDH1-R132H remained negative, the mutational hot-spots at codon 132 of IDH1 and codon 172 of IDH2 were directly sequenced as reported (*41,42*). The MGMT promoter methylation status was assessed by methylation-specific PCR, as described elsewhere (*42*).

Statistical Analyses

Descriptive statistics are provided as mean and standard deviation and/or 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 diagnostic performance of MRI for predicting a favorable PFS and OS were calculated using 2x2 contingency tables; statistical significance was determined by the Pearson's chi-squared test.

The prognostic value of the absolute ^{18}F-FET PET parameters TBR_{max}, TBR_{mean}, and MTV was assessed by receiver operating characteristic (ROC) curve analyses using a favorable PFS and OS as reference. Favorable outcome was defined as a PFS ≥ 9 months and an OS ≥ 15 months. These outcome thresholds were adopted from a previous response assessment study of our group in glioblastoma patients treated with temozolomide chemoradiation (*28*). In that study, the median PFS was 7.2 months, and the median OS 14.1 months, similar to the survival reported in the EORTC-NCIC 22981/26981 trial (PFS, 6.9 months; OS 14.6 months) (*1*). Thus, slightly higher values for PFS and OS were considered as favorable outcome thresholds. Decision cut-off was considered optimal when the product of paired values for sensitivity and specificity reached its maximum. As a measure of the test's diagnostic quality, the area under the ROC curve (AUC), its standard error, and level of significance were determined. Only patients with uncensored survival data were included in ROC analyses for the evaluation of the diagnostic performance. Additionally, the value of relative changes of TBR_{max}, TBR_{mean}, and MTV to predict a significantly longer PFS and OS as an indicator for response to adjuvant TMZ was evaluated using a threshold of ≤ 0% vs. > 0%, as reported (*28*).

Univariate survival analyses were performed using Kaplan-Meier estimates. The log-rank test was used for comparison of the median PFS and OS between the subgroups. Patients were censored if the event (progression or death) had not occurred at the time of data evaluation (April 2020). Parameters that were significant in univariate analyses were included in multivariate models. Multivariate Cox proportional hazards models were constructed to test the relationship between relative changes of ^{18}F-FET PET parameters and other strong prognostic factors (i.e., age, extent of resection, MGMT promoter methylation, and MTV or TBR_{max} at baseline) for

a favorable survival as an indicator for response to adjuvant temozolomide chemotherapy. This analysis was done for each ^{18}F-FET PET imaging parameter separately (i.e., for relative TBR_{max} and MTV change). Hazard ratios and their 95% confidence intervals were calculated.

P-values of 0.05 or less were considered significant. Statistical analyses were performed using SPSS statistics (Release 25.0, SPSS Inc., Chicago, IL, USA).

RESULTS

Patients

Forty-one patients (mean age, 52 ± 13 years; age range, 20 - 79 years; 19 females) with newly diagnosed glioma (WHO grade IV glioblastoma, IDH-wildtype, n=32; WHO grade IV glioblastoma, IDH-mutant, n=3; WHO grade IV glioblastoma, not otherwise specified, n=2; WHO grade III anaplastic astrocytoma, IDH-wildtype, n=2; WHO grade II astrocytoma, IDH-wildtype, n=1; WHO grade IV H3 K27-mutant diffuse midline glioma, n=1) were examined. Sixteen patients had a methylated MGMT promoter (39%), and in 11 patients, a complete tumor resection (27%) could be obtained. All 41 patients completed baseline ^{18}F-FET PET and MR imaging (100%). At follow-up, ^{18}F-FET PET in combination with MRI was available in 38 patients (93%). Due to subsequent clinical deterioration, 3 of 41 (7%) patients were not able to undergo follow-up ^{18}F-FET PET imaging. At the time of data evaluation, tumor progression, according to RANO criteria, had occurred in 37 patients (90%), and death in 33 patients (80%). In the whole cohort, the median PFS was 9 months (range, 3-54 months), and the median OS was 14 months (range, 5-54 months). Further details regarding the patient characteristics and neuroimaging findings at baseline and follow-up are shown in Table 1.

