Prediction of response to lomustine-based chemotherapy in glioma patients at recurrence using MRI and FET PET

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ABSTRACT

Background: We evaluated *O*-(2-[^{18}F]fluoroethyl)-L-tyrosine (FET) PET and MRI for early response assessment in recurrent glioma patients treated with lomustine-based chemotherapy.

Methods: Thirty-six adult patients with WHO CNS grade 3 or 4 gliomas (glioblastoma, 69%) at recurrence (median number of recurrences, 1; range, 1-3) were retrospectively identified. Besides MRI, serial FET PET scans were performed at baseline and early after chemotherapy initiation (not later than two cycles). Tumor-to-brain ratios (TBR), metabolic tumor volumes (MTV), the occurrence of new distant hotspots with a mean TBR >1.6 at follow-up, and the dynamic parameter time-to-peak were derived from all FET PET scans. PET parameter thresholds were defined using ROC analyses to predict PFS of ≥6 months and OS of ≥12 months. MRI response assessment was based on RANO criteria. The predictive values of FET PET parameters and RANO criteria were subsequently evaluated using univariate and multivariate survival estimates.

Results: After treatment initiation, the median follow-up time was 11 months (range, 3-71 months). Relative changes of TBR, MTV, and RANO criteria predicted a significantly longer PFS (all P≤0.002) and OS (all P≤0.045). At follow-up, the occurrence of new distant hotspots (n≥1) predicted a worse outcome, with significantly shorter PFS (P=0.005) and OS (P<0.001). Time-to-peak changes did not predict a significantly longer survival. Multivariate survival analyses revealed that new distant hotspots at follow-up FET PET were most potent in predicting non-response (P<0.001; HR, 8.578).

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Conclusions: Data suggest that FET PET provides complementary information to RANO criteria for response evaluation of lomustine-based chemotherapy early after treatment initiation.

KEYWORDS

Glioblastoma; nitrosourea; CCNU; amino acid PET

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KEY POINTS

- Both RANO criteria and FET PET metrics allow the prediction of a significantly longer survival time
- In direct comparison with FET PET metrics, response based on RANO criteria appeared to especially predict a longer OS
- The occurrence of distant and metabolically active hotspots on FET PET during lomustine-based chemotherapy proved to be the strongest predictor for non-response
- Information of both imaging modalities may improve clinical decision-making (e.g., dis- or continuation of nitrosourea-based chemotherapy)

IMPORTANCE OF THE STUDY

In glioma patients at recurrence, lomustine-based chemotherapy represents the standard care especially in the most countries of Europe, where bevacizumab is not approved. Currently, changes in conventional contrast-enhanced MRI during follow-up according to the Response Assessment in Neuro-Oncology (RANO) criteria is frequently used for response assessment. However, in randomized clinical trials with recurrent glioblastoma patients using a lomustine control arm, objective response rates, including complete and partial responses, are only around 10%. Here, we showed that RANO criteria and FET PET metrics provide complementary information for the evaluation of response to lomustine-based chemotherapy. On one hand, response based on RANO criteria appeared to especially predict a longer overall survival than FET PET imaging changes. On the other hand, the occurrence of new distant FET PET hotspots at follow-up were most potent in predicting non-response. Therefore, this may help to improve clinical decision-making (e.g., dis- or continuation of nitrosourea-based chemotherapy) since the nitrosourea lomustine is frequently associated with significant adverse events such as hematological toxicity.

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INTRODUCTION

In glioma patients at recurrence, lomustine chemotherapy represents the standard care especially in Europe, where bevacizumab is not approved (except Switzerland). Lomustine is applied either as monotherapy or in combination with procarbazine and vincristine (PCV regimen) or with procarbazine only (PC regimen) [1]. In the current guideline of the European Association of Neuro-Oncology (EANO), lomustine is recommended for use in patients with glioblastoma and WHO CNS grade 3 astrocytoma at recurrence [2]. Furthermore, lomustine is probably the critical component of the PCV regimen, which has become the standard of care for newly diagnosed patients with IDH-mutated glioma of the WHO CNS grade 2 [3].

Of note, in clinical trials using lomustine as a control arm, the progression-free survival (PFS) rate at six months was only in the range of 20%, and the overall survival (OS) ranged from 6-9 months [4]. Besides, the nitrosourea lomustine is frequently associated with significant adverse events, most frequently with hematological toxicity, e.g., thrombocytopenia. For example, in the REGOMA trial, the grade 3 or 4 hematological toxicity rate was 38% in the lomustine control arm [5].

For these reasons, assessing the response early after lomustine chemotherapy initiation is of considerable clinical importance for further treatment decisions (e.g., the continuation of lomustine). Currently, contrast-enhanced conventional MRI is the method of choice for response assessment. Precisely, changes in contrast enhancement are frequently used to assess treatment response according to the criteria defined by the Response Assessment in Neuro-Oncology (RANO) Working Group [6]. However, in randomized clinical trials with recurrent glioblastoma patients using a lomustine control arm, objective response rates, including complete and

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partial responses, are only in the range of 10% [4]. Thus, further information on treatment effects derived from other imaging modalities is desirable.

In addition to conventional MRI, PET imaging using the radiolabeled amino acid O-(2-[^{18}F]fluoroethyl)-L-tyrosine (FET) provides valuable clinical information for treatment response assessment in glioma patients in both the newly diagnosed and recurrence setting [7]. The biological mechanism responsible for FET uptake within gliomas is obtained by specific amino acid transporters especially belonging to the system of L-type amino acid transporters (LAT), particularly the subtypes LAT1 and LAT2 [8,9]. An experimental study suggested that the overexpression of LAT1 considerably facilitates the influx of FET in glioma cells [10]. On the other side, FET seems to be a poor efflux substrate of LAT1. Consequently, the entrapment of FET in glioma cells is most probably related to this asymmetry [10].

