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## 5-Aminolevulinic Acid Fluorescence-Guided Resection of <sup>18</sup>F-FET-PET Positive Tumor Beyond Gadolinium Enhancing Tumor Improves Survival in Glioblastoma

**BACKGROUND:** The value of early postoperative <sup>18</sup>F-FET-PET in patients with glioblastoma (GBM) is unclear. Five-aminolevulinic acid (5-ALA) is used for fluorescence-guided resections in these patients and previous data suggest that fluorescence and <sup>18</sup>F-FET-PET both demarcate larger tumor volumes than gadolinium enhanced magnet resonance imaging (MRI).

**OBJECTIVE:** To correlate fluorescence with enhancing volumes on postoperative MRI and <sup>18</sup>F-FET-PET tumor volumes, and determine the value of postoperative <sup>18</sup>F-FET-PET for predicting survival through observational study.

**METHODS:** GBM patients underwent fluorescence-guided resection after administration of 5-ALA followed by early postoperative MRI and <sup>18</sup>F-FET-PET for determination of residual tissue volumes. All patients were treated with standard temozolomide radiochemotherapy and monitored for progression-free and overall survival (PFS, OS).

**RESULTS:** A total of 31 patients were included. For functional reasons, residual 5-ALA derived fluorescent tissue was left unresected in 18 patients with a median <sup>18</sup>F-FET-PET volume of 17.82 cm<sup>3</sup> (interquartile range 6.50-29.19). In patients without residual fluorescence, median <sup>18</sup>F-FET-PET volume was 1.20 cm<sup>3</sup> (interquartile range 0.87-5.50) and complete resection of gadolinium enhancing tumor was observed in 100% of patients. A <sup>18</sup>F-FET-PET volume of above 4.3 cm<sup>3</sup> was associated with worse OS (logrank *P*-value ≤ .05), also in patients with no residual contrast enhancing tumor on MRI. More patients in whom fluorescing tissue had been removed completely had postoperative <sup>18</sup>F-FET-PET tumor volumes below 4.3 cm<sup>3</sup>.

**CONCLUSION:** Postoperative <sup>18</sup>F-FET-PET volumes predict OS and PFS. Resection of 5-ALA derived fluorescence beyond gadolinium enhancing tumor tissue leads to lower postoperative <sup>18</sup>F-FET-PET tumor volumes and improved OS and PFS without additional deficits.

**KEY WORDS:** Aminolevulinic acid, Fluorescence, Gadolinium, Glioblastoma, Tumor burden, (<sup>18</sup>F)fluoroethyltyrosine

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**G**lioblastoma (GBM), the most frequent malignant intrinsic brain tumor, carries a poor prognosis.<sup>1,2</sup> Current treatment standard is resection and adjuvant radiochemotherapy. The target tissue to be

resected is traditionally considered to be the gadolinium contrast enhancing (Gd+) tumor on T1 weighted magnet resonance imaging (MRI). Retrospective and prospective cohort studies have repeatedly demonstrated the

**ABBREVIATIONS:** CRET, complete resection of gadolinium enhancing tumor; F-FET, F-Fluor-Ethyl-Tyrosine; GBM, glioblastoma; HR, hazard ratios; MRI, magnet resonance imaging; MWU, Mann-Whitney U; OS, overall survival; PET, positron emission tomography; PFS, progression-free survival; SUV, standardized uptake value

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resection of Gd+ tumor to be associated with improved prognosis.<sup>3-8</sup> Removal of contrast enhancing tumor has been equated to “complete” resection.

As identification of tumor margins based on visual information or tactile tissue changes is difficult, techniques have evolved over time to overcome this issue.<sup>9</sup> One technique is fluorescence-guided resection using 5-aminolevulinic acid (5-ALA). 5-ALA leads to accumulation of fluorescent protoporphyrin IX in non-necrotic marginal GBM tissue. Using an appropriate surgical microscope, the tumor can be visualized in real time during resection.<sup>10</sup> A randomized controlled trial has shown the rates of complete resection of gadolinium enhancing tumor (CRET) to be increased, leading to approval with the European Medicines Agency as well as with the U.S. Food and Drug Administration.<sup>11</sup> Despite its widespread use, little is known regarding the tissue volume that is actually visualized by fluorescence. Early on, our group suspected fluorescence to extend beyond the region of Gd+ on MRI<sup>12,13</sup> as subsequently corroborated by others.<sup>13-15</sup> Also, nonenhancing gliomas have been observed to accumulate visibly fluorescing tissue after administration of 5-ALA, the latter in about 20% of cases.<sup>16</sup>

For surgery of high-grade gliomas, it would appear crucial to determine the expected extent of fluorescent tissue beforehand. This is important when deciding on the type of intraoperative monitoring or mapping required for safe surgery when enhancing tumor is located in proximity to eloquent brain regions.<sup>14</sup> Various MRI techniques have not yet been reliably able to predict the extent of fluorescence. Over the last years, positron emission tomography (PET) with radio labeled amid acids such as <sup>18</sup>F-Fluor-Ethyl-Tyrosine (<sup>18</sup>F-FET-PET) has been established for glioma imaging. Its use is recommended throughout all stages of diagnostics and therapy.<sup>17</sup> <sup>18</sup>F-FET tracer uptake was found to correlate with the presence of intraoperative fluorescence after administration of 5-ALA.<sup>18</sup> Similar to 5-ALA, <sup>18</sup>F-FET-PET is capable of visualizing larger tumor volumes pre- and postoperatively than MRI with gadolinium contrast alone.<sup>19,20</sup> FET-positive residual tumor volume, previously termed biological tumor volumes, correlates with worse survival in GBM patients undergoing resection and biopsy.<sup>21</sup>

Our study sought to investigate Gd+ and <sup>18</sup>F-FET-PET tumor volumes after fluorescence-guided resection in GBM patients and to correlate residual volumes with survival data. To our knowledge, an analysis of the impact of residual FET-PET volume on survival in GBM has not yet been performed in patients with maximal resective surgery.