Prognostic value of ^{18}F-FET imaging parameters as assessed by ROC analyses

The results of ROC analyses of absolute ^{18}F-FET PET parameters for predicting a favorable PFS of ≥ 9 months or an OS of ≥ 15 months are presented in Supplemental Tables 1 and 2. Predominantly all ^{18}F-FET PET parameters at baseline and follow-up significantly predicted a favorable PFS or OS (range of AUC values, 0.73 - 0.86). Highest accuracies (AUC ≥ 0.80) to predict a favorable PFS were observed for TBR_{max}

and MTV both at baseline and at follow-up, and for MTV at follow-up to predict a favorable OS. Of these significant prognostic ^{18}F-FET PET imaging parameters, parameters at baseline (before start of adjuvant TMZ therapy) were selected for univariate survival analyses.

Univariate survival analyses regarding baseline prognostic factors and ^{18}F-FET PET imaging parameters

Patients with completely resected tumors or an age ≤ 65 years had no significantly longer PFS or OS (Table 2). In contrast, patients with MGMT promoter-methylated tumors had a significantly longer PFS (12 vs. 8 months; $P = 0.010$) and OS (21 vs. 13 months; $P = 0.030$) (Table 2). Regarding ^{18}F-FET PET parameters, patients with an absolute MTV of ≤ 28.2 mL or a TBR_{max} ≤ 2.0 at baseline had an almost doubled PFS (both 11 vs. 6 months; $P < 0.001$ and $P = 0.004$, respectively). Additionally, an absolute MTV of ≤ 13.8 mL at baseline predicted a significantly longer OS (22 vs. 12 months; $P = 0.010$) (Table 2).

Univariate survival analysis regarding changes of imaging parameters during adjuvant TMZ therapy

After application of two cycles of adjuvant TMZ, relative changes of TBR_{max} and MTV predicted a significantly ($P = 0.031$ and $P = 0.007$, respectively) longer PFS (both 11 vs. 8 months) (Table 3). Relative changes of TBR_{max} and MTV after two cycles of adjuvant TMZ predicted also a significantly longer OS (24 vs. 12 months; $P = 0.032$, and 29 vs. 12 months; $P = 0.005$) (Table 3). Conversely, both the PFS and OS in responding patients on MRI (i.e., MRI findings consistent with *Stable Disease* or *Partial Response* according to RANO) was not significantly prolonged (9 vs. 10 months; $P = 0.618$, and 16 vs. 17 months; $P = 0.752$) (Figure 1, 2).

Multivariate Survival Analysis regarding changes of imaging parameters during adjuvant TMZ therapy

A TBR_{max} reduction was a significant parameter in the multivariate survival analysis (*P* = 0.012; HR, 2.920; 95% CI, 1.272 - 6.705), which predicts a significantly longer PFS (Table 2) independent of age, extent of resection, MGMT promoter methylation, and TBR_{max} at baseline. Furthermore, relative reductions of both TBR_{max} and MTV after two cycles of adjuvant TMZ predicted significantly longer OS (Table 4). A change of MTV after two cycles of adjuvant TMZ was the most significant parameter independent of age, extent of resection, MGMT promoter methylation, and MTV at baseline (*P* = 0.005; HR, 3.614; 95% CI, 1.481 - 8.820). Thus, a decrease of these ^{18}F-FET PET parameters appears to be associated with response to adjuvant temozolomide chemotherapy.

DISCUSSION

The main finding of the present study is that relative changes of MTV and TBR_{max} obtained from ^{18}F-FET PET provide valuable clinical information on tumor response to adjuvant TMZ after completion of radiotherapy with concurrent TMZ in patients with newly diagnosed glioma. Importantly, this information cannot be derived from an MRI response assessment based upon RANO criteria. In contrast to MRI, relative MTV and TBR_{max} changes predicted both a significantly longer and PFS (≥ 9 months) and OS (≥ 15 months), indicating that ^{18}F-FET PET is a powerful tool for the evaluation of treatment effects. Moreover, prediction of response to adjuvant TMZ using these ^{18}F-FET PET parameters was possible irrespective from MGMT promoter methylation and other strong prognostic factors. Thus, our data suggest that ^{18}F-FET PET is highly sensitive in the early response assessment of adjuvant TMZ, which could be useful for patient management, e.g., the diagnosis of pseudoprogression or re-evaluation of other treatment options in the case of early tumor progression (Figure 3). Furthermore, for the patient, his/her relatives, and the treating physician it is of great importance whether a favorable or unfavorable clinical course can be expected. Moreover, based on the response assessment, treatment decisions may be facilitated, e.g., an earlier change to a second-line therapy.

