Assessment of response to regorafenib in patients with glioma relapse using FET PET and MRI

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ABSTRACT

Background: Neuroimaging markers predicting response to regorafenib in patients with glioma relapse remain scarce; we evaluated whether early changes in amino acid PET and MRI are associated with overall survival (OS).

Methods: Twenty adult patients with CNS WHO grade 3 or 4 gliomas at relapse (glioblastoma, 85%) were treated according to the REGOMA trial. Amino acid PET using the tracer *O*-(2-[^{18}F]fluoroethyl)-L-tyrosine (FET) and MRI were performed at baseline and after two cycles. From these imaging data, tumor-to-brain ratios (TBR), metabolic tumor volumes (MTV), the dynamic parameters time-to-peak and slope, and apparent diffusion coefficients (ADC) were obtained. Parameter thresholds to predict OS≥6 months as a surrogate for response were defined using ROC analyses. In addition, RANO criteria for MRI and PET were used to evaluate response. The association of imaging parameters with OS was evaluated using univariate and multivariate survival estimates.

Results: Patients received a median of three regorafenib cycles (range, 2-16 cycles). The median follow-up was 10.3 months (range, 3.2-27.6 months). A decline in mean TBR values by ≥10% was significantly associated with longer OS (10.4 vs. 5.3 months; *P*=0.027). Other FET PET parameters, RANO criteria for MRI and PET, and ADC values were not associated with OS (*P*>0.05). At follow-up, TBR_{mean} ≤2.0 was associated with longer OS (10.6 vs. 4.5 months; *P*=0.009). Multivariate survival analyses revealed that changes in mean TBR values were independently associated with longer OS (*P*=0.006; HR, 0.200) and a lower TBR_{mean} at follow-up were strongly prognostic (*P*<0.001; HR, 0.030).

Conclusion: FET PET parameters are clinically valuable for identifying responders to regorafenib early after treatment initiation.

KEYWORDS

brain tumor; multikinase inhibitor; diffusion-weighted imaging; metabolic response; multimodal imaging

INTRODUCTION

For patients with glioblastoma relapse, the prognosis remains devastating despite various treatment options, including surgery, re-irradiation, re-challenge using alkylating chemotherapy, antiangiogenic therapy, targeted therapies, other experimental approaches, and combinations thereof (*1*). In recent years, regorafenib, an orally available small-molecule multikinase inhibitor, has gained attention as a further treatment option. Regorafenib targets various molecular pathways involved in angiogenesis, oncogenesis, and maintenance of the tumoral microenvironment (*2,3*). A promising sign for efficacy of regorafenib has been provided by the randomized phase-2 REGOMA trial, showing a significant improvement in overall survival (OS) for patients with glioblastoma at first relapse compared to the control group treated with lomustine (7.4 vs. 5.6 months, *P*=0.0009; hazard ratio, 0.5) (*4*). Based on these results, regorafenib has been included in the treatment guidelines of the Italian Medicines Agency (AIFA). Although other monocentric studies suggested its efficacy in patients with glioblastoma relapse (*5-7*), the regorafenib arm in the phase-2/-3 GBM AGILE trial was halted after an interim analysis showed limited potential for significant OS improvement (*8*). Additionally, the occurrence of grade 3 or 4 adverse events associated with regorafenib warrants careful consideration in clinical practice. Consequently, there is an urgent need to identify patients who may respond to regorafenib shortly after treatment initiation. Although its effects on prolonging OS may seem modest, it is still unclear whether a subset of patients, also potentially identifiable by imaging biomarkers, might experience a more substantial positive effect.

For response assessment, it is recommended to evaluate changes in contrast enhancement on MRI following treatment using the Response Assessment in Neuro-Oncology (RANO) criteria (*9,10*). Notably, in the REGOMA trial, only 5% of the patients

showed complete or partial responses according to the RANO criteria for MRI, even though regorafenib improved OS (*4*). Therefore, predictive neuroimaging markers that identify patients who benefit from regorafenib are needed.

A growing body of literature suggests that serial amino acid PET provides valuable additional information for response assessment compared to anatomical MRI. In more detail, several studies reported that changes in parameters derived from amino acid PET following local (e.g., radiotherapy with concurrent alkylating chemotherapy) and systemic treatment options (e.g., alkylating and antiangiogenic agents, targeted therapies) predicted a significantly longer survival than in metabolic non-responders and even in MRI responders (*11-15*).

Up to now, only preliminary data suggest that PET using the tracer *O*-(2-[^{18}F]fluoroethyl)-L-tyrosine (FET) is helpful in identifying responders to regorafenib (*15-17*). Besides, diffusion-weighted imaging (DWI) and other MRI approaches have been investigated for the assessment of response to regorafenib (*16,18,19*).

To this end, we compared anatomical MRI, diffusion-weighted MRI, and FET PET to predict early response to regorafenib in patients with glioma relapse.

PATIENTS AND METHODS

Patients

We retrospectively identified patients referred to our site between 2019 and 2022 diagnosed with histomolecularly defined CNS WHO grade 3 and 4 gliomas according to the fifth edition of the WHO Classification of Tumors of the Central Nervous System(*20*) at relapse. Further search criteria were patients who had (i) completed at least one line of pretreatment including resection, radiotherapy, alkylating chemotherapy, or combinations thereof, (ii) radiologically confirmed tumor relapse according to the RANO criteria (*9,10*), (iii) undergone a regorafenib therapy, and (iv) undergone serial MR and FET PET imaging for response assessment (i.e., at baseline and after the second cycle). Patients were not stratified for age or sex. A patient inclusion and exclusion flow chart is provided in Supplemental Figure 1. The extent of resection was defined according to the residual tumor after resection using the RANO resect classifier (*21*).

All patients were treated with regorafenib outside of clinical trials, were previously discussed in our local interdisciplinary neurooncological tumor board, and had exhausted standard treatment options. Regorafenib was administered following the REGOMA trial, with 160 mg given once daily during the first three weeks of each four-week cycle, with individual dosage adjustments based on adverse effects (*4*). Median time between FET PET baseline and regorafenib treatment initiation was 2 weeks (range, 0-7 weeks).

The local ethics committee approved the retrospective neuroimaging data analysis. 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.

Follow-Up

Patients underwent clinical assessments, including neurological examinations and Karnofsky Performance Score evaluations at baseline and every 8-12 weeks. After the follow-up FET PET scan, contrast-enhanced conventional MRI scans were performed every 8-12 weeks. FET PET imaging was repeated if the advent of equivocal MRI findings prompted suspicion of treatment-related changes. Progression-free survival (PFS) was defined as the duration from the start of regorafenib to tumor progression, characterized by clinical deterioration and MRI findings consistent with *Progressive Disease* according to the RANO criteria (*9,10*). OS was defined as the duration from the initiation of regorafenib to death.