## METHODS

### Study Design and Patients

From April 8, 2015, 31 adult patients with Gd+ GBM and subsequent fluorescence-guided resection were included in this exploratory prospective observational single-center study. Surgical aim was safe complete resection of enhancing tumor or cytoreductive surgery in

tumors extending into crucial cortex or white matter tracts. Early postoperative MRI was obtained within 48 h after surgery. Early postoperative <sup>18</sup>F-FET-PET was performed at the earliest time point possible (median 6 d, interquartile range 4-7 d, average 6.25 d; SD 3.78) but within 15 d after surgery as previously recommended.<sup>19</sup> All patients were treated with concomitant radiochemotherapy with temozolomide followed by sequential chemotherapy with temozolomide alone.<sup>1</sup> Patients were followed up every 3 mo with serial MRI and clinical examination. Progression was assessed in accordance with the criteria defined by the Response Assessment in Neuro-Oncology Working Group.<sup>22</sup> The last visit for survival analysis was on August 2, 2018. Median follow-up was 2.5 yr (1.7-3.3 CI). No patient was lost to follow-up. Full ethical approval was obtained at the regional ethical review committee. This study is reported according to the STROBE guidelines for reporting observational studies.<sup>23</sup>

### Fluorescence-Guided Resection

Intraoperatively, neuronavigation, ultrasound, and 5-ALA derived fluorescence were used. 5-ALA (Gliolan<sup>®</sup>, Medac, Wedel, Germany) was administered at 20 mg per kilogram b.w. orally 4 h prior to induction of anesthesia. Amicroscope with appropriate filters (Pentero with BLUE400 filter option, Zeiss Meditec, Oberkochen, Germany) was used for fluorescence visualization. Surgical goal was the complete resection of visible fluorescence while respecting eloquent brain. Residual fluorescence at the end of surgery was documented in the surgical report. Functional MRI and fiber tracking studies were obtained as necessary, as well as methods of intraoperative mapping and monitoring including awake craniotomies for language mapping.<sup>24</sup> Three primary neuro-oncological surgeons including the senior author were involved in patient treatment. All resections were finalized by the senior author. Preoperatively, patients received 4 mg dexamethasone 3 times a day for duration of 3 d, which was tapered postoperatively.

### MRI Protocol

Early postoperative MRI was obtained within 48 h after surgery including contrast enhanced sequences. Images were segmented for volumetric analysis using an established semiautomatic technique (Medical Imaging Toolkit, German Cancer Research Center, Division of Medical Image Computing, Heidelberg, Germany, open source, [www.mitk.org](http://www.mitk.org)) by a neuroradiologist (P.S.) blinded to survival data and extent of resection. Residual enhancing tumor volumes of less than .175 cm<sup>3</sup> were considered to represent “complete” resections in accordance with previous publications.<sup>11,14</sup>

### <sup>18</sup>F-FET-PET Protocol

Patients were studied after overnight fasting and after informed consent. <sup>18</sup>F-FET PET-CT was performed as previously described in 12 patients with minor modifications.<sup>25</sup> Static images were acquired 20 to 40 min after intravenous injection of 3 MBq <sup>18</sup>F-FET/kg body weight using a hybrid PET-computed tomography device (mCT, Siemens, Erlangen, Germany). Images were processed and reconstructed with software as supplied by the manufacturer, including homogeneous attenuation correction of the head based on contours extracted from the emission data. In 19 patients, 40 min dynamic FET-PET acquisition was started immediately after injection of the tracer using list mode and a MR-PET device (Siemens, Erlangen, Germany). These images were reconstructed as four 10 min frames the third and fourth frame comparable with the static images. PET images were coregistered with

early postoperative MRI using Syngo.via software (Siemens, Erlangen, Germany). Volumes of interest were placed on the tumor to detect the maximal standardized uptake value (SUV). A standardized circular reference region encompassing grey and white matter was drawn on axial slices at the level above the side ventricles in the unaffected hemisphere or in the anterior of posterior half of the brain in case of bilateral tumors. Uptake ratios were calculated as tumor maximum SUV/reference mean SUV. To assess for tumor volume, a spherical volume of interest was defined covering all visible tumor lesions and carefully avoiding structures with physiological uptake like venous sinuses and the scalp or the base of the skull. Within this volume only voxels exceeding the threshold of 1.8 x mean activity of the reference ROI were used to calculate the <sup>18</sup>F-FET-PET positive tumor volume.<sup>21</sup> Analyses were performed by a nuclear medicine specialist (M.W.) blinded to survival data and extent of resection according to other modalities.

Due to logistical reasons, mainly short-term availability that would not interfere with the timing of urgent surgery in GBM, the number of patients with preoperative <sup>18</sup>F-FET-PET was too low to allow meaningful analysis.