Our findings are in line with a previous study assessing the evaluation of response to radiotherapy in glioblastoma patients. That prospective study evaluated the predictive value of early TBR changes of ^{18}F-FET uptake after postoperative radiotherapy with concurrent TMZ in patients with newly diagnosed glioblastoma (*28,30*). ^{18}F-FET PET was performed at baseline (before chemoradiation) and early after chemoradiation completion (i.e., after 7 - 10 days, and 6 - 8 weeks later). One main finding of that study was that a relative decrease of TBRs related to radiotherapy

with concurrent TMZ was associated with a significantly longer survival (i.e., PFS and OS). Furthermore, and consistent with our findings, the authors observed that ^{18}F-FET PET tumor volume changes (MTV) relative to baseline were also associated with a significantly longer OS. However, in that study, the value of ^{18}F-FET PET for the evaluation of effects to adjuvant TMZ after chemoradiation completion was not assessed. In addition to the latter study evaluating the effects of radiotherapy with concurrent TMZ on ^{18}F-FET PET parameters and survival (*28,30*), we here observed the additional value of relative MTV change for the prediction of response to adjuvant TMZ chemotherapy.

The value of the relative MTV change has also been reported for the evaluation of the effects of other neurooncological treatment options such as antiangiogenic therapy. In a prospective study by Schwarzenberg and colleagues, predominantly heavily pretreated progressive glioma patients underwent bevacizumab and irinotecan therapy. They were examined using standard MRI and 3,4-dihydroxy-6-[^{18}F]fluoro-L-phenylalanine (^{18}F-FDOPA) amino acid PET at baseline and early after starting the therapy (i.e., after 2 weeks, and after 6 weeks) (*43*). Consistent with our study, the relative ^{18}F-FDOPA MTV change relative to baseline following bevacizumab and irinotecan predicted a significantly prolonged OS. Additionally, a prospective study by our group has also highlighted the value of MTV for the evaluation of response to bevacizumab plus lomustine (*44*). In that study, IDH-wildtype glioblastoma patients at first progression were treated with bevacizumab plus lomustine. Contrast-enhanced MRI and ^{18}F-FET PET were performed at baseline and follow-up after 8-10 weeks. Again, relative MTV changes enabled an OS prediction early after treatment initiation.

Furthermore, the predictive value of relative MTV changes has also been reported in patients with non-enhancing WHO grade II or III glioma treated with alkylating chemotherapy (TMZ or lomustine plus procarbacine) (*26*).

In summary, ^{18}F-FET PET-derived imaging parameters can be used to predict response to adjuvant TMZ chemotherapy and may thus provide important information concerning the patient's PFS and OS. In particular, parameters derived from ^{18}F-FET PET, such as relative MTV changes, appear to be a powerful tool for identifying responders to adjuvant TMZ early after treatment initiation irrespective from MGMT promoter methylation. Our results suggest that ^{18}F-FET PET is a valuable diagnostic tool for treatment monitoring including response assessment and justifies its use in clinical routine. An important next step to evaluate the additional clinical value of ^{18}F-FET PET is the monitoring of newer treatment options such as targeted therapy or immunotherapy, ideally in a prospective setting.

COMPETING INTERESTS

The authors declare that they have no competing interests.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All subjects gave prior written informed consent for their participation in the ^{18}F-FET PET study and evaluation of their data for scientific purposes. The local ethics committee approved the evaluation of retrospectively collected neuroimaging data. All procedures performed in studies involving human participants followed the national ethical standards and the Declaration of Helsinki.

KEY POINTS

Question: Is ^{18}F-FET PET superior to conventional MRI for predicting a significantly longer survival early after adjuvant temozolomide chemotherapy initiation?