Anatomical MR Imaging Acquisition

Following the International Standardized Brain Tumor Imaging Protocol (*22*), MR imaging was conducted using either a 1.5 T or 3.0 T MRI scanner equipped with a standard head coil, both before and after the administration of a gadolinium-based contrast agent (0.1 mmol/kg body weight). The imaging protocol consisted of acquiring 3D isovoxel T1-weighted, 2D T2-weighted, and 2D fluid-attenuated inversion recovery sequences.

Diffusion-weighted MRI Acquisition and Parameter Determination

DWI was conducted using a 1.5 T Intera or 3.0 T Ingenia MRI system from Philips Healthcare (Best, The Netherlands). The protocol included b-values of 0 s/mm² and 1000 s/mm². Sequence details for the 1.5 T system are: 30 slices, a slice thickness of

5 mm, a field-of-view of 23 cm, an acquired matrix size of 112 x 90 pixels, and a reconstructed matrix size of 228 x 228 pixels. The sequence applied at 3.0 T consisted of 30 slices with a slice thickness of 5 mm, a field-of-view of 25 cm, an acquired matrix size of 168 x 111 pixels, and a reconstructed matrix size of 320 x 320 pixels. ADC maps were calculated using the vendor-provided software. The Picture Archiving and Communication System (IMPAX EE, Agfa Healthcare, Bonn, Germany) was used for data evaluation. Regions-of-interest (ROI) analyses were performed by two board-certified neuroradiologists (C.K. and L.G.) as reported previously (*23*). Two-dimensional ROI analyses were performed on T1-weighted post-contrast images corresponding to the entire measurable enhancing portion of the lesion on the section with maximum lesion extent suspicious of tumor relapse, excluding areas of necrosis or cysts. Subsequently, ROI were transferred to the coregistered ADC maps for the calculation of mean and minimum ADC values.

Next, a three-dimensional approach was applied to obtain more detailed and reliable insights into signal-reduced tumor regions in ADC maps. The two readers (C.K., L.G.) segmented all the ADC-reduced tumor components on each slice of the ADC map. For each segment, mean and minimum ADC values were determined. To compute the overall mean ADC value of all signal-reduced components, the mean ADC of each segment was weighted by its respective area. Intra- and interreader reliability were assessed by calculating the intraclass correlation coefficient (ICC). For the evaluation of ADC values, the mean of the two readers' measurements was used.

FET PET Acquisition

The amino acid tracer FET was produced and applied as described previously (*24*). 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 and after the second cycle of regorafenib. PET imaging was performed either on an ECAT Exact HR+ PET scanner in 3-dimensional mode (n=36 scans; Siemens, Erlangen, Germany) or simultaneously with 3T MR imaging using a BrainPET insert (n=4 scans; Siemens, Erlangen, Germany) (*25,26*). 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 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 it employed a template-based approach (*25*). The reconstructed dynamic data sets consisted of 16 time frames (5 x 1 minute; 5 x 3 minutes; 6 x 5 minutes) 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. This filter kernel demonstrated sufficient comparability between PET data obtained from the ECAT HR+ PET and the BrainPET scanner in phantom experiments using spheres of different sizes to simulate lesions (*27*).

Determination of FET PET Parameters

FET PET scans were evaluated following the current practice guidelines (*28*). In brief, summed PET images from 20-40 minutes post-injection were analyzed using the software PMOD (Version 4.3, PMOD Technologies Ltd., Switzerland). The mean reference tracer uptake was assessed using a crescent-shaped volume-of-interest positioned in the hemisphere contralateral to the lesion in healthy appearing brain tissue including grey and white matter. The metabolic tumor volume (MTV) was determined by a three-dimensional auto-contouring process using a tumor-to-brain

ratio (TBR) of 1.6 or more. This cutoff is based on a biopsy-controlled study of cerebral gliomas, in which a lesion-to-brain ratio of 1.6 best separated tumoral from peritumoral tissue (*29*). Maximum and mean TBR were calculated by dividing the maximum and mean uptake value of the tumor region by the mean uptake value of the reference region.

Time-activity curves of the mean FET uptake were generated by centering a spherical 2 mL volume-of-interest on the voxel with the maximum tracer uptake. To evaluate time-activity curves, the time-to-peak (TTP; time in minutes from the beginning of the dynamic acquisition up to the maximum uptake) was determined (*30*). In cases with steadily increasing FET uptake without identifiable peak uptake, the end of the dynamic PET acquisition was defined as TTP. The slope of the time-activity curves was assessed by fitting a linear regression line to the late phase of the curve (20-50 minutes post-injection). The slope was expressed as the standardized uptake value (SUV) change per hour.

Evaluation of MRI Patterns Following Regorafenib

As reported in an earlier study (*18*), we further evaluated the occurrence and the predictive value of a T2-dominant MRI pattern characterized by a considerable decrease of contrast enhancement combined with a simultaneous distinct increase in T2 hyperintensity as an imaging marker for response to regorafenib.

Data Analyses Including Statistics

Descriptive statistics are provided as mean and standard deviation or median and range. The Student’s t-test was used to compare the two groups. The Mann-Whitney

rank-sum test or Wilcoxon matched-pairs signed rank test were used when variables were not normally distributed.

Changes in MRI findings at the first follow-up compared to the baseline scan were evaluated by a board-certified neuroradiologist (C.K.) according to the RANO criteria (*9,10*). The criteria *Stable Disease*, *Partial Response*, and *Complete Response* were considered as the response to regorafenib. Changes in FET PET findings at follow-up compared to the baseline scan were evaluated according to the PET RANO 1.0 criteria (*31*). The criterion PET-based *Stable Disease*, PET-based *Partial Response*, and PET-based *Complete Response* were considered as response to regorafenib. The diagnostic performance of the RANO criteria for MRI and PET for predicting a favorable OS was calculated using 2x2 contingency tables with the Fisher's exact test to determine statistical significance. Based on a previously reported median survival of 6.2 months following regorafenib (*6*), a favorable outcome was defined as an OS ≥ 6 months. Besides, receiver operating characteristic (ROC) curve analyses were performed to define the decision cut-off values for static and dynamic FET PET and diffusion-weighted MRI parameters using favorable OS ≥ 6 months as reference. The decision cut-off was considered optimal at maximum product of paired values for sensitivity and specificity. The area under the ROC curve (AUC), its standard error, and level of significance were determined to measure the test's diagnostic quality. Univariate survival analyses were performed using Kaplan-Meier estimates and the log-rank test to compare the median OS between subgroups.

Univariate Cox proportional hazards models were constructed to test the association between FET PET parameters and other decisive prognostic and predictive factors for a favorable survival as an indicator for response to regorafenib. Hazard ratios (HR)

and their 95%-confidence intervals (CI) were calculated. For multivariate Cox models, a maximum of three degrees of freedom was prespecified to limit events-per-variable and overfitting (*32*). Among clinically plausible covariates, two variables with the strongest univariable signals were included together with the most robust FET PET parameter, yielding an events-per-variable-constrained, parsimonious model.