### Histopathological and Molecular Analysis

Tissue diagnoses were obtained locally in accordance with the 2016 World Health Organization classification of tumors of the central nervous system.<sup>2</sup>

### Survival Data Analysis and Statistics

Overall survival (OS) was defined as time from day of surgery until death from any cause. Patients alive were censored at time of last contact. Progression-free survival (PFS) was defined as time from day of surgery until diagnosis of progressive disease according to MRI criteria or death from any cause. Patients without such event were censored at last contact. Standard descriptive analyses were performed. Categorical variables are shown as absolute and relative frequencies and continuous variables are presented as mean ± standard deviation or median and interquartile range (IQR, 25% quartile-75% quartile). Fisher's exact test was calculated to compare categorical variables and two-

sided non-parametric Mann-Whitney *U* (MWU) test were performed for continuous variables. Wilcoxon signed rank test was used to compare related samples. OS and PFS were analyzed using time-to-event methods: Kaplan-Meier estimates, log-rank tests, and univariate Cox regressions.<sup>26,27</sup> Results are reported as hazard ratios (HR) and corresponding 95% confidence interval (95% CI). Exploratory cut-off point determination of residual <sup>18</sup>F-FET-PET volumes was performed to predict OS and PFS based on the maximally selected standardized log rank statistics using the R package *maxstat*.<sup>28</sup> Exact *P*-values ( $p_{max}$ ) for these maximally selected Gauss statistics were calculated.<sup>29</sup> Additionally, time-dependent ROC curves from censored event data using Kaplan-Meier method were estimated using the R package *survivalROC*<sup>28</sup> for the continuous variable residual <sup>18</sup>F-FET-PET.<sup>30</sup> Area under the curve (AUC), sensitivity and specificity were calculated to predict 1-yr OS or PFS. As sensitivity analysis, another definition of PFS was analysed, where patients alive without progression were censored at day of their last MRI without progression. Statistical analyses were performed using R 3.5.1,<sup>28</sup> SAS software version 9.4 for Windows (SAS Institute, Cary, North Carolina) and IBM SPSS Statistics for Windows, version 25 (IBM Corp., Armonk, New York). Inferential statistics such as *P*-values and confidence intervals were intended to be exploratory and not confirmatory. *P*-values represent only an unadjusted metric measure of evidence against the respective null hypothesis and were used only to generate new hypotheses. Therefore, neither global nor local significance levels were determined, and no adjustment for multiplicity was applied. *P*-values ≤ .05 were considered statistically noticeable.

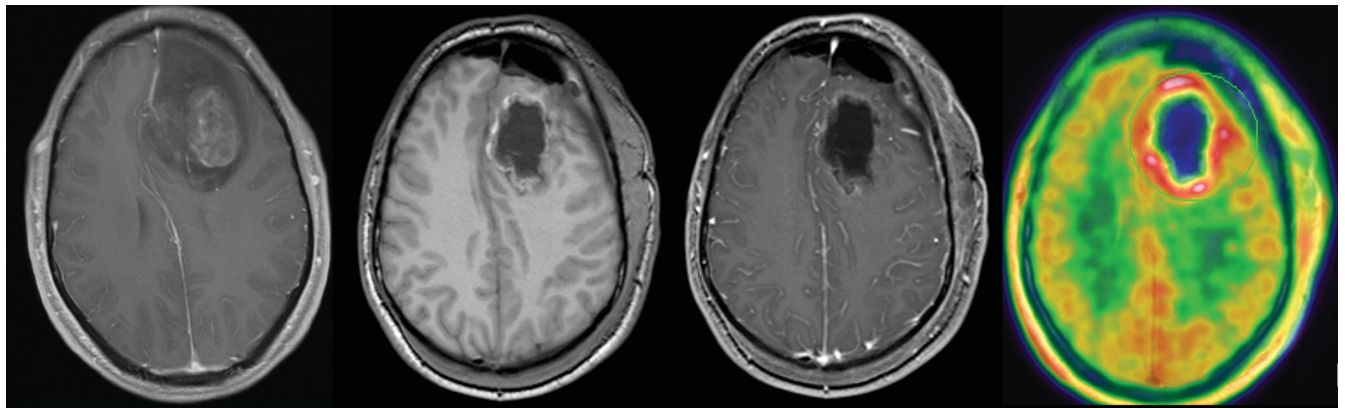
## RESULTS

A total of 31 patients (18 females, 13 males) were analyzed. Median age was 66.8 yr (interquartile range 51.2-71.5 yr). CRET was achieved in 18 (58%) patients. Complete resection of all fluorescing tissue was achieved in 13 (41.9%) patients, all of which with CRET. One patient (3%) suffered permanent hemiparesis from surgery (see Table 1 for patient characteristics).

**TABLE 1. Patient Characteristics**

Characteristic	Overall	<sup>18</sup> F-FET-PET Volume ≤ 4.3 cm <sup>3</sup>	<sup>18</sup> F-FET-PET Volume > 4.3 cm <sup>3</sup>	<i>P</i> value
Number of cases	31	11	20	–
Age in years	66.8 (51.2 - 71.5)	55.1 (41.74 - 68.67)	67.2 (57.99 - 74.33)	.064 <sup>†</sup>
Female/Male (n,%)	18/13 (58.1%/41.9%)	7/4 (63.6%/36.4%)	11/9 (55%/45%)	.718 <sup>#</sup>
MGMT Methylation (n,%)	18 (58.1%)	8 (72.7%)	10 (55.6%)	.276 <sup>#</sup>
IDH Mutation (n,%)	2 (6.5%)	2 (18.2%)	0 (0%)	.118 <sup>#</sup>
Residual Fluorescence (n,%)	18 (58%)	2 (18.2%)	16 (80%)	<b>.002<sup>#</sup></b>
No Residual Fluorescence (n,%)	13 (41.9%)	9 (81.8%)	4 (20%)	
Early Postoperative Gd+ Volume in cm <sup>3</sup>	0 (0 - 1.1)	0 (0 - 0.02)	0.51 (0.04 - 1.99)	<b>.005<sup>†</sup></b>
CRET (%)	18 (58%)	11 (100%)	7 (35%)	<b>&lt; .001<sup>#</sup></b>
Early Postoperative <sup>18</sup> F-FET-PET Volume in cm <sup>3</sup>	7.3 (2.2 - 25.3)	1.07 (0.22 - 2.91)	13.98 (7.46 - 28.55)	–
Surgically acquired permanent motor or language deficit (n,%)	1 (3%)	0 (0%)	1 (5%)	> .99 <sup>#</sup>

Data are presented median (25% quantile-75% quantile) or frequencies (percentages). Statistically noticeable differences between <sup>18</sup>F-FET-PET Volume ≤ 4.3 cm<sup>3</sup> and > 4.3 cm<sup>3</sup> are marked in bold (*P* ≤ .05). CRET complete resection of enhancing tumor; Gd+ gadolinium contrast enhancing tumor; IDH isocitrate dehydrogenase; IQR interquartile range; MGMT O<sup>6</sup>-methylguanine-DNA methyltransferase; <sup>†</sup>Mann-Whitney *U* test, <sup>#</sup>Fisher's exact.