Its added clinical value has been demonstrated for various treatment regimens, including radiotherapy with concomitant temozolomide [11], adjuvant temozolomide [12], bevacizumab-based therapies [13-15], or experimental therapies, e.g., regorafenib [16]. A recent study suggested that FET PET is also valuable in assessing response to immunotherapy using checkpoint inhibitors in patients with brain metastases [17]. Importantly, irrespective of the treatment regimen, metabolic responders identified using FET PET had a significantly longer PFS [11-13,17].

Except for an initial study [18], data on the FET PET value for evaluating response to lomustine-based chemotherapy remain scarce. Therefore, we evaluated the response to lomustine-based chemotherapy using FET PET compared to contrast-

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enhanced MRI in patients with WHO CNS grade 3 or 4 gliomas at recurrence to identify the optimal parameter to predict an early response.

PATIENTS AND METHODS

Patients

From 2015-2021, we retrospectively identified patients with histomolecularly characterized glioma who (i) had completed at least one line of pretreatment including resection, radiotherapy, alkylating chemotherapy, or combinations thereof, (ii) had progressive MRI findings according to the RANO criteria [6], (iii) were treated with a lomustine-based chemotherapy, and (iv) underwent serial MR and FET PET imaging for response assessment (i.e., at baseline and after the second cycle, or in the case of clinical disease progression and/or early progression on MRI before the second cycle).

Lomustine-based chemotherapy consisted of lomustine as monotherapy (90-110 mg/m^2 body surface area on day 1 of a 42-day cycle) or the combination of lomustine with procarbazine (PC regimen; lomustine 90-110 mg/m^2 body surface area on day 1, and procarbazine 60 mg/m^2 body surface area on days 8-21 of a 56-day cycle). FET PET was performed not later than 7-10 days after MR imaging and not later than seven days before treatment initiation.

The local ethics committee approved the retrospective analysis of neuroimaging data. There was no conflict with the Declaration of Helsinki. Before PET imaging, all patients had given written informed consent for the PET investigation and data usage for scientific purposes.

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Follow-Up

Patients were assessed clinically by neurological examination and the Karnofsky Performance Score at baseline, every 8-12 weeks during the treatment, and every 8-12 weeks after treatment completion. After the last FET PET scan, a contrast-enhanced conventional MRI was performed every 8-12 weeks. The PFS was defined as the time interval between initiation of lomustine-based chemotherapy and tumor progression with clinical deterioration and MRI findings consistent with *Progressive Disease* according to RANO criteria [6]. The latter situation prompted discontinuation of lomustine-based chemotherapy. The OS was defined as the time interval between the initiation of lomustine-based chemotherapy and death.

Conventional MR Imaging

Following the International Standardized Brain Tumor Imaging Protocol (BTIP) [19], 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 imaging protocol comprised 3D isovoxels acquired in 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 by an experienced neuroradiologist (C.K.) according to the RANO criteria [6]. The criteria for *Stable Disease*, *Partial Response*, and *Complete Response* were considered as response to lomustine-based chemotherapy.

FET PET Imaging

As described previously, the amino acid 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% [20]. According to

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international guidelines for brain tumor imaging using radiolabeled amino acid analogues [21], patients fasted for at least four hours before the PET measurements. All patients underwent a dynamic PET scan from 0 to 50 minutes after injection of 3 MBq of FET per kg of body weight at baseline (within seven days before starting lomustine-based chemotherapy) and after the second cycle of lomustine-based chemotherapy. 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=8 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 [22]. Iterative reconstruction parameters were 16 subsets, 6 iterations using the OSEM algorithm for the ECAT HR+ PET scanner and two subsets, and 32 iterations using the OPOSEM 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 [22]. 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 [23].

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FET PET Data Analysis

For evaluating FET data, summed PET images from 20-40 minutes after injection were used. FET metabolic tumor volumes (MTV) and mean tumoral FET uptake were determined by a three-dimensional auto-contouring process using a threshold of 1.6 using the software PMOD (Version 4.3, PMOD Technologies Ltd.). This cut-off was based on a biopsy-controlled study in glioma patients and differentiated best between tumoral and peritumoral tissue [24]. Using a regions-of-interest (ROI) analysis, maximum and mean tumor-to-brain ratios (TBR_{max}, TBR_{mean}) 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 [21].

To evaluate new hotspot regions at follow-up, every lesion with a TBR_{mean} > 1.6 without spatial connection to (distant to) the tumor ROI at baseline was defined as a hotspot.

As described previously [25], time-activity curves (TACs) of FET uptake (mean SUV) in the tumor were generated by the application of a spherical volume-of-interest (VOI) with a volume of 2 mL centered on the voxel with the maximum tumor uptake and the reference ROI as described above to the entire dynamic dataset. A reference TAC was generated by placing a reference ROI in the unaffected brain tissue as reported [25]. For TAC evaluation, the time-to-peak (TTP; time in minutes from the beginning of the dynamic acquisition up to the maximum SUV of the lesion) was determined [25]. In cases with steadily increasing FET uptake without identifiable peak uptake, we defined the end of the dynamic PET acquisition as TTP.

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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 2021 [26]. 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. To assess 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 [27,28]. If immunostaining for IDH1-R132H remained negative, the mutational hotspots at codon 132 of IDH1 and codon 172 of IDH2 were directly sequenced as reported [29,30]. The MGMT promoter methylation status was assessed by methylation-specific PCR, as described elsewhere [30].

Statistical Analyses

Descriptive statistics are provided as mean and standard deviation 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 was calculated using 2x2 contingency tables; The Pearson's chi-squared test determined statistical significance.

The values of relative changes of the PET parameters TBR_{max}, TBR_{mean}, MTV, and TTP to predict a significantly longer PFS and OS as an indicator for response to lomustine-based chemotherapy were assessed by receiver operating characteristic (ROC) curve analyses using a favorable PFS and OS as reference. A favorable

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outcome was defined as a PFS ≥ 6 months and an OS ≥ 12 months. These thresholds were adopted from studies investigating lomustine and other agents in patients with recurrent glioblastoma [4,31,32]. 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.