P-values of 0.05 or less were considered statistically significant. For comparison between baseline and follow-up imaging parameters, a correction for multiple testing was performed using the Holm-Šídák method. Given the hypothesis-generating design of the study, no correction for multiple testing was performed for ROC analyses (*33*). Statistical analyses were performed using GraphPad Prism (RRID:SCR_002798; release 10.3.0, GraphPad Software Inc., La Jolla, CA, USA). The ICC of ADC measurements were calculated using the software DATAtab (DATAtab e.U., Graz, Austria). Data from this project may be shared at reasonable request to the corresponding author.

RESULTS

Patients

The study included twenty patients (median age, 51 years; range, 30-72 years; 35% female sex) who met the search criteria and had CNS WHO grade 3 or 4 gliomas at relapse (glioblastoma, 85%). Initial diagnoses were distributed as follows: CNS WHO grade 4 glioblastoma, IDH-wildtype, n=17; CNS WHO grade 3 astrocytoma, IDH-mutant, n=2; CNS WHO grade 4 astrocytoma, IDH-mutant, n=1. The rate of gliomas with methylated *MGMT* promotor was 60%. The median number of relapses was 2 (range, 1-4). Most patients (n=13, 65%) had two or more pretreatment lines, and 7 patients (35%) were treated with regorafenib at first relapse. The median Karnofsky Performance Status score before initiation of regorafenib was 80% (range, 70-100%). The rate of patients receiving dexamethasone before initiation of regorafenib was 45% (range, 0-8 mg). The median dose of regorafenib was 160 mg (range, 120-160 mg). The patients were treated with a median of three regorafenib cycles (range, 2-16 cycles). Seven patients (35%) with adverse effects had their regorafenib dose reduced to 120 mg. Further clinical details are summarized in Supplemental Tables 1 and 2.

At the time of data evaluation, all but one patient had discontinued regorafenib therapy. Tumor progression had occurred in 19 patients, and death in 18 patients (Figure 1). One patient (#12) was lost to follow-up 20.6 months after regorafenib initiation. The median PFS after initiation of regorafenib therapy was 3.6 months (range, 1.7-11.9 months), and the median OS was 7.9 months (range, 3.2-27.3 months).

MR and PET Imaging Changes Following Regorafenib

Following two cycles of regorafenib, 12 patients (60%) had *Progressive Disease* according to the RANO criteria, in 6 patients (30%) MRI changes were consistent with

Stable Disease, and two patients (10%) had a *Partial Response* (Figure 1). DWI and T2-weighted imaging were unavailable for one patient. There was a considerably high interreader ICC for mean and minimum ADC value calculated from two-dimensional ROI with 0.74 (95% CI, 0.52 - 0.86) and 0.84 (95% CI, 0.71 - 0.92). The three-dimensional approach resulted in higher ICC with 0.79 (95% CI, 0.63 - 0.89) and 0.93 (95% CI, 0.86 - 0.96) for mean and minimum ADC values, respectively. Intrareader ICC were 0.77 (95% CI, 0.60 - 0.88) for ADC_{mean}, and 0.78 (95% CI, 0.50 - 0.90) for ADC_{min}, respectively. Given the higher interreader reliability, ADC values from three-dimensional measurements were used for the further evaluations.

Both ADC_{mean} and ADC_{min} values were reduced in 13 (72%) of 18 patients. At follow-up, mean and minimum ADC values were significantly lower than at baseline (ADC_{mean} [$\times 10^{-3}$ mm^2/s], 0.70 ± 0.1 vs. 0.91 ± 0.2, P=0.005; ADC_{min} [$\times 10^{-3}$ mm^2/s], 0.43 ± 0.2 vs. 0.65 ± 0.2; P=0.002). A T2-dominant MRI pattern was observed in 6 out of 19 patients (32%).

The mean values of TBR_{mean} and TBR_{max} at follow-up were significantly lower compared to baseline (TBR_{mean}, 1.9 ± 0.4 vs. 2.2 ± 0.2; P=0.006; TBR_{max}, 3.1 ± 1.0 vs. 3.6 ± 0.8; P=0.005). The mean MTV changed insignificantly (MTV, 41.9 mL ± 38 mL vs. 44.2 mL ± 61 mL; P=0.498). Likewise, the averaged TTP and slope did not change significantly compared to the baseline (TTP, 28.4 ± 2.2 minutes vs. 25.4 ± 2.1 minutes, P=0.163; slope, 0.04 ± 0.3 SUV/h vs. -0.24.4 ± 0.2 SUV/h, P=0.164). According to the PET RANO 1.0 criteria, five patients (25%) had a PET-based *Progressive Disease*. In one patient (5%), findings were consistent with PET-based *Stable Disease*, and 14 patients (70%) had a PET-based *Partial Response.* PET-based *Complete Response* was not observed. After correcting for multiple testing, the changes of TBR and ADC

values remained statistically significant (P<0.05). Further details are summarized in Supplemental Tables 3, 4, and 5.

Discrepant Findings in MRI and FET PET

Although 6 of the 12 patients had *Progressive Disease* on MRI (patients #2, #11, #12, #17, #18, and #19), changes in FET PET were consistent with PET-based *Partial Response* according to PET RANO 1.0 criteria and had a reduction of TBR_{mean} by at least 10% (range, 10-19%) in these 5 patients (Figure 2). Furthermore, after two cycles of regorafenib, metabolic response was associated with a favorable survival outcome (median OS, 11.6 months; range, 8.0-25.8 months).

Univariate Survival Analysis for Prediction of Response to Regorafenib

The results of the ROC analyses revealed that a reduction of the static FET PET parameter TBR_{mean} by at least 10% identified a response, defined as OS ≥ 6 months (sensitivity, 79%; specificity, 83%; P=0.012). Patients with a metabolic response according to the relative change of TBR_{mean} had an almost two-fold longer OS than non-responders (10.4 vs. 5.3 months; P=0.027) (Figure 3). Changes in other static or dynamic FET PET parameters and the PET RANO 1.0 criteria were not significant regarding the prediction of OS ≥ 6 months (Figure 3; Supplemental Table 6).

MRI changes (i.e., *Stable Disease* or *Partial Response* compared to *Progressive Disease* according to the RANO criteria for MRI) were not predictive of longer OS (7.4 vs. 10.0 months; P=0.672) (Figure 3). In addition, changes in mean and minimum ADC values and the occurrence of a T2-dominant MRI pattern were not significant in predicting an OS ≥ 6 months (Supplemental Table 7). There was no difference between

the OS of patients with and without the occurrence of a T2-dominant MRI pattern (9.2 vs. 9.9 months; *P*=0.965).

