OPEN

The Value of 5-Aminolevulinic Acid in Low-grade Gliomas and High-grade Gliomas Lacking Glioblastoma Imaging Features: An Analysis Based on Fluorescence, Magnetic Resonance Imaging, ^{18}F-Fluoroethyl Tyrosine Positron Emission Tomography, and Tumor Molecular Factors

Mohammed Jaber, MD*‡
Johannes Wölfer, MD*‡
Christian Ewelt, MD‡
Markus Holling, MD‡
Martin Hasselblatt, MD§
Thomas Niederstadt, MD¶
Tarek Zoubi, MD¶
Matthias Weckesser, MD‖
Walter Stummer, MD‡

‡Department of Neurosurgery, University Hospital Münster, Münster, Germany; §Institute of Neuropathology, University Hospital Münster, Münster, Germany; ¶Institute for Clinical Radiology, University Hospital of Münster, Münster, Germany; ‖Department of Nuclear Medicine, University Hospital of Münster, Münster, Germany

*These authors have contributed equally to this article.

Correspondence:
Walter Stummer, MD,
Neurochirurgische Klinik,
Universitätsklinikum Münster,
Albert-Schweitzer-Campus 1,
Gebäude A1, 48149 Münster,
Germany.
E-mail: walter.stummer@ukmuenster.de

Received, May 31, 2015.
Accepted, August 1, 2015.
Published Online, September 11, 2015.

BACKGROUND: Approximately 20% of grade II and most grade III gliomas fluoresce after 5-aminolevulinic acid (5-ALA) application. Conversely, approximately 30% of nonenhancing gliomas are actually high grade.

OBJECTIVE: The aim of this study was to identify preoperative factors (ie, age, enhancement, 18F-fluoroethyl tyrosine positron emission tomography [^{18}F-FET PET] uptake ratios) for predicting fluorescence in gliomas without typical glioblastomas imaging features and to determine whether fluorescence will allow prediction of tumor grade or molecular characteristics.

METHODS: Patients harboring gliomas without typical glioblastoma imaging features were given 5-ALA. Fluorescence was recorded intraoperatively, and biopsy specimens collected from fluorescing tissue. World Health Organization (WHO) grade, Ki-67/MIB-1 index, IDH1 (R132H) mutation status, O^6-methylguanine DNA methyltransferase (MGMT) promoter methylation status, and 1p/19q co-deletion status were assessed. Predictive factors for fluorescence were derived from preoperative magnetic resonance imaging and ^{18}F-FET PET. Classification and regression tree analysis and receiver-operating-characteristic curves were generated for defining predictors.

RESULTS: Of 166 tumors, 82 were diagnosed as WHO grade II, 76 as grade III, and 8 as glioblastomas grade IV. Contrast enhancement, tumor volume, and ^{18}F-FET PET uptake ratio >1.85 predicted fluorescence. Fluorescence correlated with WHO grade ($P < .001$) and Ki-67/MIB-1 index ($P < .001$), but not with MGMT promoter methylation status, IDH1 mutation status, or 1p19q co-deletion status. The Ki-67/MIB-1 index in fluorescing grade III gliomas was higher than in nonfluorescing tumors, whereas in fluorescing and nonfluorescing grade II tumors, no differences were noted.

CONCLUSION: Age, tumor volume, and ^{18}F-FET PET uptake are factors predicting 5-ALA-induced fluorescence in gliomas without typical glioblastoma imaging features. Fluorescence was associated with an increased Ki-67/MIB-1 index and high-grade pathology. Whether fluorescence in grade II gliomas identifies a subtype with worse prognosis remains to be determined.

KEY WORDS: 5-ALA, Anaplastic glioma, Astrocytoma, FET-PET, Fluorescence-guided resections, Glioblastoma, Glioma

Neurosurgery 78:401–411, 2016 DOI: 10.1227/NEU.0000000000001020 www.neurosurgery-online.com

ABBREVIATIONS: 5-ALA, 5-aminolevulinic acid; **CRT,** classification and regression tree; **^{18}F-FET PET,** 18F-fluoroethyl tyrosine positron emission tomography; **FLAIR,** fluid-attenuated inversion recovery; **GBM,** glioblastoma multiforme; **O^6-MGMT,** methylguanine DNA methyltransferase; **ROC,** receiver-operating characteristic; **SUV,** standardized uptake value; **WHO,** World Health Organization