Univariate survival analyses were performed using Kaplan-Meier estimates. The log-rank test was used to compare the median PFS and OS between the subgroups. Patients were censored if the event (tumor progression or death) had not occurred at the time of data evaluation (March 2022). 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 FET PET parameters and other decisive prognostic and predictive factors (i.e., RANO criteria, the extent of resection, MGMT promoter methylation, and IDH mutation status) for a favorable survival as an indicator for response to lomustine-based chemotherapy. This analysis was performed for each FET PET imaging parameter separately. 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 28.0.1.0, SPSS Inc., Chicago, IL, USA).

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RESULTS

Patients

According to the search criteria, we identified 36 adult patients (mean age, 54 ± 13 years; age range, 21 - 82 years; 16 females) with WHO CNS grade 3 or 4 gliomas at recurrence (glioblastoma, 69%) and a Karnofsky performance status ≥ 70%. Initial glioma diagnoses were distributed as follows: WHO CNS grade 4 glioblastoma, IDH-wildtype, n=25; WHO CNS grade 4 H3 K27-mutant diffuse midline glioma, n=1; WHO CNS grade 4 astrocytoma, IDH-mutant, n=2; WHO CNS grade 3 astrocytoma, IDH-mutant, n=2; WHO CNS grade 3 oligodendroglioma, IDH-mutant, 1p/19q co-deletion, n=6. Eighteen patients (50%) had a methylated MGMT promoter. The median number of recurrences before initiating lomustine-based chemotherapy was 1 (range, 1-3). Most patients (n=25; 69%) were treated with lomustine-based chemotherapy at first recurrence. 33% of the patients (n=12) were treated with lomustine monotherapy, and 68% (n=24) with the PC regimen. The rate of patients with two and three recurrences was 25% (n=9) and 6% (n=2), respectively. Further details on demographics, neuropathological diagnoses, pretreatment, clinical follow-up parameters, and the patients' survival are listed in Supplemental Tables 1 and 2.

All 36 patients completed FET PET and MR imaging at baseline and follow-up. Static and dynamic FET PET parameters at baseline and after two cycles of lomustine-based chemotherapy and their relative changes are listed in Supplemental Table 3. At the time of data evaluation, tumor progression had occurred in 32 patients (89%) and death in 31 patients (86%). The median PFS after initiation of lomustine-based chemotherapy was three months (range, 1-71 months), and the median OS was 11 months (range, 3-71 months).

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Imaging Changes Following Lomustine-based Chemotherapy

According to RANO criteria, two patients (6%) had a *Partial Response*, in 13 patients (36%) MRI findings were consistent with *Stable Disease*, and 21 patients (58%) had a *Progressive Disease* (Supplemental Table 3). None of the patients had a *Complete Response* on MRI. In patients with *Partial Response* or *Progressive Disease* on MRI, FET PET findings (i.e., TBR_{max} or MTV changes) were highly congruent (100% and 91%, respectively).

Notably, in three patients with *Stable Disease* on MRI after two cycles of lomustine-based chemotherapy and a favorable OS of ≥ 12 months (patients #17, #28, #29), the metabolic activity on FET PET, as assessed by TBR_{max}, decreased in the range of 6-21% (Figure 1). In 2 of 21 patients with *Progressive Disease* on MRI (patients #26 and #35), FET PET findings were discrepant. In these patients, reductions either of TBR_{max} or MTV after two cycles of lomustine-based chemotherapy were associated with an OS of ≥ 12 months (Figure 1). In two further patients (patients #1, #22) with a relative increase of TBR_{max} and an OS < 12 months, MRI changes were consistent with *Stable Disease* (Figure 1).

Univariate Survival Analysis Regarding Changes in Imaging Parameters During Lomustine-based Chemotherapy

The results of the ROC analyses regarding changes in FET PET parameters during lomustine-based chemotherapy for predicting a favorable PFS of ≥ 6 months or an OS of ≥ 12 months are presented in Supplemental Tables 4 and 5. Relative changes in static FET PET parameters TBR_{mean} (6.0 vs. 2.9 months; $P = 0.002$), TBR_{max} (6.5 vs. 2.6 months; $P < 0.001$), and MTV (6.5 vs. 2.6 months; $P < 0.001$) predicted a significantly longer PFS (Supplemental Table 6; Figures 2 and 3). Relative changes

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in TBR_{mean}, TBR_{max}, and MTV after two cycles of lomustine-based chemotherapy also predicted a significantly longer OS (TBR_{mean}, 17.1 vs. 9.7 months; *P* = 0.035; TBR_{max}, 13.7 vs. 9.1 months; *P* = 0.015; and MTV, 12.8 vs. 8.8 months; *P* = 0.045) (Supplemental Table 6; Figures 2 and 3). Changes in the dynamic FET PET parameter TTP were not significant regarding the prediction of both PFS and OS. Furthermore, the occurrence of any new FET hotspot at follow-up predicted both a shorter PFS (2.1 vs. 4.0 months; *P* = 0.005) and OS (6.8 vs. 13.1 months; *P* < 0.001) (Supplemental Table 6; Figures 2 and 3).

MRI changes according to RANO criteria (i.e., MRI findings consistent with *Stable Disease, Partial Response*, or *Complete Response* compared to *Progressive Disease*) predicted a significantly longer PFS and OS (PFS, 8.0 vs. 2.9 months; *P* < 0.001; and OS, 26.5 vs. 8.8 months; *P* = 0.002) (Supplemental Table 6; Figures 3 and 4).

In the univariate survival analysis for the subgroup of patients with IDH-wildtype glioblastoma, changes of static FET PET parameters (i.e., TBR_{max}, TBR_{mean}, MTV), the dynamic FET PET parameter TTP, and MRI changes according to RANO criteria predicted a significant longer PFS. In addition, RANO criteria and the absence of new hotspots at follow-up on FET PET predicted a significantly longer OS (*P* = 0.013 and *P* = 0.001, respectively) (Supplemental Table 7).