**FIGURE 1.** Complete resection (center left without and center right with contrast) of a gadolinium contrast enhancing left frontal glioblastoma (far left image). Early postoperative  $^{18}\text{F}$ -FET-PET (far right image) showed residual tumor volume of more than  $4.3\text{ cm}^3$ .

### Early Postoperative Tumor Volumes

Median residual Gd+ tumor volume was  $0\text{ cm}^3$  (IQR  $0\text{-}1.1\text{ cm}^3$ ) and residual  $^{18}\text{F}$ -FET-PET+ volume  $7.3\text{ cm}^3$  (IQR  $2.2\text{-}25.3\text{ cm}^3$ ;  $P < .0001$ , Wilcoxon signed rank test).  $^{18}\text{F}$ -FET-PET positive volumes exceeded Gd+ volumes in all patients. Figure 1 depicts a case of complete resection of enhancing right temporal GBM, whereas  $^{18}\text{F}$ -FET-PET showed a residual tumor volume of more than  $4.3\text{ cm}^3$ .

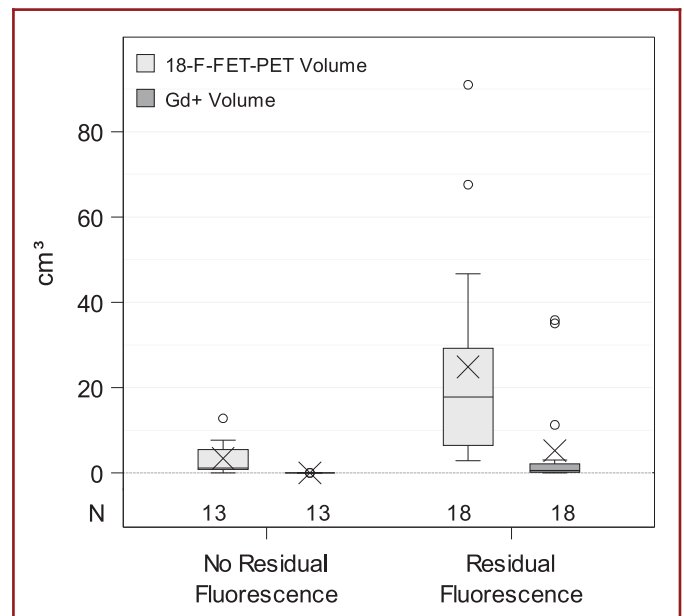
Stratifying for residual fluorescence, fluorescence-guided resection lead to 100% CRET when no residual fluorescence was noted at the end of surgery. The median Gd+ volume was  $0\text{ cm}^3$  (IQR  $0\text{-}0\text{ cm}^3$ ). In the group with residual fluorescence, CRET was achieved in 5/18 (28%) patients. In this group, median Gd+ volume was  $0.55\text{ cm}^3$  (IQR  $0.15\text{-}2.34\text{ cm}^3$ ;  $P < .001$ , MWU test). All patients with residual fluorescence showed  $^{18}\text{F}$ -FET tracer uptake. Median  $^{18}\text{F}$ -FET-PET volume in patients with residual fluorescence was  $17.82\text{ cm}^3$  (IQR  $6.25\text{-}33.14\text{ cm}^3$ ) and larger than the median volume of  $1.2\text{ cm}^3$  (IQR  $0.55\text{-}6.38\text{ cm}^3$ ) in patients without residual fluorescence ( $P < .001$ , MWU test).

Figure 2 compares  $^{18}\text{F}$ -FET-PET and Gd+ tumor volumes according to residual or no residual intraoperative fluorescence. The distributions of Gd+ and  $^{18}\text{F}$ -FET-PET tumor volumes are additionally illustrated as a scatter plot in Figure 3. All data points were located above the bisectrix, illustrating  $^{18}\text{F}$ -FET-PET tumor always to exceed Gd+ volumes.

We also stratified tumors by their localization according to the system proposed by Sawaya et al. into grade I to III tumors to determine whether location influenced residual tumor volumes.<sup>31</sup> We found no significant influence regarding the distribution of grades I to III on residual  $^{18}\text{F}$ -FET-PET volumes.

### Survival Analyses

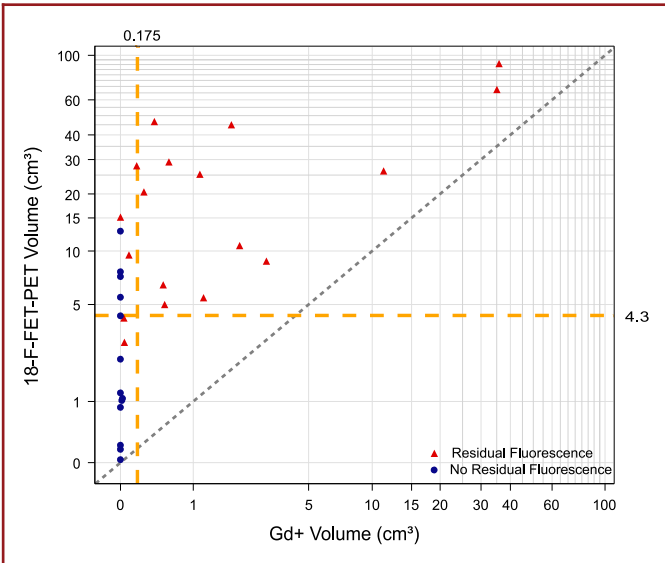
A synopsis of different resection target volumes and their impact on OS is displayed in Figure 4. Stratifying for residual



**FIGURE 2.** Boxplots showing the distribution of  $^{18}\text{F}$ -FET-PET and Gd+ tumor volumes according to residual or no residual intraoperative fluorescence noted at the end of the operation. An X denotes the mean.