Pertinent findings: The response to adjuvant temozolomide chemotherapy was evaluated in 41 newly diagnosed and histomolecularly defined glioma patients using ^{18}F-FET PET and contrast-enhanced MRI. Already after two cycles, uni- and multivariate survival analyses revealed that a reduction of ^{18}F-FET PET parameters compared to the baseline scan predicted a significantly longer progression-free and overall survival while standard MRI response criteria were not significant.

Implications for patient care: In contrast to conventional MRT, changes of ^{18}F-FET PET parameters appear to be helpful for identifying responders after two cycles of temozolomide chemotherapy, which could be useful for patient management such as the diagnosis of pseudoprogression or re-evaluation of other treatment options.

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FIGURE LEGENDS

Figure 1: Kaplan-Meier curves for PFS separated by relative changes of the maximum tumor-to-brain ratio (TBR_{max}) on ^{18}F-FET PET (top) and RANO criteria for MRI (bottom) after 2 cycles of adjuvant temozolomide. Responders on ^{18}F-FET PET defined by any decrease or an unchanged TBR_{max} at follow-up compared to baseline had a significantly longer PFS than non-responders (i.e., patients with an increase of TBR_{max} at follow-up compared to baseline) (11 vs. 8 months; P = 0.031). On the other hand, the PFS of responders according to RANO criteria regarding MRI was not significantly longer than in non-responders.

Figure 2: Kaplan-Meier curves for OS separated by the relative metabolic tumor volume (MTV) changes on ^{18}F-FET PET (top) and RANO criteria for MRI (bottom) after 2 cycles of adjuvant temozolomide. Responders on ^{18}F-FET PET defined by any decrease or an unchanged MTV at follow-up compared to baseline had a significantly 2.4-fold longer OS than patients with an increase of MTV at follow-up compared to baseline (29 vs. 12 months; P = 0.005). In contrast, the OS of responders according to RANO criteria regarding MRI was not significantly longer than in non-responders.

Figure 3: Patient with an IDH-wildtype glioblastoma (GBM) with an unfavorable survival (patient #8). After two cycles of adjuvant temozolomide chemotherapy, the contrast-enhancing lesion on MRI is slightly enlarged (criterion *Progressive Disease* according to RANO criteria not fulfilled) compared to the baseline MRI (upper row). In contrast, the corresponding ^{18}F-FET PET at follow-up shows relative to the baseline scan (bottom row) an increase of the metabolic activity as assessed by the maximum tumor-to-brain ratio (TBR_{max}) and metabolic tumor volume (MTV) (relative increase,

8% and 88%, respectively). The patient had an unfavorable outcome with a PFS of 5 months, and an OS of 12 months.