Multivariate Survival Analysis Regarding Changes in Imaging Parameters During Lomustine-based Chemotherapy

Relative changes in the static FET PET parameters TBR_{max} and MTV predicted a significantly longer PFS (*P* = 0.029 and *P* = 0.001, respectively) independent of the extent of resection, MGMT promoter methylation, IDH mutation status, and MRI

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changes according to RANO criteria (Table 4). In contrast, none of the static FET PET parameters were independent predictors of OS ($P > 0.05$). On the other hand, the absence of any new FET hotspot after two cycles of lomustine-based chemotherapy predicted a significantly longer PFS ($P = 0.019$) and OS ($P < 0.001$) and was the most significant parameter independent of the extent of resection, MGMT promoter methylation, IDH mutation status, and MRI changes according to RANO criteria. MRI changes according to RANO criteria were significant predictors for both PFS ($P = 0.002$) and OS ($P = 0.013$) independent of the extent of resection, MGMT promoter methylation, IDH mutation status. Supplemental Table 8 provides a summary of all results of the multivariate analysis.

In the multivariate survival analysis for the subgroup of patients with IDH-wildtype glioblastoma only, the absence of any new FET hotspot after two cycles of lomustine-based chemotherapy also predicted a significantly longer OS ($P = 0.005$) and was the most significant parameter (RANO criteria, $P = 0.034$) (Supplemental Table 9).

DISCUSSION

One main finding of the present study is that metabolic changes of imaging parameters derived from FET PET early after initiation of lomustine-based chemotherapy for treating patients with recurrent gliomas of the WHO CNS grades 3 or 4 seem to be of clinical value in predicting the patient's response to therapy and outcome. In particular, relative reductions of static FET PET parameters such as the MTV or TBR_{max} could identify metabolic responders with a significantly longer PFS and OS than non-responders (Figures 2 and 5). Additionally, the occurrence of new distant hotspots on FET PET after initiation of lomustine-based chemotherapy had the highest significance level in predicting non-response and an unfavorable

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outcome. Moreover, these FET PET parameters' predictive values appear to be independent of other strong prognostic and predictive factors such as the extent of resection at initial glioma diagnosis, MGMT promoter methylation status, IDH mutation status, and RANO criteria.

In direct comparison with FET PET metrics, response based on RANO criteria appeared to especially predict a longer OS. On the other hand, the occurrence of new distant FET PET hotspots at follow-up were most potent in predicting non-response. Thus, RANO criteria and FET PET metrics provide complementary information for the evaluation of response to lomustine-based chemotherapy (Figures 3 and 6) and may help to improve clinical decision-making (e.g., dis- or continuation of nitrosourea-based chemotherapy) since the nitrosourea lomustine is frequently associated with significant adverse events such as hematological toxicity.

Similar findings were reported in studies evaluating FET PET for assessing response to alkylating chemotherapy (predominantly temozolomide) in patients with non-enhancing gliomas, usually with a WHO CNS grade 2 [18,33,34]. The main finding of these studies is that a more substantial reduction of the MTV (range of decrease, 10-25%) predicted a significantly longer survival or a favorable clinical course, i.e., an improvement of seizure control [34]. In contrast, our study shows that an unchanged MTV at follow-up after start of lomustine-based chemotherapy was already associated with a prolonged PFS and OS. Reasons for that remain unclear and are most probably related to various factors such as a different metabolic behavior on amino acid PET of the treated WHO CNS grade 2 gliomas [35,36], the applied chemotherapy regimen, and the disease stage (i.e., newly diagnosed tumor or at recurrence). Furthermore, in two of these studies [18,34], response evaluation using

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MRI according to the RANO criteria for low-grade gliomas [37] was not significant in predicting a significantly longer PFS or OS.

The value and robustness of relative changes of the FET PET-derived parameters MTV and TBR_{max} for response assessment have already been shown for radiotherapy with concomitant temozolomide chemotherapy [11], adjuvant temozolomide maintenance chemotherapy [12], and bevacizumab plus lomustine chemotherapy [13]. However, in these studies, mainly performed in newly diagnosed glioblastoma patients, MRI response to treatment according to RANO criteria for high-grade gliomas was not significant in predicting either PFS or OS [6]. Even a lesser reduction of the contrast enhancement in one study (i.e., ≤ -25%) could not predict a significantly longer survival [11].

In contrast to these studies, response assessment according to RANO criteria for high-grade gliomas [6] proved to be a strong predictor in the present study although only 6% of the patients had objective responses (two patients had a *Partial Response*, none of the patients had a *Complete Response*). In contrast, in more than the half of the patients (4 of 7 patients; 57%) with *Stable Disease* on MRI after two cycles of lomustine-based chemotherapy and a favorable OS of ≥ 12 months, the metabolic activity on FET PET decreased considerably, thereby allowing a more objective response assessment. In addition, two patients with an OS of ≥ 12 months had *Progressive Disease* on MRI, whereas the metabolic activity on FET PET decreased. Importantly, that more intuitive reduction of metabolic activity as an additional sign of response - already on a purely visual level - may improve clinical decision-making.

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A possible explanation for the predictive value of RANO criteria is that radiotherapy was not part of the treatment for the patients at recurrence included in the present study. In these patients, pretreatment with radiotherapy was already performed at least six months before the recurrence. Thus, radiotherapy-induced changes such as pseudoprogression, which typically occurs weeks within the first 12 weeks after radiotherapy completion [38] and considerably hampers MRI-based response assessment, were less likely to occur.

Another notable finding of this study is the outstanding predictive value of new hotspots on FET PET following lomustine-based chemotherapy for an unfavorable PFS and OS, highlighting its value for treatment response evaluation, particularly by identifying non-responders (Figure 6). Notably, new hotspot occurrence remained the most significant parameter in the multivariate analysis. To our knowledge, the predictive value of new distant hotspots on FET PET has not been reported yet. Of note, most patients with glioblastoma at initial diagnosis have MRI-defined local disease and maintain this pattern notwithstanding multiple recurrences irrespective of the applied treatment [39,40]. Thus, the occurrence of distant new hotspots on FET PET during treatment may help diagnose disease progression earlier.