Gd+ volumes, a residual Gd+ volume  $< 0.175\text{ cm}^3$  (CRET) prolongs OS (Figure 5) as expected. The same was observed for PFS (logrank  $P$ -value .008; HR 3.37, 95% CI 1.31-8.70), see Supplemental Digital Content. Exploratory cut-off point determination of residual  $^{18}\text{F}$ -FET-PET volumes based on maximally selected standardized logrank statistics found  $4.3\text{ cm}^3$  to be the cut-off point for OS and PFS with adjusted exact  $P$ -values  $p_{\max}$  of .002 for OS and .09 for PFS. Using  $^{18}\text{F}$ -FET-PET volume as continuous variable, time dependent AUC, sensitivity, and specificity were calculated to predict 1-yr OS or PFS. AUC, sensitivity,





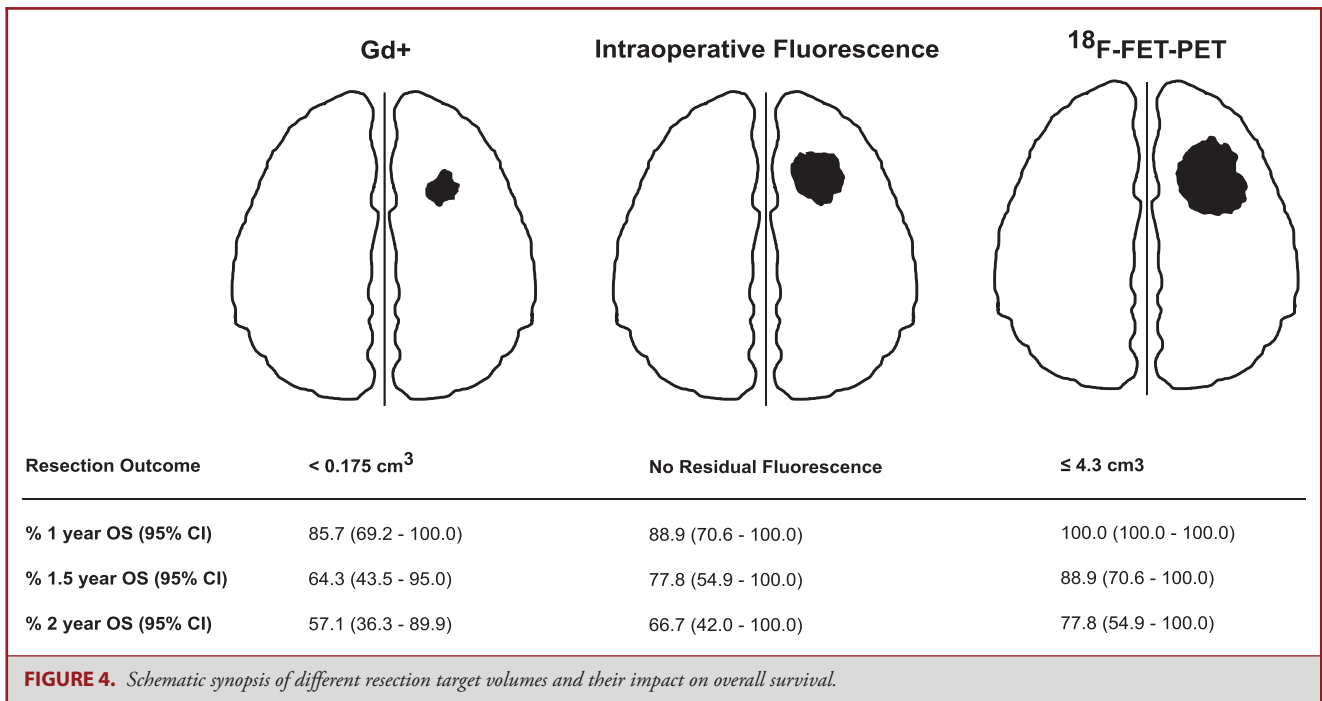
**FIGURE 3.** Scatter plot of Gd+ and <sup>18</sup>F-FET-PET volumes of each patient. Dotted bisectrix refers to perfect matching absolute values of Gd+ and <sup>18</sup>F-FET-PET volumes. Intersected lines at x and y axes refer to cut-off values of 0.175 cm<sup>3</sup> for Gd+ (indicating a “complete” resection by MRI standards) and 4.3 cm<sup>3</sup> for <sup>18</sup>F-FET respectively, the cut-off found for survival with this modality. For the group with no residual fluorescence median Gd+ volume was 0 cm<sup>3</sup> (IQR 0-0) and median <sup>18</sup>F-FET-PET volume was 1.2 cm<sup>3</sup> (IQR 0.05-6.08). In the group with residual fluorescence Gd+ volume was 0.55 cm<sup>3</sup> (IQR 0.15-2.34) and <sup>18</sup>F-FET-PET volume was 17.82 cm<sup>3</sup> (IQR 6.25-33.14). The graph indicates “complete” resections based on visible fluorescence to extend well into the residual <sup>18</sup>F-FET-PET volume.

and specificity were 0.75, 100%, and 55.8% for 1-yr OS and 0.70, 86.6%, and 63.2% for 1-yr PFS, respectively. This analysis again indicated 4.3 cm<sup>3</sup> to be the relevant cut off. To examine the robustness of this result, a sensitivity analysis was performed using an alternate definition of PFS, where patients alive without progression were censored at day of last MRI without progression. Cut-off point determination led to the same results (data not shown). Extent of tumor resection leading to an early postoperative <sup>18</sup>F-FET-PET volume of ≤4.3 cm<sup>3</sup> noticeably prolonged OS (Figure 6).