Table 1: Patient characteristics and neuroimaging findings

#	Gender	Age at diagnosis	MGMT promoter methylation	IDH mutation	Diagnosis	EoR	MTV baseline (mL)	MTV follow-up (mL)	TBR_{mean} baseline	TBR_{mean} follow-up	TBR_{max} baseline	TBR_{max} follow-up	MRI response	PFS (months)	OS (months)
1	M	66	meth	wt	GBM	B	42.8	20.8	2.3	2.2	3.1	2,5	PD	16	29
2	M	47	meth	wt	GBM	B	10.7	20.2	2.0	2.2	2.0	2.6	SD	16	21
3	M	62	meth	wt	GBM	CR	5.2	4.3	1.9	1.9	1.9	1.9	SD	11	31
4	M	76	meth	wt	GBM	B	95.5	n.a.	2.1	n.a.	2.8	n.a.	PD	3	5
5	M	69	not meth	wt	GBM	CR	3.4	4.1	1.8	1.8	1.8	1.8	SD	9	34
6	M	69	meth	wt	GBM	B	37.8	n.a.	1.9	n.a.	2.3	n.a.	SD	4	5
7	M	44	not meth	wt	GBM	PR	18.4	14.2	1.9	1.8	1.9	1.8	PD	11	17
8	F	50	meth	wt	GBM	B	8.4	15.8	1.9	1.9	2.4	2.6	SD	5	12
9	F	49	not meth	wt	GBM	PR	26.8	53.7	1.9	1.9	2.0	2.1	PD	11	12
10	F	58	meth	wt	GBM	B	4.3	4.3	2.0	1.9	2.0	1.9	SD	54*	54*
11	M	30	not meth	wt	GBM	PR	17.8	18.9	2.1	2.2	2.8	3.1	SD	8	14
12	M	54	not meth	wt	GBM	PR	60.7	101.0	1.8	2.1	2.0	2.3	PD	8	10
13	F	66	not meth	wt	GBM	B	103.2	137.1	2.6	2.2	4.4	3.2	PD	10	11
14	M	44	meth	mut	GBM	B	13.8	8.4	1.8	1.9	2.0	2.1	PD	15	54*
15	M	58	meth	wt	GBM	CR	43.6	82.1	1.9	2.0	2.1	2.3	SD	5	11
16	F	61	not meth	NOS	GBM	CR	44.1	44.5	2.3	2.4	2.8	3.2	PD	8	50
17	M	61	not meth	wt	GBM	B	29.7	38.3	2.2	2.2	2.5	3.1	PD	4	5
18	F	26	meth	wt	GBM	CR	2.4	0.7	1.9	1.8	1.9	1.8	SD	37	47*
19	F	51	not meth	wt	GBM	CR	1.8	4.5	1.8	1.8	1.8	1.8	PD	12	22
20	F	50	not meth	wt	GBM	CR	14.2	10.7	1.8	1,8	1.8	1.8	n.a.	10	10
21	M	59	not meth	NOS	GBM	PR	10.8	24.9	2.0	1.9	2.1	2.3	SD	7	10
22	M	39	not meth	wt	GBM	B	3.2	11.5	2.0	2.2	2.0	2.8	PD	5	10
23	F	54	meth	wt	AA	B	0.0	0.0	1.7	1.3.	1.7	1.3.	SD	30	31*
24	F	32	meth	mut	GBM	PR	3.6	14.9	2.2	1.8	2.2	1.8	PD	12	24
25	F	20	not meth	wt	H3K27M	PR	1.4	0.6	1.8	1.7	1.8	1.7	PD	18	31
26	M	66	meth	wt	AA	B	0.6	3.2	1.7	1.8	1.7	1.8	SD	11	16
27	M	46	not meth	wt	GBM	CR	12.7	14.7	1.9	2.0	1.9	2.1	SD	9	16
28	F	66	meth	wt	GBM	CR	13.5	9.3	1.8	1.8	1.9	1.9	PD	8	17
29	M	48	meth	mut	GBM	CR	14.6	5.6	1.9	1.8	2.1	1.8	PD	24*	24*
30	F	49	not meth	wt	GBM	CR	7.2	14.9	1.8	2.0	1.8	2.3	PD	11	22
31	M	38	not meth	wt	GBM	PR	75.5	40.2	1.8	1.9	2.3	2.5	SD	6	12
32	F	52	not meth	wt	GBM	PR	48.9	48.5	2.5	2.3	3.5	3.0	PD	4	13
33	M	46	meth	wt	GBM	B	2.6	n.a.	1.8	n.a.	1.8	n.a.	SD	4*	8
34	F	68	not meth	wt	GBM	PR	41.6	26.3	2.0	1.8	2.0	1.8	PD	7	15
35	M	79	not meth	wt	GBM	B	58.0	59.0	2.3	2.1	3.2	2.4	PD	6	6
36	F	43	not meth	wt	GBM	B	67.0	54.0	2.1	2.0	3.3	2.6	PD	7	14*
37	M	58	not meth	wt	GBM	B	6.0	7.0	1.9	2.0	1.9	2.0	PD	9*	12
38	F	54	not meth	wt	GBM	B	2.1	2.6	1.7	1.7	1.7	1.7	SD	9	9*
39	F	62	not meth	wt	GBM	PR	69.2	102.6	2.1	2.3	2.4	3.3	SD	7	9
40	M	42	n.a.	wt	A	B	10.8	15.4	2.1	2.1	2.3	2.2	PD	6	11*
41	F	49	not meth	wt	GBM	PR	15.6	6.9	1.9	1.8	1.9	1.8	PD	12	14