Potential limitations of our study might be the retrospective character and treatment heterogeneity, i.e., treatment with lomustine monotherapy or the PC regimen. Further multicenter trials, especially in a prospective setting to evaluate chemotherapy effects in glioma patients, are warranted to validate our data.

In summary, FET PET-derived imaging parameters provide complementary information to RANO criteria to assess the response to lomustine-based chemotherapy in glioma patients at recurrence and may predict the patients' outcome

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early after treatment initiation. RANO criteria and relative changes in static FET PET parameters such as MTV and TBR_{max} can be used to identify responders, whereas the occurrence of new hotspots on FET PET following lomustine-based chemotherapy strongly correlates with non-response. An important next step to confirm our initial results on FET PET for response assessment of lomustine-based chemotherapy in a higher number of patients, ideally in a prospective setting.

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FIGURE LEGENDS

Figure 1: Waterfall plot of responses based on changes of the static FET uptake parameter maximum tumor-to-brain ratio (TBR_{max}) in relation to MRI responses according to RANO criteria. Relative changes of TBR_{max} after two cycles of lomustine-based chemotherapy are plotted on the y-axis. In relation to the individual metabolic response on FET PET, patient columns on the x-axis are color-coded assigned to the respective MRI changes according to RANO criteria (i.e., green = *Partial Response*; blue = *Stable Disease*; orange = *Progressive Disease*). Basically, the most patients with *Progressive disease* on MRI showed an increase in TBR_{max}, and those with *Partial response* had a decrease in TBR_{max}. Notably, considerable discrepancies, i.e., increasing metabolic activity on FET PET and unchanged MRI (e.g., patients #1, #22), and decreasing metabolic activity and stable (e.g., patients #17, #28, #29) or progressive MRI changes (patients #26 and #35) could be observed.

Figure 2: Kaplan-Meier curves for PFS and OS separated by relative changes of the maximum tumor-to-brain ratio (TBR_{max}) (top row), metabolic tumor volume (MTV) (middle row), and the of occurrence of new hotspots (bottom row) on FET PET after two cycles of lomustine-based chemotherapy. Responders on FET PET defined by any decrease or an unchanged TBR_{max} and/or MTV at follow-up compared to baseline had a significantly longer PFS (both 6.5 vs. 2.6 months; $P < 0.001$) and OS (TBR_{max}, 13.7 vs. 9.1 months; $P = 0.015$; MTV, 12.8 vs. 8.8 months; $P = 0.045$) than non-responders (i.e., patients with an increase of TBR_{max} and/or MTV at follow-up compared to baseline). Non-responders on FET PET defined by the occurrence of any new distant hotspot at follow-up compared to baseline had a significantly shorter

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PFS (2.1 vs. 4.0 months, $P = 0.005$) and OS (6.8 vs. 13.1 months, $P < 0.001$) than patients without any new distant hotspots at follow-up.

Figure 3: Swimmer plots of each individual patient after initiation of lomustine-based chemotherapy. Patient bars are sorted by the OS and are color-coded based on changes in RANO criteria (top row), maximum tumor-to-brain ratio (TBR_{max}) (middle row), and the occurrence of new hot spots on FET PET (bottom row). Of note, the occurence of new hotspots are present only in patients with an OS < 12 months, indicating its high positive predictive value for OS.

Figure 4: Kaplan-Meier curves for PFS and OS separated by MRI changes according to RANO criteria after two cycles of lomustine-based chemotherapy. Responders (i.e., MRI changes consistent with *Complete/Partial Response* or *Stable Disease* according to RANO criteria) had a significantly longer PFS (8.0 vs. 2.9 months; $P < 0.001$) and OS (26.5 vs. 8.8 months; $P = 0.002$) than non-responders (i.e., *Progressive Disease* according to RANO criteria).

Figure 5: Contrast-enhanced MRI and FET PET of a 31-years-old female patient (patient #29) with an astrocytoma (IDH-mutant, WHO CNS grade 3, MGMT promoter methylated) treated with lomustine-based chemotherapy. After two cycles, the contrast-enhancing lesion progressed slightly. In contrast, the follow-up FET PET showed, relative to the baseline scan, a considerably decreased metabolic activity of 21% as assessed by a relative reduction of maximum tumor-to-brain ratios (baseline, 4.3; follow-up, 3.4) and indicated metabolic response. Without clinical deterioration or

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treatment change, the patient had a favorable PFS and OS (both not reached; 71.8 months at the time of data evaluation).

Figure 6: Patient with a right parietal glioblastoma with an unfavorable survival (patient #22). After two cycles of lomustine chemotherapy, the contrast-enhancing lesion on MRI appeared unchanged (*Stable Disease* according to RANO criteria) compared to the baseline MRI scan. In contrast, the corresponding FET PET at follow-up showed, relative to the baseline scan, an increased maximum tumor-to-brain ratio (TBR_{max}) and metabolic tumor volume (MTV) (relative increase, 21% and 14%, respectively). Additionally, a new distant hotspot lesion at follow-up was observed in the left temporal lobe on FET PET but not MRI. The patient had an unfavorable outcome with a PFS of 1.7 months and an OS of 9.1 months.