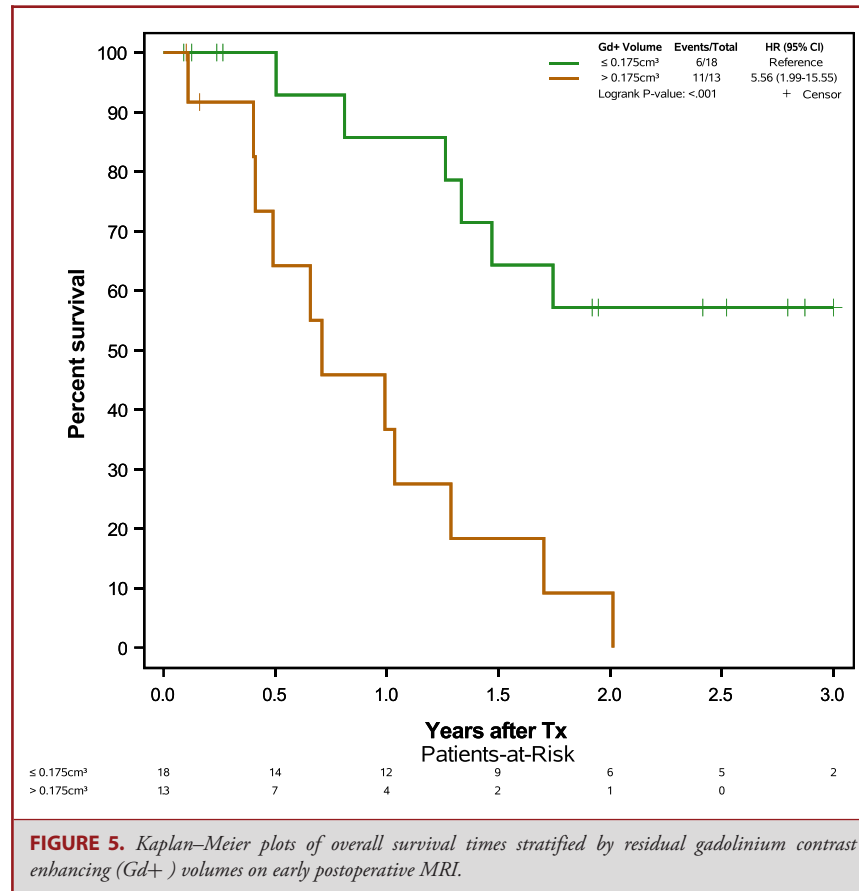
In univariable Cox regressions, MGMT status, age, and <sup>18</sup>F-FET-PET volume were predictive for OS (Table 2). Volume of gadolinium contrast enhancing tumor, <sup>18</sup>F-FET-PET volume, and residual fluorescence were collinear, as expected from Gd+ and <sup>18</sup>F-FET-PET volume distributions. However, a meaningful multivariable analysis of these variables could not be performed, given of the small number of patients in this study.

We additionally analyzed *only patients with CRET* to determine whether resecting <sup>18</sup>F-FET-PET positive, but gadolinium negative marginal tumor influenced outcome. In this subgroup of 18 patients with CRET, residual <sup>18</sup>F-FET-PET volume ≤4.3 cm<sup>3</sup> was similarly associated with prolonged OS (Figure 7). Complete resection of fluorescent tissue resulted in a larger proportion of patients with postoperative <sup>18</sup>F-FET-PET tumor burdens of <4.3 cm<sup>3</sup> compared to incomplete resections (P = .002, Fisher).

All patients were treated by surgery and concomitant radiochemotherapy with temozolomide followed by adjuvant temozolomide. We did not note significant differences in



**FIGURE 4.** Schematic synopsis of different resection target volumes and their impact on overall survival.



secondary therapies after tumor recurrence in patients with initial CRET regarding re-operation, second line chemotherapeutic strategies or re-irradiation strategies. Approximately one third of these patients received secondary therapies and the numbers were too low to allow meaningful statistical corroboration.

In univariable Cox regression, it was found that per 1 cm<sup>3</sup> residual <sup>18</sup>F-FET-PET volume the risk of death increased by a factor of 2. Univariate Cox-Regression found a hazard ratio of 1.04 (95% CI 1.01-1.06) for worse OS with every 1 cm<sup>3</sup> residual <sup>18</sup>F-FET-PET volume. For PFS the hazard ratio was found to be 1.02 (0.988%-1.054 95% CI).