5-Aminolevulnic acid (5-ALA) is well established for intraoperative fluorescence-guided resection of malignant gliomas.[1-10] The application of 5-ALA for lesions regarded as low-grade gliomas is less accepted, with only 10% to 20% of these tumors showing visible porphyrin accumulation, which can be used for resection.[10,11] On the

other hand, if magnetic resonance imaging (MRI) is suggestive of low-grade glioma, anaplastic foci can be found in 44% to 55% of cases.[12,13] These might be identified by amino acid positron emission tomography and intraoperative fluorescence,[14-16] the latter phenomenon being regarded as helpful for directing tissue sampling during surgery and avoiding undertreatment, if anaplastic foci were not specifically sampled.

Generally, administering 5-ALA to all glioma patients without clear glioblastoma imaging features, irrespective of assumed histology, requires further justification. Thus, the aim of our study was to determine which patients with suspected gliomas benefit from preoperative 5-ALA administration, for instance, by disclosing useful tumor fluorescence after administration of 5-ALA, which in turn might help to reveal high-grade pathologies in apparently low-grade, non- or indistinctly contrast-enhancing gliomas. Patients with unambiguous imaging features for glioblastomas were not included (ie, central necrosis with a rim of nodular enhancement and edema). The current analysis was based on factors typically available before surgery, mainly from imaging. In addition, we used the opportunity to determine whether 5-ALA–induced fluorescence, if observed, had any relationship to World Health Organization (WHO) grading and routinely assessed molecular markers.

METHODS

This study included consecutive patients from the prospectively compiled tumor patient database of the University of Münster Brain Tumor Center. Ethics committee approval was obtained for this analysis.

A total of 166 glioma patients were identified from our database who did not display typical imaging features of glioblastoma on preoperative scans, with no enhancement, weak or patchy enhancement, or strong homogeneous enhancement, and were operated on at our institution using 5-ALA between January 2010 and December 2013. Of these patients, 143 additionally underwent preoperative 18F-fluoroethyl tyrosine positron emission tomography (^{18}F-FET PET) imaging as is the general standard at our institution for all suspected nonglioblastoma multiforme (GBM) gliomas to identify possible "hot spots" for focusing on for histology during surgery.

MAGNETIC RESONANCE IMAGING

Preoperative MRI was performed within a time frame of maximally 3 weeks before surgery on one of two 1.5-T scanners (Achieva and Intera 1.5 T; Philips, Best, the Netherlands) using a 6-channel SENSE head in all patients with multiple sequences. Axial gradient-echo T1-weighted sequences before administration of contrast agent and gradient-echo T1-weighted multiplanar sequence after administration of weight-adapted, intravenous gadolinium-based contrast agent were used for determining enhancement characteristics.

Enhancement was characterized according to 3 categories[17]: no enhancement (Figure 1A), patchy (Figure 1B) or weak enhancement (Figure 1C), or strong, homogeneous enhancement (Figure 1D).

In tumors without enhancement, the diameters for calculating volume were determined from axial fluid-attenuated inversion recovery (FLAIR) images (Figure 1A). These were also used for tumors with patchy, contrast-enhancing regions embedded in