Uni- and Multivariate Cox Regression Analyses

After the second regorafenib cycle, a reduction in TBR_{mean} of 10% was significantly associated with longer overall survival (HR, 0.328; *P*=0.036). The number of completed regorafenib cycles was significantly associated with longer overall survival (HR, 0.731; *P*=0.004). As this post-baseline variable is time-dependent, it was not included in the primary multivariable model. Besides those two parameters, no other clinical parameter, the RANO 2.0 criteria, or ADC metrics reached statistical significance in univariate models.

The association of reduction in TBR_{mean} of 10% remained significant in the multivariate model (HR, 0.200; *P*=0.006) with a Harrell's C-index of 0.766 (95%CI, 0.653-0.879), confirming the significant association of this PET parameter with OS. The multivariate model included the change in the Karnofsky Performance Score after two regorafenib cycles and age, which had the strongest signals in the univariate cox regression analyses. Of note, both parameters reached statistical significance in the multivariate model (Supplemental Table 8).

Survival Analysis in Patients with IDH-wildtype Glioblastoma

To address biological differences between IDH-mutant gliomas and glioblastomas, subgroup analyses were performed. In patients with IDH-wildtype glioblastoma (n=17) only, a ≥ 10% reduction in TBR_{mean} values remained significantly associated with longer

OS (median OS 9.9 vs. 5.3 months; *P*=0.049). Kaplan-Meier curves for OS in patients with IDH-wildtype glioblastoma are presented in the Supplemental Figure 2.

Prognostic Value of Absolute PET Parameters After Two Regorafenib Cycles

At follow-up FET PET, TBR_{mean} values ≤ 2.0 were prognostic (sensitivity, 86%; specificity, 83%; *P*=0.019). Patients with mean TBR values ≤ 2.0 at follow-up had significantly longer OS (10.6 vs. 4.5 months; *P*=0.009) (Figure 3). TBR_{mean} values at follow-up remained statistically significant in the multivariate analysis (*P*<0.001; HR, 0.030), that again included age and Karnofsky Performance Score at follow-up. As in the previous uni- and multivariate analyses regarding changes in FET PET parameters, both age and Karnofsky Performance Score at follow-up were not prognostic in the univariate analysis (*P*=0.100 and *P*=0.755, respectively). Of note, age at follow-up showed a significant association in the multivariate analysis, albeit with a smaller effect size than TBR_{mean} values ≤ 2.0 (HR=1.151; *P*>0.001). In direct comparisons, TBR_{mean} at follow-up ≤2.0 (Harrell's C-index of 0.668; 95%CI, 0.560-0.775) was more strongly associated with OS than age (Harrell's C-index of 0.609; 95%CI, 0.518-0.701). (Supplemental Tables 7-10).

DISCUSSION

One of the study's key findings suggests that a decrease in metabolic activity after two cycles of regorafenib in patients with CNS WHO grade 3 or 4 gliomas at relapse has a clinically valuable association with longer OS. Of note, the association of FET PET parameter changes (i.e., TBR_{mean}) with survival seem to be independent from other prognostic and predictive factors. Moreover, we show that the decrease in metabolic activity remained significantly associated with longer overall survival within the subgroup of patients with IDH-wildtype glioblastoma.

The reduction of TBR_{mean} by at least 10% to identify metabolic responders, as confirmed by ROC analysis in this study, is in line with the threshold of the criterion PET-based *Partial Response* defined by the PET RANO 1.0 criteria (*31*). Counterintuitively, when using the parameter TBR_{mean} at the same threshold to assess response to regorafenib in combination with other amino acid PET parameters postulated by the PET RANO 1.0 criteria, an improved OS could not be predicted. This discrepancy may be related to the definition of PET-based *Partial Response*. To fulfill this criterion, a decrease in either TBR_{mean} (10%), TBR_{max} (30%), or MTV (40%) at follow-up suffices, if the other parameters remain stable. Using one of the other FET PET parameter to identify responders may negatively affect the prediction of responders. For example, in contrast to TBR_{mean}, a PET-based *Partial Response* related to changes in the parameters TBR_{max} or MTV identified two patients as responders, who had an OS of less than 6 months, suggesting different predictive power among these parameters. This suggests that changes in mean TBR values may be the most robust parameter. Nevertheless, generalizability of this parameter warrants further validation in a higher number of patients.

The results of the present study differ from those of another study evaluating response to regorafenib after two cycles using FET PET (*16*). In that study, relative MTV changes in FET PET and MRI according to the RANO criteria, but not TBR values, predicted a significantly longer OS. Most probably, the differences are related to the methodology (e.g., the use of another predefined threshold in comparison to the present study, which used a survival time-based ROC analysis). Moreover, lower MTV values at baseline in that study compared to the present findings (mean MTV, 14.2 mL vs. 41.9 mL) may account for the discrepant results, given the higher susceptibility of lower volumes to percentage changes.

Further findings of the present study suggested an association between TBR_{mean} at follow-up FET PET after two cycles of regorafenib and OS. Notably, a lower metabolic activity (i.e., a mean TBR ≤ 2.0) at that time identified patients with more than a two-fold longer OS, and showed a stronger association with survival than other clinical prognostic factors (i.e., age). This finding aligns with the results of earlier amino acid PET studies, in which lower metabolic activity observed early after treatment initiation, including bevacizumab, was prognostic (*13,34*).

Regarding MRI, a few ADC-based approaches have been used to assess response to regorafenib in patients with glioma relapse(*16,18*). Here, we evaluated mean and minimum ADC values, a method potentially helpful to predict response to bevacizumab in patients with glioblastoma at relapse (*35*) and distinguish glioma relapse from treatment-related changes (*23*). However, in our study, as well as in the study by Martucci et al. (*19*), these ADC parameters did not predict response to regorafenib. A possible explanation is that regorafenib-induced effects, such as coagulative necrosis, may have impaired response assessment (*17*). Furthermore, we observed that the

occurrence of a T2-dominant MRI pattern was not helpful in predicting response to regorafenib, as reported previously (*18*). That study predicted a longer OS in patients showing a T2-dominant MRI pattern following regorafenib (27 vs. 10 weeks, $P = 0.003$). In contrast, in our study, a prediction of a significantly longer OS was not observed, and the occurrence of the T2-dominant MRI pattern was lower than previously reported (32% vs. 52%).

From a clinical perspective, regorafenib may provide an OS benefit at relapse in a subset of patients, with limited radiographic response rates, while clinically relevant grade 3-4 toxicities are not uncommon (*4,6*). Furthermore, FET PET-based early identification of responders after two cycles may have direct clinical implications. For example, in metabolic non-responders, discontinuation or switching of treatment may reduce exposure to toxicity and preserve quality of life. In metabolic responders, continuation of treatment is encouraged despite ambiguous MRI changes, e.g., coagulative necrosis following regorafenib (*17*).

Besides the retrospective design, a few limitations of the present study warrant discussion. The relatively small number of patients may seem a limitation. On the other hand, the number of glioma patients treated with regorafenib at relapse is inherently low. This scarcity also explains why treatment was not limited to patients with glioblastomas but extended to patients with CNS WHO grade 3 and 4 astrocytomas. While this heterogeneity might be considered a limitation, from a clinical perspective, the treatment of these patients at advanced disease stages is comparable, and both groups require optimized treatment monitoring, including response assessment.