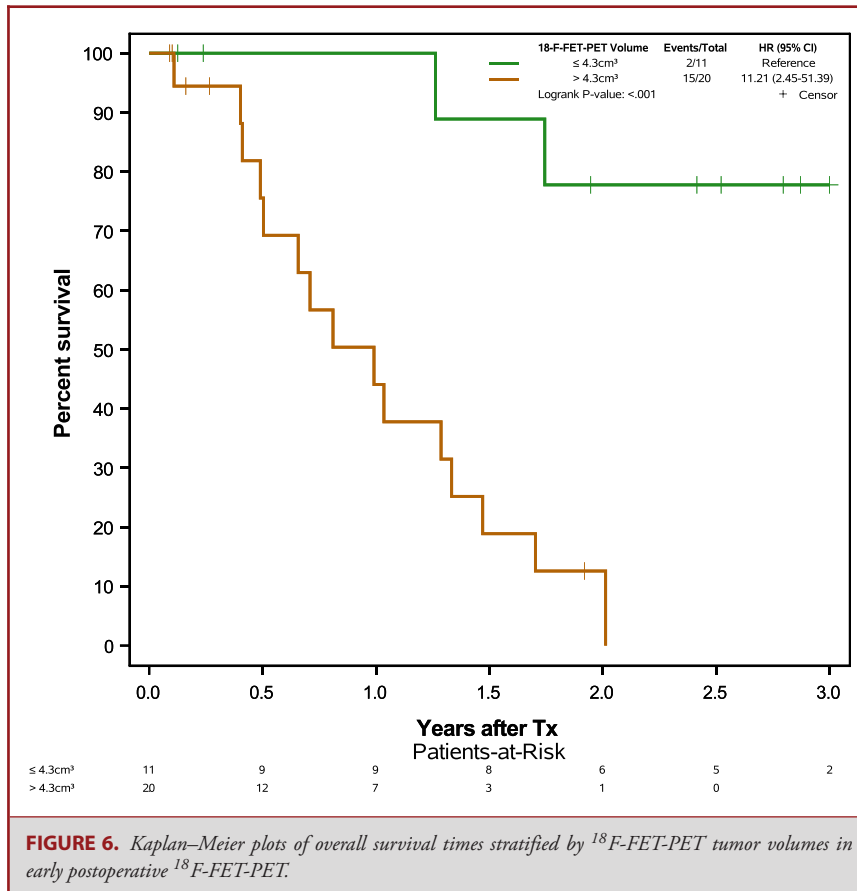
## DISCUSSION

Currently, MRI is the imaging modality of choice for GBM, and gadolinium enhancing tumor is the resection target volume. Even so, it is well known that infiltrating tumor cells by far exceed Gd+ tumor volumes, leading to inevitable recurrence at the margin of the resection.<sup>32</sup> 5-ALA derived fluorescence has been shown to mark tumor tissue volume exceeding Gd+ tumor volume.<sup>3,12,33,34</sup> Studies correlating completeness of tumor resection with outcome have largely examined the

percentage of tumor removal. Even though this concept has been popularized, it does not represent the biological reality of the tumor burden.<sup>22</sup> Furthermore, residual tumor volumes on MRI appear to predict survival in a continuous but not “all or nothing” relationship.<sup>35</sup> In accordance with previous studies, we found that postoperative <sup>18</sup>F-FET-PET tumor volumes exceed Gd+ volumes and that fluorescence-guided resection using 5-ALA can be relied upon for identifying tissue that is a part of the Gd+ volume, but also part of the <sup>18</sup>F-FET volume, exceeding the Gd+ volume. Several histopathological sampling studies have demonstrated that tumor tissue exceeds 5-ALA derived fluorescence.<sup>34,36</sup> Removing all fluorescing tissue reduced the residual density of tumor cells to about 10%.<sup>37</sup>

We demonstrate that by resecting beyond the Gd+ volume, the residual <sup>18</sup>F-FET-PET volume can be further reduced, which affects survival. To our knowledge, this is the first demonstration that supramarginal resections as quantified by early postoperative <sup>18</sup>F-FET-PET serve to improve survival in patients with GBM.

Similarly, previous work found postoperative <sup>18</sup>F-FET-PET volumes to influence survival independent of age or MGMT promoter methylation, although the cut-off was more than twice as high as in our patients.<sup>21</sup> However, almost half of patients had only received biopsies. Therefore, while this study indicates a



**TABLE 2. Univariable Cox Regression Analysis of Prognostic Factors of Overall Survival**

Independent variables	Univariable Cox regression	
	HR (95% CI)	P value
Age	1.02 (1.02-1.118)	.005
Sex	0.60 (0.23-1.604)	.310
Gd+ volume	1.09 (1.02-1.163)	.012
<sup>18</sup> F-FET-PET volume	1.03 (1.01-1.059)	.006
Residual fluorescence	5.77 (1.59-20.96)	.008
MGMT methylation	0.47 (0.18-1.24)	.125