A = astrocytoma (WHO grade II); **AA** = anaplastic astrocytoma**; B** = biopsy; **CR** = complete resection; **EOR** = extent of resection; **F** = female; **GBM** = glioblastoma; **H3K27** = H3K27-mutant diffuse midline glioma; **M** = male; **meth** = MGMT promoter methylated; **MGMT** = O^6-methylguanine-DNA methyltransferase; **MTV** = metabolic tumor volume; **mut** = mutant; **n.a.** = not available; **NOS** = not otherwise specified; **OS** = overall survival; **PD** = "Progressive Disease" according to RANO criteria; **PFS** = progression-free survival; **PR** = partial resection / "Partial Response" according to RANO criteria; **SD** = "Stable Disease" according to RANO criteria; $\mathbf{TBR_{max}}$**,** $\mathbf{TBR_{mean}}$ = maximum and mean tumor-to-brain ratio; **wt** = wildtype; ***** = censored

Table 2: Univariate survival analyses regarding general prognostic factors and ^{18}F-FET PET imaging parameters

Parameter	Threshold	Univariate PFS analysis		Threshold	Univariate OS analysis	
		***P*-value**	**PFS (months)**		***P*-value**	**OS (months)**
MGMT promoter	meth vs. not meth	0.010	12 vs. 8	meth vs. not meth	0.030	21 vs. 13
EOR	CR vs. PR / B	0.458	10 vs. 8	CR vs. PR / B	0.127	22 vs. 13
Age	≤ 65 vs. > 65 years	0.120	10 vs. 8	≤ 65 vs. > 65 years	0.174	16 vs. 15
TBR_{mean} at baseline	1.9	0.173	11 vs. 7	1.8	0.557	22 vs. 14
TBR_{max} at baseline	2.0	0.004	11 vs. 6	1.9	0.328	17 vs. 12
MTV at baseline	28.2 mL	< 0.001	11 vs. 6	13.8 mL	0.010	22 vs. 12

B = biopsy; **CR** = complete resection; **EOR** = extent of resection; **meth** = MGMT promoter methylated; **MGMT** = O^6-methylguanine-DNA methyltransferase; **MTV** = metabolic tumor volume; **OS** = overall survival; **PFS** = progression-free survival; **PR** = partial resection; **TBR_{max}, TBR_{mean}** = maximum and mean tumor-to-brain ratio

Table 3: Univariate survival analysis regarding changes of imaging parameters during adjuvant temozolomide therapy

Parameter	Threshold	Univariate PFS analysis		Univariate OS analysis	
		***P*-value**	**PFS (months)**	***P*-value**	**OS (months)**
RANO criteria	SD / PR / CR vs. PD	0.618	9 vs.10	0.752	16 vs.17
TBR_{mean} change	≤ 0% vs. > 0%	0.217	10 vs. 8	0.328	17 vs. 14
TBR_{max} change	≤ 0% vs. > 0%	0.031	11 vs. 8	0.032	24 vs. 12
MTV change	≤ 0% vs. > 0%	0.007	11 vs. 8	0.005	29 vs. 12

CR = "Complete Response" according to RANO criteria; **MTV** = metabolic tumor volume; **OS** = overall survival; **PD** = "Progressive Disease" according to RANO criteria; **PFS** = progression-free survival; **PR** = "Partial Response" according to RANO criteria; **SD** = "Stable Disease" according to RANO criteria; **TBR_{max}, TBR_{mean}** = maximum and mean tumor-to-brain ratio

Table 4: Multivariate Survival Analysis of changes of ^{18}F-FET PET imaging parameters

Parameter	Multivariate PFS analysis			Multivariate OS analysis		
	Hazard ratio	**95% confidence interval**	***P*-value**	**Hazard ratio**	**95% confidence interval**	***P*-value**
TBR_{max} change	2.920	1.272 - 6.705	0.012*	2.660	1.144 - 6.189	0.023*
MTV change	1.925	0.842 - 4.404	0.121**	3.614	1.481 - 8.820	0.005**

MTV = metabolic tumor volume; **OS** = overall survival; **PFS** = progression-free survival; **PR** = partial response / partial resection; **TBR_{max}** = maximum tumor-to-brain ratio; * = compared to age, EOR, MGMT promoter methylation status, and TBR_{max} baseline; ** = compared to age, EOR, MGMT promoter methylation status, and MTV baseline