Additionally, the exploratory nature of the analyses involving multiple imaging parameters raises the risk of false-positive results. To mitigate this risk, multiple measures were implemented. Although multiple imaging parameters were explored, multiplicity was handled for paired baseline-follow-up comparisons (Holm-Šídák method) and ROC analyses were considered exploratory. To mitigate overfitting in the survival models, parsimonious Cox models with ≤3 degrees of freedom were prespecified. These steps may help to reduce, but cannot eliminate the risk of small-sample optimism and imprecision. Phantom data from prior studies were used to justify the harmonization approach between the two PET scanners.

Furthermore, since patients had to complete two cycles of regorafenib to undergo follow-up imaging, the study design may introduce the risk of an immortal-time bias, potentially affecting survival estimates. To address this, OS was measured from treatment initiation, and a detailed flowchart of patient identification and selection is provided in Supplemental Figure 1. Nevertheless, immortal-time bias seems to be unlikely in the present study as nearly all patients (90%) who completed two cycles of regorafenib ultimately died during follow-up, and none of the patients with only one completed cycle of regorafenib died within the imaging interval.

To support the biological relevance of the observed ≥ 10% reduction in TBR_{mean}, the minimal detectable change in the PET scans (i.e., the smallest change in a measurement that is statistically significant and not likely due to random error or measurement variability) was calculated based on prior reproducibility data and the variance observed in the current study (*36*). This value was determined to be 0.09, indicating the smallest change that can be confidently distinguished from measurement variability. The 10%-reduction threshold used in this study corresponds to an absolute

change of approximately 0.2, well above the minimal detectable change, suggesting that the observed changes in TBR_{mean} likely reflect a true biological response rather than a random event.

In summary, our results suggest that FET PET parameters are clinically valuable for identifying responders to regorafenib in glioma patients at relapse (Figure 4). Identifying response early after treatment initiation using FET PET is of particular clinical relevance in pretreated patients receiving therapy with potentially considerable adverse events, such as regorafenib. In contrast, the RANO criteria, PET RANO 1.0 criteria, and changes in diffusion MRI metrics had limited value in predicting the response to regorafenib. Moreover, absolute FET PET parameters at follow-up after two cycles of regorafenib provide prognostic information. These initial results warrant further confirmation, ideally in a prospective setting.

KEY POINTS

QUESTION: Can changes in amino acid PET imaging parameters predict response to regorafenib in patients with glioma relapse?

PERTINENT FINDINGS: In this retrospective study of 20 patients with CNS WHO grade 3 or 4 gliomas at relapse, a ≥10% reduction in the mean tumor-to-brain ratio on FET PET after two cycles of regorafenib was associated with significantly longer overall survival (10.4 vs. 5.3 months). Both RANO criteria for MRI and parameter changes derived from diffusion-weighted MRI did not predict response to regorafenib

IMPLICATIONS FOR PATIENT CARE: FET PET imaging may enable early identification of responders to regorafenib in glioma relapse, aiding in treatment decisions and potentially minimizing unnecessary exposure to toxic therapy.

REFERENCES

1. Weller M, van den Bent M, Preusser M, et al. EANO guidelines on the diagnosis and treatment of diffuse gliomas of adulthood. *Nat Rev Clin Oncol.* 2021;18:170-186.

2. Abou-Elkacem L, Arns S, Brix G, et al. Regorafenib inhibits growth, angiogenesis, and metastasis in a highly aggressive, orthotopic colon cancer model. *Mol Cancer Ther.* 2013;12:1322-1331.

3. Wilhelm SM, Dumas J, Adnane L, et al. Regorafenib (BAY 73-4506): a new oral multikinase inhibitor of angiogenic, stromal and oncogenic receptor tyrosine kinases with potent preclinical antitumor activity. *Int J Cancer.* 2011;129:245-255.

4. Lombardi G, De Salvo GL, Brandes AA, et al. Regorafenib compared with lomustine in patients with relapsed glioblastoma (REGOMA): a multicentre, open-label, randomised, controlled, phase 2 trial. *The Lancet Oncology.* 2019;20:110-119.

5. Lombardi G, Caccese M, Padovan M, et al. Regorafenib in Recurrent Glioblastoma Patients: A Large and Monocentric Real-Life Study. *Cancers (Basel).* 2021;13.

6. Werner JM, Wolf L, Tscherpel C, et al. Efficacy and tolerability of regorafenib in pretreated patients with progressive CNS grade 3 or 4 gliomas. *J Neurooncol.* 2022;159:309-317.

7. Ruda R, Bruno F, Pellerino A, et al. Observational real-life study on regorafenib in recurrent glioblastoma: does dose reduction reduce toxicity while maintaining the efficacy? *J Neurooncol.* 2022;160:389-402.

8. Wen P, Alexander B, Berry D, et al. CTNI-85. GBM AGILE PLATFORM TRIAL FOR NEWLY DIAGNOSED AND RECURRENT GBM: RESULTS OF FIRST EXPERIMENTAL ARM, REGORAFENIB. *Neuro Oncol.* 2023;25:v97-98.

9. Wen PY, van den Bent M, Youssef G, et al. RANO 2.0: Update to the Response Assessment in Neuro-Oncology Criteria for High- and Low-Grade Gliomas in Adults. *J Clin Oncol.* 2023;41:5187-5199.

10. Wen PY, Macdonald DR, Reardon DA, et al. Updated response assessment criteria for high-grade gliomas: response assessment in neuro-oncology working group. *J Clin Oncol.* 2010;28:1963-1972.

11. Ceccon G, Lohmann P, Werner JM, et al. Early Treatment Response Assessment Using (18)F-FET PET Compared with Contrast-Enhanced MRI in Glioma Patients After Adjuvant Temozolomide Chemotherapy. *J Nucl Med.* 2021;62:918-925.

12. Wollring MM, Werner JM, Bauer EK, et al. Prediction of response to lomustine-based chemotherapy in glioma patients at recurrence using MRI and FET PET. *Neuro Oncol.* 2023;25:984-994.

13. Schwarzenberg J, Czernin J, Cloughesy TF, et al. Treatment Response Evaluation Using 18F-FDOPA PET in Patients with Recurrent Malignant Glioma on Bevacizumab Therapy. *Clin Cancer Res.* 2014;20:3550-3559.

14. Galldiks N, Langen K, Holy R, et al. Assessment of treatment response in patients with glioblastoma using [18F]Fluoroethyl-L-Tyrosine PET in comparison to MRI. *J Nucl Med.* 2012;53:1048-1057.

15. Galldiks N, Werner JM, Tscherpel C, Fink GR, Langen KJ. Imaging findings following regorafenib in malignant gliomas: FET PET adds valuable information to anatomical MRI. *Neurooncol Adv.* 2019;1:vdz038.