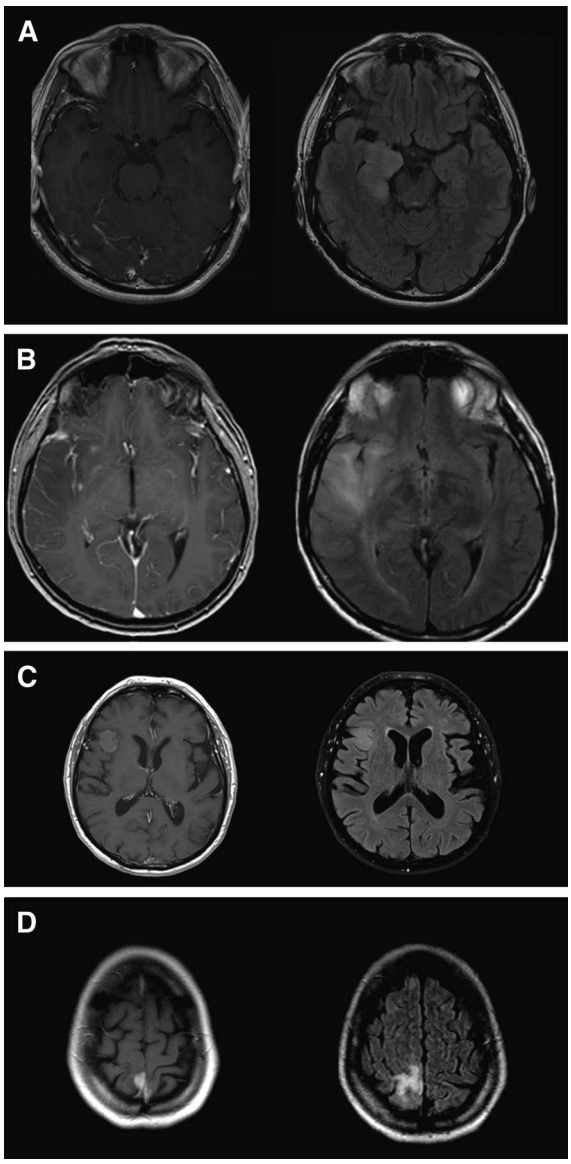

FIGURE 1. *Examples of gliomas without imaging features characteristic for glioblastomas and the different types of enhancement.* **A**, *no enhancement: glioma WHO grade II; Ki-67/MIB-1 index, 5%; IDH1; 1p/19q status not determined; no MGMT promoter methylation; ^{18}F-FET PET uptake ratio, 2.0; ALA+ with a weak patch of fluorescence in hot spot.* **B**, *patchy enhancement: anaplastic astrocytoma WHO grade III; Ki-67/MIB-1 index, 40%; no 1p/19q co-deletion; no MGMT promotor methylation; IDH1, wild type; PET uptake ratio, 2:6; ALA+ (patchy fluorescence).* **C**, *weak enhancement: anaplastic astrocytoma grade III; Ki-67/MIB-1 index, 30%; no MGMT promotor methylation; IDH1, wild type; ^{18}F-FET PET uptake ratio, 3.7; ALA weak homogeneous fluorescence.* **D**, *strong enhancement: anaplastic astrocytoma; Ki-67/MIB-1 index, 30%; IDH1, wild type; 1p19q-, IDH1-, no MGMT promotor methylation; ^{18}F-FET PET uptake ratio, 4.2; ALA+ with strong fluorescence. ALA, aminolevulinic acid; ^{18}F-FET PET, 18F-fluoroethyl tyrosine positron emission tomography; MGMT, O^6-methylguanine DNA methyltransferase; WHO, World Health Organization.*

larger low-grade tumors (Figure 1B) or weakly enhancing tumors without obvious edema (Figure 1C). In patients with strong contrast enhancement and edema, the diameters of the contrast-enhancing tumor regions were measured on the T1-weighted images (Figure 1D).

Case selection, measurement of tumor size, and assessment of contrast enhancement were performed by 2 investigators working together (W.S., M.J.). Tumor size was determined as previously described.[18] Any discrepancies between the assessments of either investigator were resolved by professional discussion.

PET WITH ^{18}F-FET

^{18}F-FET PET was performed as previously described[19] using a hybrid PET–computed tomography (Biograph 16 or mCT, Siemens, Erlangen, Germany). Static images were acquired 20 to 40 minutes after intravenous injection of 3 MBq ^{18}F-FET/kg body weight. Low-dose computed tomography images were acquired for attenuation correction, and images were processed and reconstructed with the standard software as supplied by the manufacturer. PET images were coregistered with MRI using either *Syngo* or *Syngo*.via software (Siemens), and volumes of interest were placed on the tumor to detect the maximal standardized uptake value (SUV). A standardized reference region 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 (Figure 2).

INTRAOPERATIVE ASSESSMENT OF FLUORESCENCE

Patients were pretreated with ALA (Gliolan; Medac, Wedel, Germany) at a dose of 20 mg/kg body weight administered 4 hours before induction of anesthesia. Surgery was performed using an OPMI Pentero (Zeiss, Oberkochen, Germany) equipped with BLUE 400 for visualizing fluorescence in conjunction with neuronavigation. Only 2 dedicated surgeons certified for fluorescence-guided surgery performed resections (J.W., W.S.). Frozen sections were obtained for all cases to confirm glioma histology before to biopsies from regions of interest and definitive resection.