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Figure 1

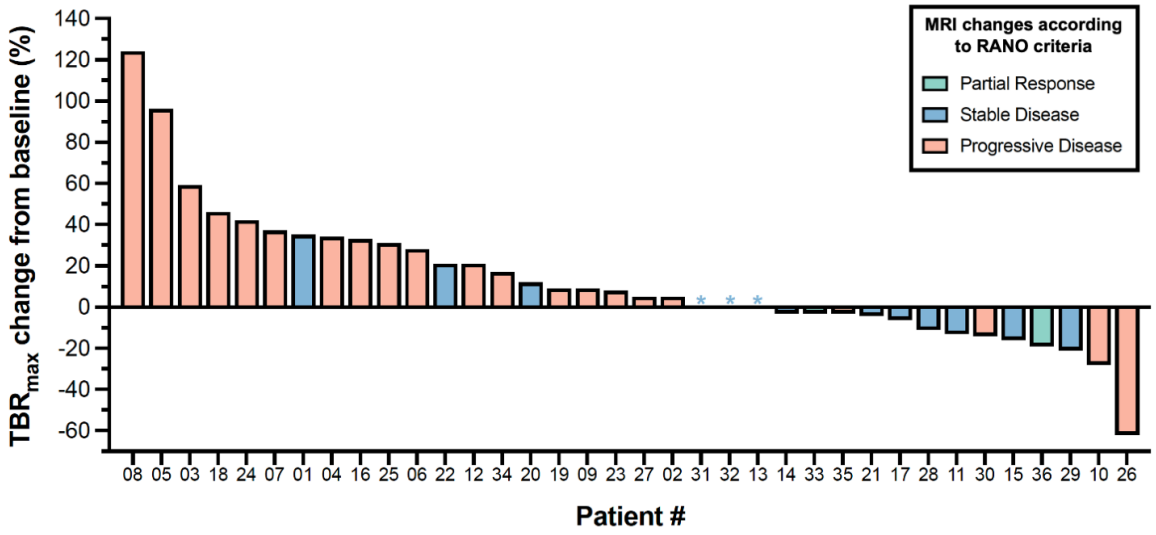

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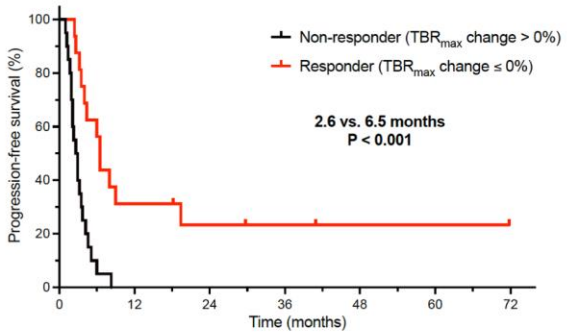
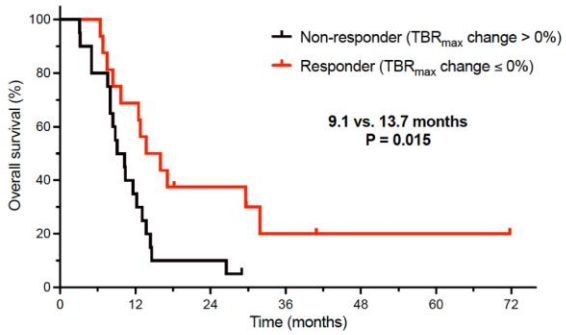
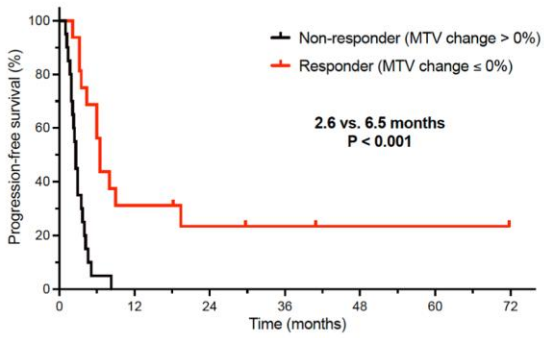
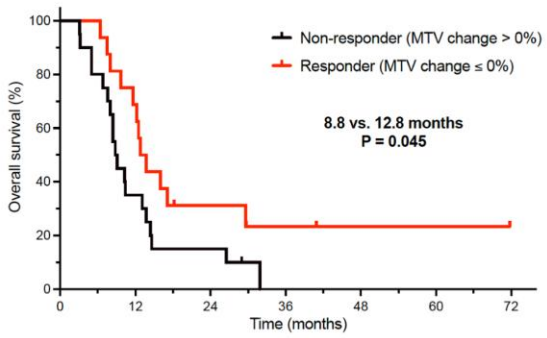
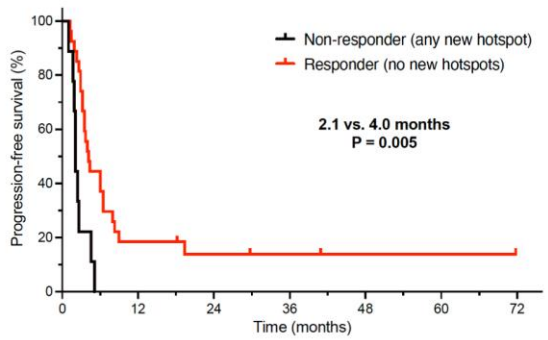
Figure 2