16. Lombardi G, Spimpolo A, Berti S, et al. PET/MR in recurrent glioblastoma patients treated with regorafenib: [(18)F]FET and DWI-ADC for response assessment and survival prediction. *Br J Radiol.* 2022;95:20211018.

17. Werner JM, Wollring MM, Tscherpel C, et al. Multimodal imaging findings in patients with glioblastoma with extensive coagulative necrosis related to regorafenib. *Neuro Oncol.* 2023;25:1193-1195.

18. Zeiner PS, Kinzig M, Dive I, et al. Regorafenib CSF Penetration, Efficacy, and MRI Patterns in Recurrent Malignant Glioma Patients. *J Clin Med.* 2019;8.

19. Martucci M, Ferranti AM, Schimperna F, et al. Magnetic resonance imaging-derived parameters to predict response to regorafenib in recurrent glioblastoma. *Neuroradiology.* 2023;65:1439-1445.

20. Louis DN, Perry A, Wesseling P, et al. The 2021 WHO Classification of Tumors of the Central Nervous System: a summary. *Neuro Oncol.* 2021;23:1231-1251.

21. Karschnia P, Young JS, Dono A, et al. Prognostic validation of a new classification system for extent of resection in glioblastoma: A report of the RANO resect group. *Neuro Oncol.* 2023;25:940-954.

22. Ellingson BM, Bendszus M, Boxerman J, et al. Consensus recommendations for a standardized Brain Tumor Imaging Protocol in clinical trials. *Neuro Oncol.* 2015;17:1188-1198.

23. Werner JM, Stoffels G, Lichtenstein T, et al. Differentiation of treatment-related changes from tumour progression: a direct comparison between dynamic FET PET and ADC values obtained from DWI MRI. *Eur J Nucl Med Mol Imaging.* 2019;46:1889-1901.

24. Hamacher K, Coenen HH. Efficient routine production of the 18F-labelled amino acid O-2-18F fluoroethyl-L-tyrosine. *Appl Radiat Isot.* 2002;57:853-856.

25. Herzog H, Langen KJ, Weirich C, et al. High resolution BrainPET combined with simultaneous MRI. *Nuklearmedizin.* 2011;50:74-82.

26. Caldeira L, Rota Kops E, Yun SD, et al. The Jülich Experience With Simultaneous 3T MR-BrainPET: Methods and Technology. *IEEE Transactions on Radiation and Plasma Medical Sciences.* 2019;3:352-362.

27. Lohmann P, Herzog H, Rota Kops E, et al. Dual-time-point O-(2-[(18)F]fluoroethyl)-L-tyrosine PET for grading of cerebral gliomas. *Eur Radiol.* 2015;25:3017-3024.

28. Law I, Albert NL, Arbizu J, et al. Joint EANM/EANO/RANO practice guidelines/SNMMI procedure standards for imaging of gliomas using PET with radiolabelled amino acids and [(18)F]FDG: version 1.0. *Eur J Nucl Med Mol Imaging.* 2019;46:540-557.

29. Pauleit D, Floeth F, Hamacher K, et al. O-(2-[18F]fluoroethyl)-L-tyrosine PET combined with MRI improves the diagnostic assessment of cerebral gliomas. *Brain.* 2005;128:678-687.

30. Galldiks N, Stoffels G, Filss C, et al. The use of dynamic O-(2-18F-fluoroethyl)-l-tyrosine PET in the diagnosis of patients with progressive and recurrent glioma. *Neuro Oncol.* 2015;17:1293-1300.

31. Albert NL, Galldiks N, Ellingson BM, et al. PET-based response assessment criteria for diffuse gliomas (PET RANO 1.0): a report of the RANO group. *The Lancet Oncology.* 2024;25:e29-e41.

32. Peduzzi P, Concato J, Kemper E, Holford TR, Feinstein AR. A simulation study of the number of events per variable in logistic regression analysis. *J Clin Epidemiol.* 1996;49:1373-1379.

33. Bender R, Lange S. Adjusting for multiple testing--when and how? *J Clin Epidemiol.* 2001;54:343-349.

34. Wirsching HG, Roelcke U, Weller J, et al. MRI and (18)FET-PET Predict Survival Benefit from Bevacizumab Plus Radiotherapy in Patients with Isocitrate Dehydrogenase Wild-type Glioblastoma: Results from the Randomized ARTE Trial. *Clin Cancer Res.* 2021;27:179-188.

35. Pope WB, Qiao XJ, Kim HJ, et al. Apparent diffusion coefficient histogram analysis stratifies progression-free and overall survival in patients with recurrent GBM treated with bevacizumab: a multi-center study. *J Neurooncol.* 2012;108:491-498.

36. Gutsche R, Scheins J, Kocher M, et al. Evaluation of FET PET Radiomics Feature Repeatability in Glioma Patients. *Cancers (Basel).* 2021;13.