Using Brainlab neuronavigation (Brainlab AG, Feldkirchen, Germany), ^{18}F-FET PET images were fused with preoperative MRI. Target areas were defined on ^{18}F-FET PET and specifically interrogated using neuronavigation as early as possible during resection to minimize inaccuracies caused by brain shift (eg, for the tumor in Figure 2). When fluorescence was encountered, tissue samples were specifically harvested for pathology before tumor resection.

Fluorescence was categorized as no visible fluorescence; weak fluorescence, either patchy or homogeneous throughout the tumor; or strong fluorescence.

Fluorescence categorization was based on the subjective assessment of both surgeons.

NEUROPATHOLOGY

All tumors were assessed neuropathologically according to current WHO criteria[20] by senior neuropathologists unaware of the intraoperative fluorescence findings. Ki-67/MIB-1 proliferation index and IDH1 (R132H) mutation status[21] were determined using immunohistochemistry. O^6-methylguanine DNA methyltransferase (MGMT) promoter methylation status was determined in all malignant gliomas (WHO grades III and IV). Briefly, after isolation and bisulfite conversion (EZ DNA Methylation-Gold Kit; Zymo Research, Orange, California), DNA from representative formalin-fixed, paraffin-embedded material was subjected to methylation-specific polymerase chain

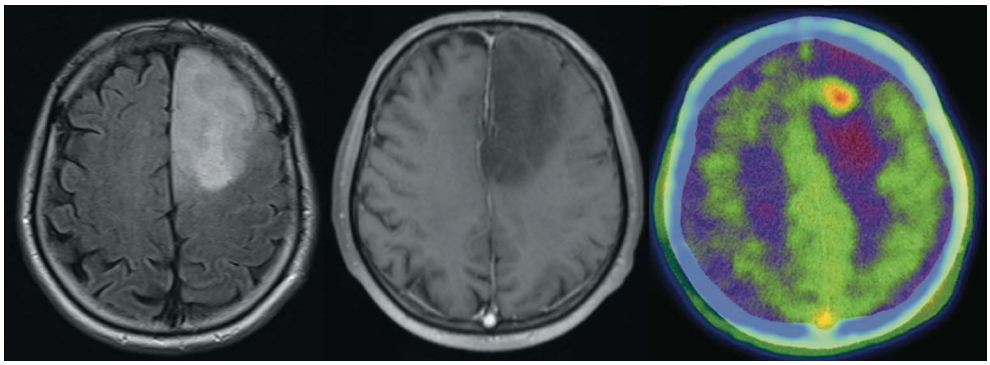

FIGURE 2. *Example of a GBM without typical imaging features of a GBM. This particular tumor showed no enhancement. The ^{18}F-FET PET hot spot, which was located with neuronavigation, showed strong fluorescence (Ki-67/MIB-1 index, 25%; IDH1, wild type; 1p/19q status not determined; no O^6-methylguanine DNA methyltransferase promotor methylation; ^{18}F-FET PET uptake ratio, 3.7). ^{18}F-FET PET, 18F-fluoroethyl tyrosine positron emission tomography; GBM, glioblastoma multiforme. Color version available online only.*

reaction, as described previously.[22] Controls included clones representing methylated and unmethylated bisulfite-converted DNA, tumor samples with known methylation status, as well as negative controls (H_2O). Determination of 1p/19q co-deletion status was performed using quantitative polymerase chain reaction, as described previously[23] in all tumors, in which an oligodendroglial differentiation was suspected histopathologically.

STATISTICAL METHODS

Data were analyzed by commercially available software (SPSS version 21.0; SPSS Inc, Chicago, Illinois). Statistical significance was considered at $P < .05$. For contingency tables, the Pearson χ^2 test for categorical data with Bonferroni correction was used. The significance of differences in the mean value between multiple independent groups was determined using analysis of variance with post hoc Scheffé test. For 2 independent groups, the t test was used. Classification and regression tree (CRT) analysis was used for identifying predictors for intraoperative fluorescence. Receiver-operating characteristic (ROC) curves were generated to test the influence of ^{18}F-FET PET uptake ratio thresholds on the sensitivity and specificity of predicting fluorescence based on PET.