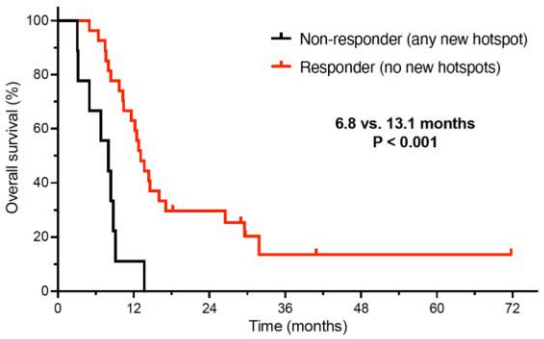


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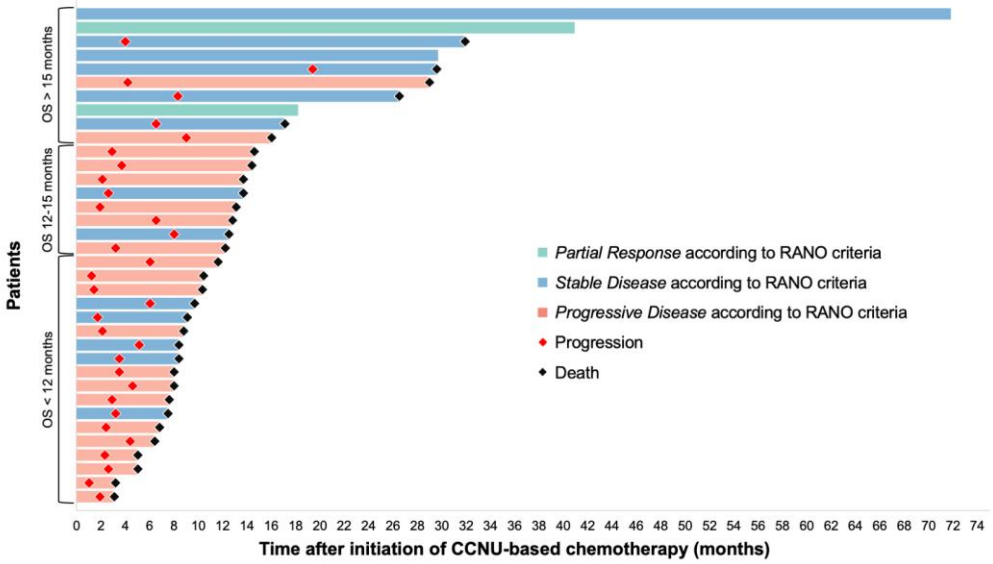
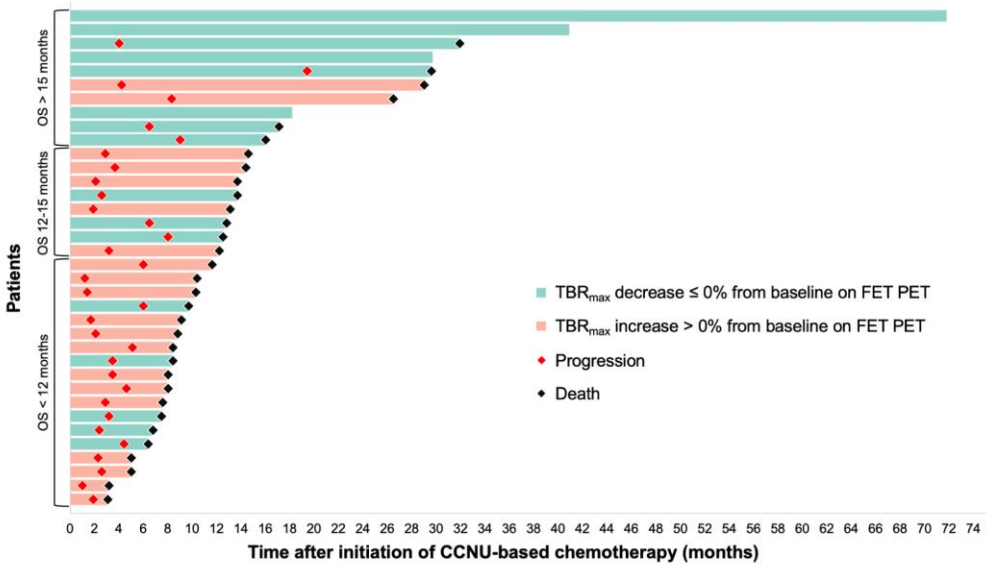
Figure 3





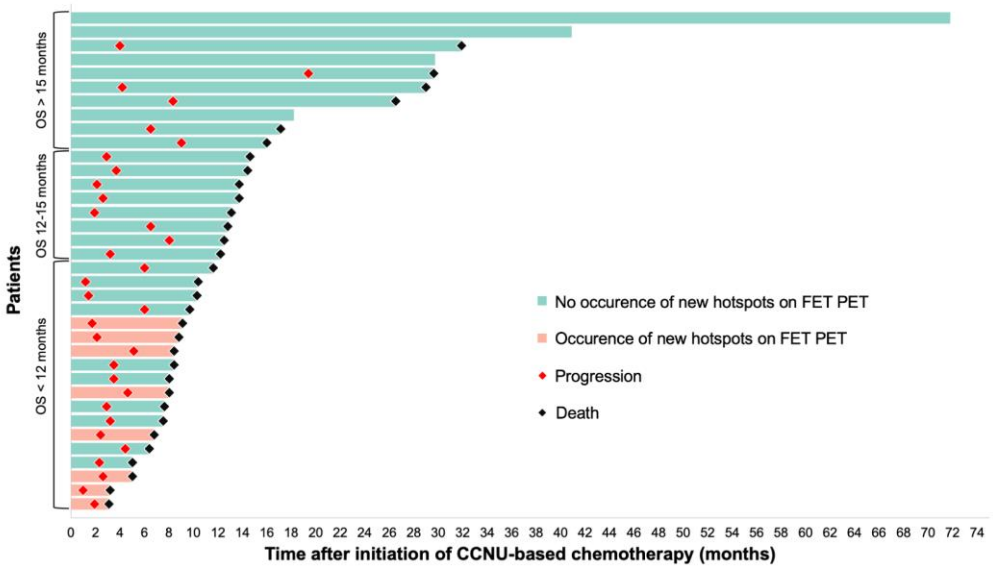


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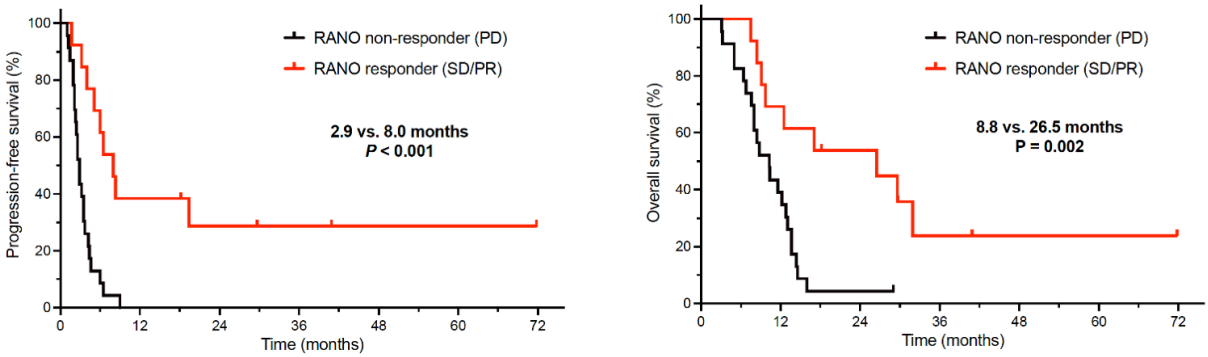
Figure 4