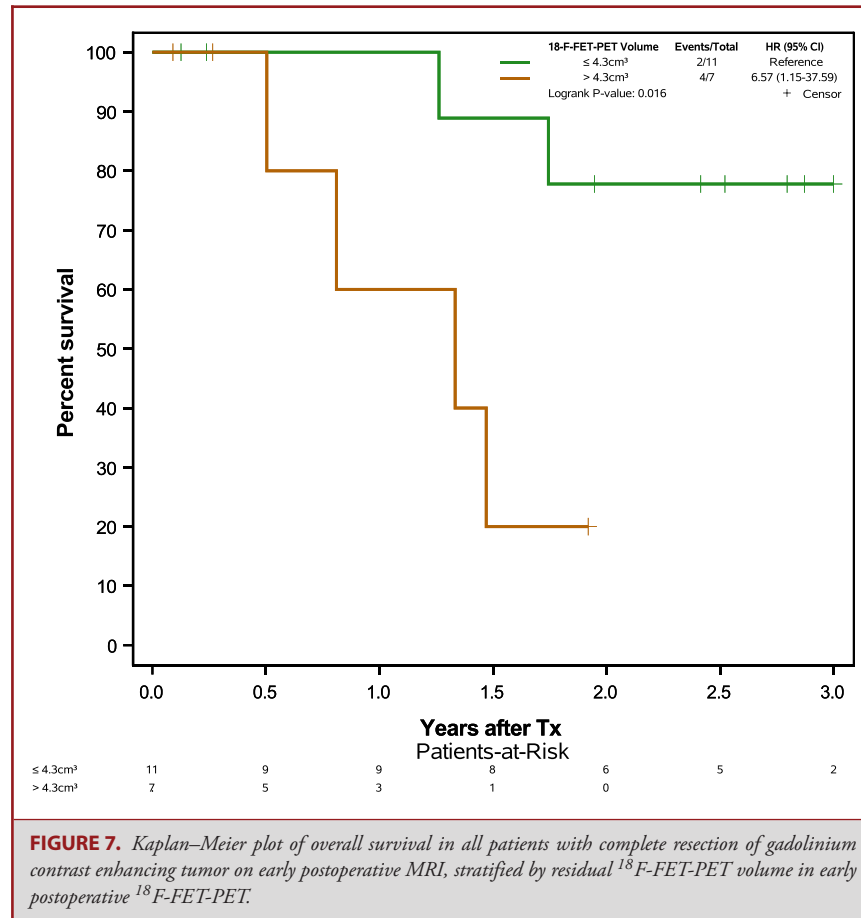
Results are reported as hazard ratios, 95% confidence intervals and P-values from Wald-tests. Gd+ gadolinium contrast enhancing tumor; MGMT O<sup>6</sup>-methylguanine-DNA methyltransferase.

principle value of <sup>18</sup>F-FET-PET for predicting prognosis, it does not assess whether resections outside the gadolinium enhancing portions of GBM confers an additional survival advantage, provided this is done safely. With our observations we demonstrate this to be the case.

The knowledge, that residual postoperative <sup>18</sup>F-FET-PET volumes after surgery in patients with GBM will affect PFS and OS might affect the overall management of patients. We show

that the use of 5-ALA-induced porphyrins can help reduce the residual <sup>18</sup>F-FET-PET tumor volume, underlining the importance of using fluorescence for malignant glioma surgery. Apart from mere surgical aspects, our observations demonstrate <sup>18</sup>F-FET-PET to be useful for estimating residual tumor burden and prognosis on a more biological level. This might translate into decisions regarding adjuvant therapies, such as extended course of temozolomide. Gadolinium enhancement indicates blood-brain barrier breakdown or increased vascular volume in GBM, rather than tumor cell infiltration. Accordingly, <sup>18</sup>F-FET-PET has been proposed as an additional tool in resection planning and for defining additional tissue volumes to be incorporated in radiotherapy planning.<sup>38,39</sup>

From a surgical perspective, we observed that complete resection of fluorescence allows additional tumor removal beyond Gd+. This volume, visualized by <sup>18</sup>F-FET-PET may be used as a new definition of resection target volume. The concept of “supramarginal” resections, ie, beyond the contrast enhancing part of the tumor, has previously been put forward,<sup>40-42</sup> using FLAIR, DTI abnormalities or functional limits to define the borders of resection, provided such extensions of resection can be achieved safely. Deficits from surgery in GBM patients have been associated with decreased survival.<sup>43</sup> The judicious use of intraoperative



monitoring in conjunction with intraoperative fluorescence has already proved for achieving CRET.<sup>14</sup> Future studies are needed to investigate safety and feasibility of extended <sup>18</sup>F-FET-PET based resections.

### Limitations

One limitation of this study is the small number of patients. Since the resulting effect measures are to some extent uncertain, leading to large confidence intervals, the reported results should be interpreted carefully. The determination of the cut-off value was explorative and due to the distribution of the few observed <sup>18</sup>F-FET-PET volumes more precise cut-off estimation was not possible. Due to the sample size, it was also not possible to perform multivariable analyses including relevant covariates and confounders. Consequently, a prospective study is required to confirm results and to validate the cut-off value. Further, the mode of acquiring <sup>18</sup>F-FET-PET needs to be further investigated, eg, regarding timing or the definition of the optimal tumor to brain ratio when calculating <sup>18</sup>F-FET-PET volumes. Authors have used different ratios of 1.6-2.0 resulting in variations in tumor volumes.<sup>44</sup>

Importantly, PET volumetry depends on the definition of thresholds. We conducted our study at a tumor to brain ratio of 1.8, in accordance with the largest previous study<sup>21</sup> and a consensus publication of nuclear medicine and neuro-oncological societies.<sup>45</sup>

Finally, our assessment of residual fluorescence was subjective, which is inherent to this method. Residual fluorescence hidden under blood or behind overhanging brain margins can never be ruled out completely. 5-ALA fluorescence-guided resection is an optical but not spectroscopic method. However, all resections in this study were finalized by the senior author having profound experience with the method. To further overcome these limitations we are in the active planning stage for a multicentric approach to prospectively analyzing the relationship between CRET, 5-ALA derived fluorescence and <sup>18</sup>F-FET-PET and their usefulness for driving resection, looking at safety and outcomes.

### CONCLUSION

Postoperative <sup>18</sup>F-FET-PET volumes correlate with OS and PFS. Resection of 5-ALA derived fluorescence of GBM tissue leads to lower postoperative <sup>18</sup>F-FET-PET tumor volumes and



a higher number of patients below a calculated survival cut off of 4.3 cm<sup>3</sup>. <sup>18</sup>F-FET-PET may potentially serve to better define the target volume, which should be resected in patients with GBM.

## Disclosures

Dr Stummer reports consultant and lecture activities activity for medac (Wedel, Germany), Carl Zeiss Meditech (Oberkochen, Germany), and NxDC (Lexington, Kentucky). The other authors have no personal, financial, or institutional interest in any of the drugs, materials, or devices described in this article.

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**Supplemental Digital Content. Figures.** Kaplan–Meier plots.

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