FIGURES

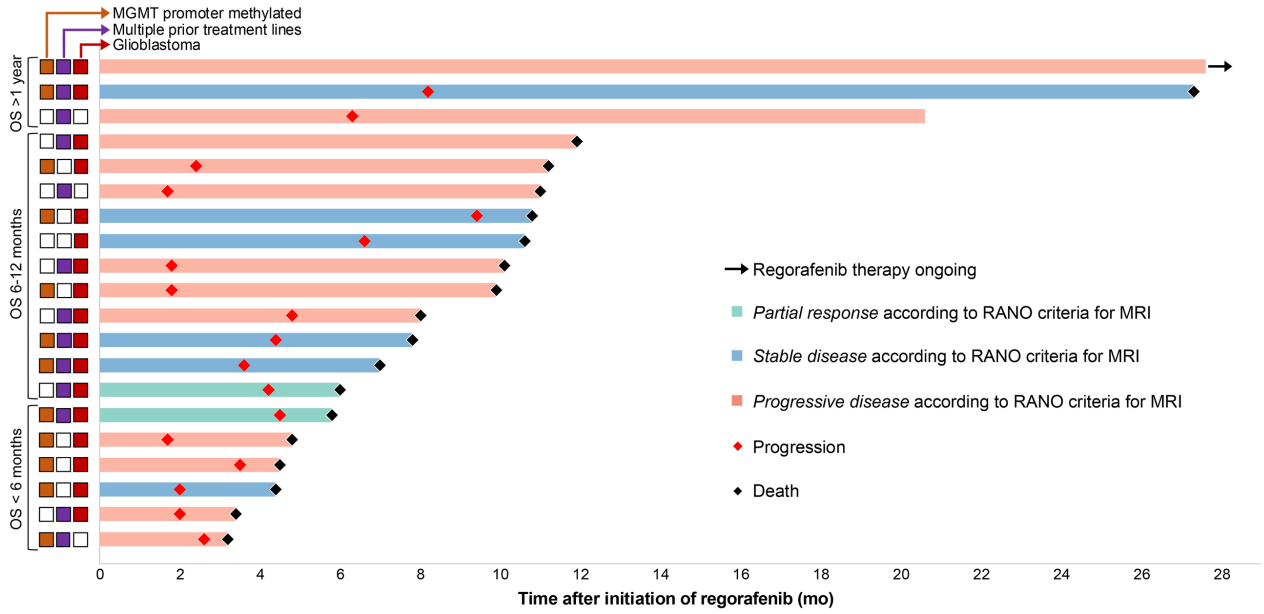

Figure 1: Swimmer plot of all 20 patients, sorted by overall survival after initiation of regorafenib. The time to progression ranged from 1.7 to 11.9 months. Patient bars are color-coded based on the RANO criteria. All but two patients (90%) had died, while regorafenib therapy was still ongoing in one patient (patient #19), and another patient was lost to follow-up (patient #12). Of note, the only two patients with a *Partial Response* according to the RANO criteria (patients #9 and #10) had a shorter overall survival (5.8 and 6.0 months, respectively) compared to the median overall survival of 7.9 months.

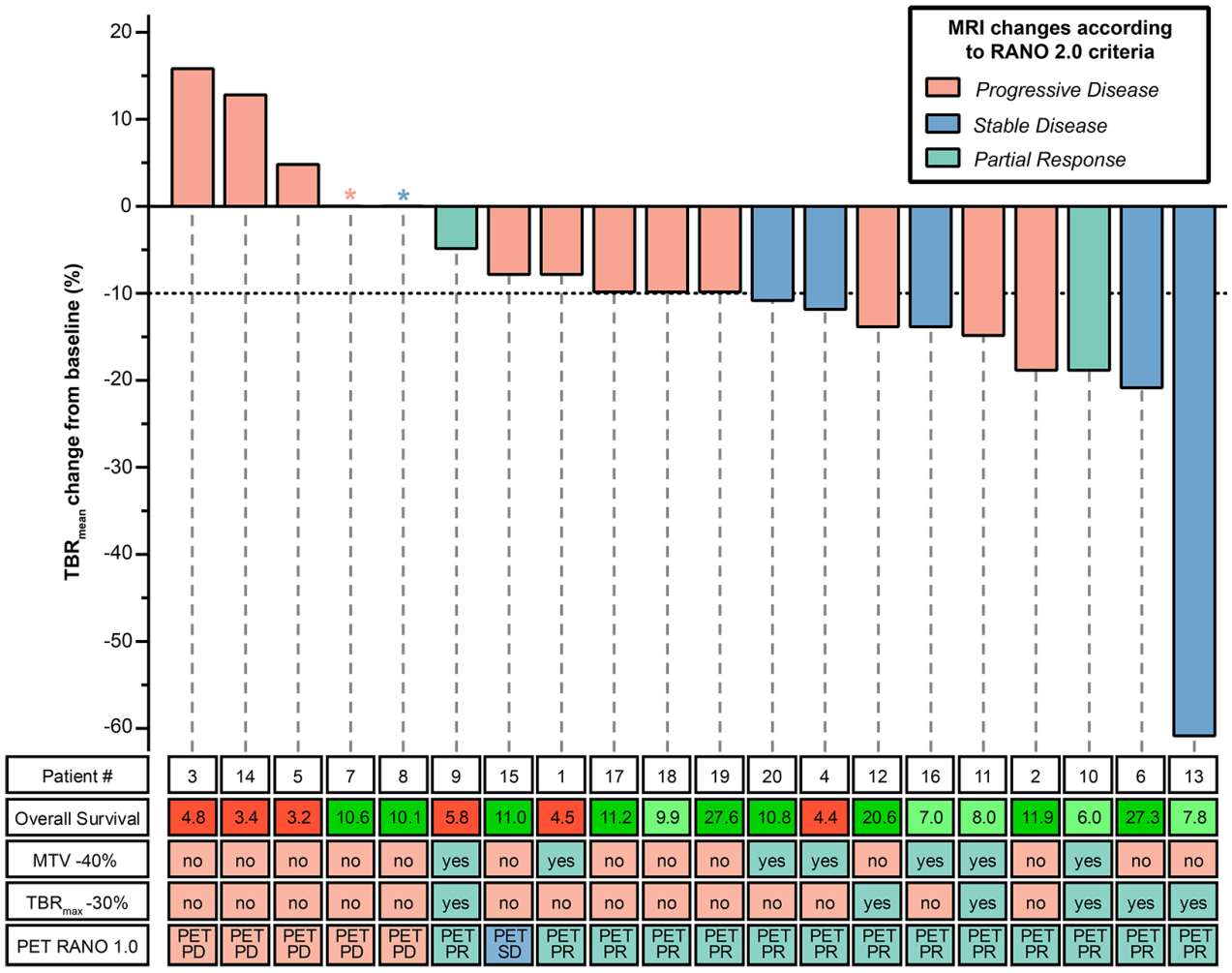

Figure 2: Waterfall plot of responses based on relative changes of the mean tumor-to-brain ratios (TBR_{mean}) in relation to MRI responses according to the RANO criteria. Relative changes of TBR_{mean} are plotted on the y-axis, and patient columns (x-axis) are color-coded corresponding to the respective MRI changes according to the RANO criteria (i.e., green = *Partial Response*; blue = *Stable Disease*; orange = *Progressive Disease*). In total, 15 patients (75%) showed a decrease in TBR_{mean}. Notably, discrepancies in metabolic response on FET PET and progressive MRI according to the RANO criteria were observed in several patients with prolonged overall survival (e.g., patients #2, #11, #12). Additionally, some patients who did not respond in terms of reduction of TBR_{mean} by at least 10% and had a short overall survival of 4.5 and 5.8 months (patients #1 and #9) were classified as having a PET-based *Partial Response* according to the PET RANO 1.0

criteria due to a relative decline in TBR_{max} and/or MTV according to the proposed thresholds (i.e., TBR_{max}, 30%; MTV, 40%).