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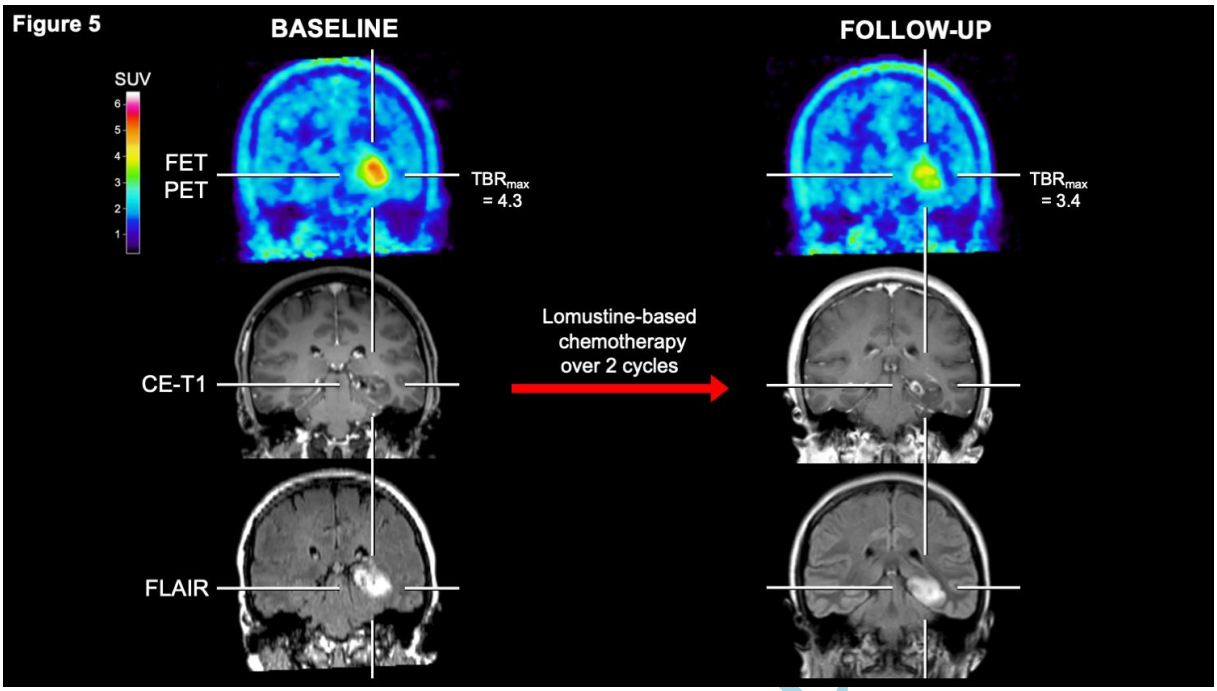

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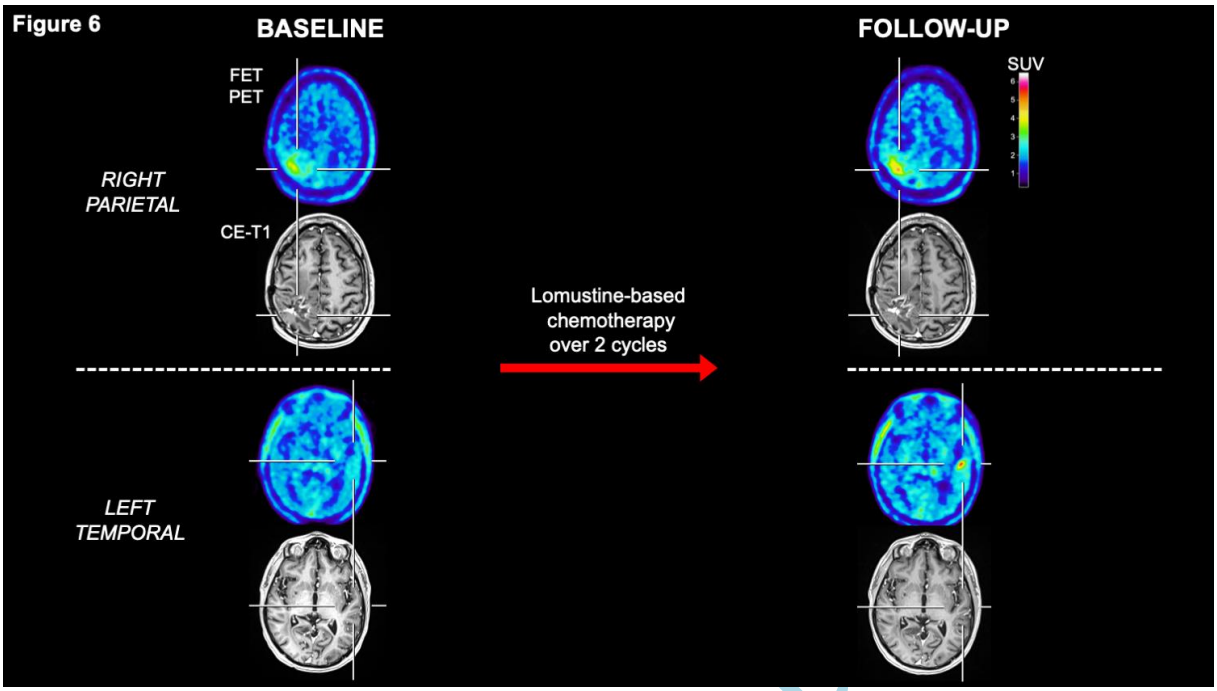

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