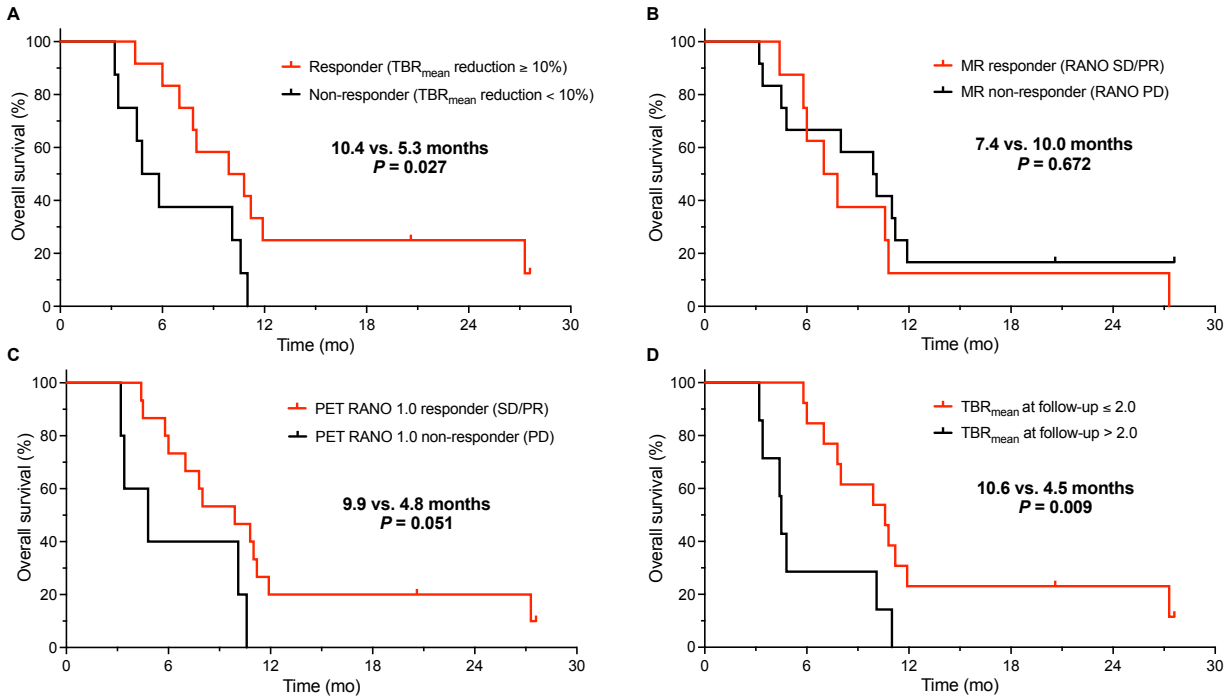

Figure 3: Kaplan-Meier curves for overall survival separated by relative changes in mean tumor-to-brain ratios (TBR_{mean}) on FET PET (A), MRI changes according to the RANO criteria (B), PET changes according to the PET RANO 1.0 criteria (C) after two cycles of regorafenib, and separated by TBR_{mean} at follow-up (D). Responders on FET PET defined by a decrease in TBR_{mean} by at least 10% compared to baseline had a significantly longer OS (10.4 vs. 5.3 months; $P = 0.027$) than non-responders (i.e., patients with an increase in TBR_{mean} or unchanged FET uptake at follow-up compared to baseline). In contrast, changes according to the RANO and PET RANO 1.0 criteria did not predict significantly longer overall survival. At follow-up, patients with a $TBR_{mean} \leq 2.0$ had a significantly longer OS than those with a $TBR_{mean} > 2.0$ (10.6 vs. 4.5 months; $P = 0.009$)

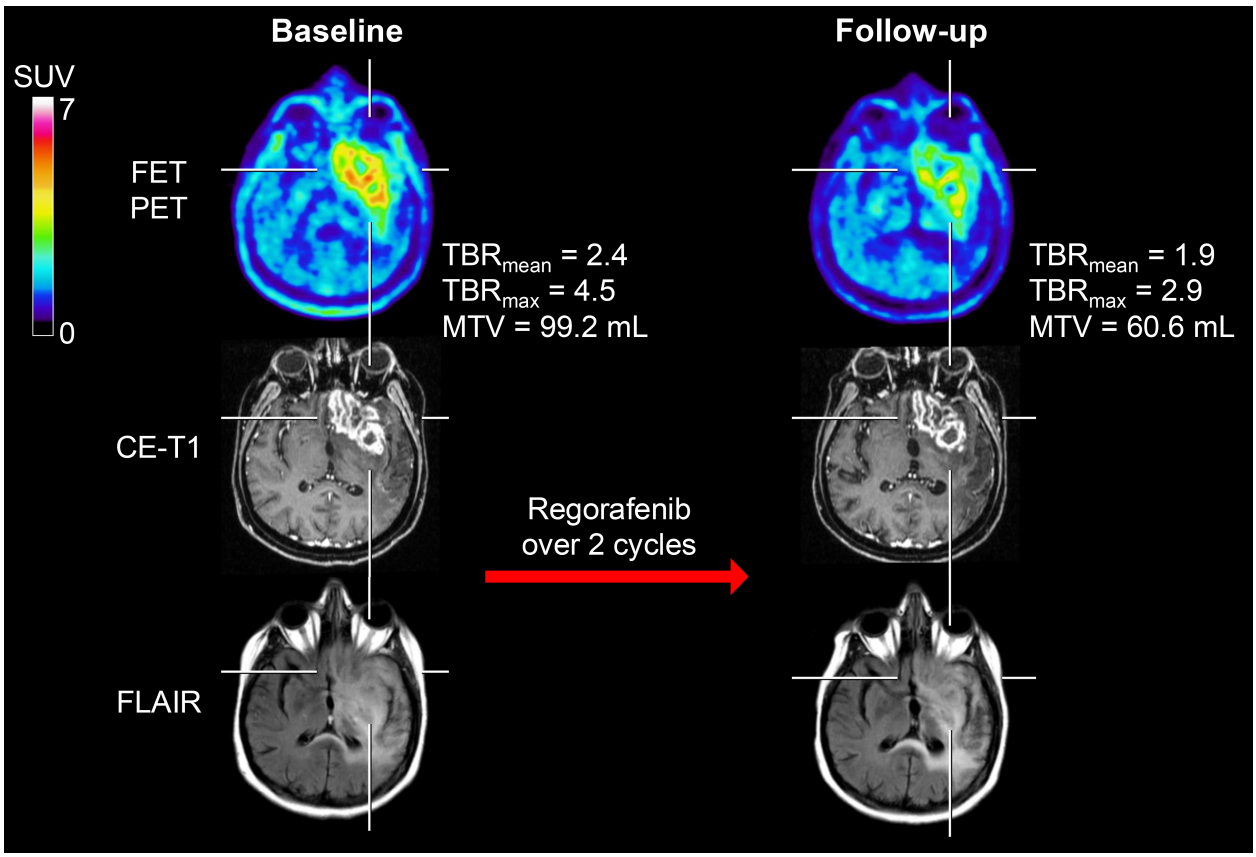

Figure 4: MRI and FET PET of a 44-year-old glioblastoma patient (patient #6) at baseline and after 2 cycles of regorafenib. Following regorafenib, FET PET at follow-up revealed a substantial reduction of metabolic activity compared to the baseline scan, i.e., a decrease of TBR_{mean} by 21%, TBR_{max} by 36%, and MTV by 39%, also fulfilling the criteria for a PET-based *Partial Response* according to the PET RANO 1.0 criteria. In contrast, the contrast-enhancing lesion on MRI remained unchanged. The patient received eight regorafenib cycles and had a favorable overall survival of 27 months after initiation of regorafenib.