RESULTS

Preoperative Patient and Imaging Characteristics

Preoperative characteristics of tumors without imaging features typical of GBMs are summarized in Table 1. Eighty-two were diagnosed as WHO grade II, 76 as WHO grade III (17 anaplastic oligodendrogliomas or oligoastrocytoma, 59 anaplastic astrocytomas), and 8 as WHO grade IV (Table 1).

WHO grade was associated with significant differences in age (median age for grade II tumors, 40.8 years; 48.4 years for grade III tumors; and 58.5 years for grade IV tumors). Differences were also found on imaging, with only 9% of grade II tumors showing any form of contrast enhancement compared with 86.8% of grade III tumors and 87.5% of our preselected grade IV tumors. Tumor volumes were greatest in grade III tumors. Preselection in our GBMs (ie, those with non-GBM imaging features) was also reflected by their volumes, which were similar to our grade II tumor volumes and their peak uptake ratios, which were again similar to the values obtained for grade II gliomas. The peak ^{18}F-FET PET uptake ratio was highest in grade III tumors.

Intraoperative Findings

Of 82 tumors designated as WHO grade II as final histology, 15.9% revealed fluorescence intraoperatively. In 4.9% of cases, this fluorescence was considered strong. Of grade III tumors, 77.6% displayed intraoperative fluorescence, which was considered strong in 21% of cases. The subgroup of GBM with non-GBM imaging features showed fluorescence in all cases, considered strong in 75% of cases (Table 2).

Importantly, of 10 grade III gliomas that did not show enhancement on preoperative MRI, 7 (70%) revealed intraoperative fluorescence (Table 3). This was similar to enhancing grade III gliomas, of which 55 of 66 (83.3%) showed intraoperative fluorescence.

TABLE 1. Patient Data Stratified by WHO Grade[a]

	Grade II	Grade III	Grade IV	*P* Value
No.	82	76	8	
Age, yr, avg. ± SD	43.3 ± 14.6	50.5 ± 15.4	60.7 ± 10.4	.00 (1-way ANOVA)
Median	40.8	48.4	58.5	
Range	16.4-84.6	19.5-83.2	50.3-80.33	
	$P = .01$ vs III°	$P = .129$ vs IV°		
	$P = .01$ vs IV°, (post hoc Scheffé)			
Sex, no. (%)				
Female	33 (40.2)	47 (61.8)	3 (37.5)	.096 (χ^2 test)
Male	49 (59.8)	29 (38.2)	5 (62.5)	
MRI enhancement, no. (%)				
None	63 (76.9)	10 (13.2)	1 (12.5)	.00 (χ^2 test)
Weak, patchy	12 (14.6)	47 (61.8)	3 (37.5)	
Strong	7 (8.54)	19 (25)	4 (50.0)	
	$P < .05$ (after Bonferroni) vs III°, IV°			
Volume, cm^3				
Avg. ± SD	9.65 ± 11.8	20.63 ± 32.0	11.6 ± 9.11	.02 (1-way ANOVA)
	$P = .02$ vs III°, (post hoc Scheffé)			
^{18}F-FET PET SUV				
Peak ± SD	2.60 ± 1.43	3.38 ± 1.59	2.66 ± 1.55	.02 (1-way ANOVA)
	$P = .02$ vs III°, (post hoc Scheffé)			

[a] °, grade; WHO, World Health Organization; avg., average; ANOVA, analysis of variance; MRI, magnetic resonance imaging; ^{18}F-FET PET SUV, 18F-fluoroethyl tyrosine positron emission tomography standardized uptake value.

TABLE 2. Intraoperative Fluorescence Findings and Molecular Pathology Stratified by WHO Grade[a]

	Grade II	Grade III	Grade IV	*P* Value
No.	82	76	8	
MIB index, avg. ± SD	5.13 ± 5.35	13.7 ± 5.35	27 ± 13.5	.000 (1-way ANOVA)
Median	3	10	30	
Range	1-30	5-60	5-40	
	P = .00 vs III°, IV° (post hoc Scheffé)	*P* = .00 vs IV° (post hoc Scheffé)		
Subgroups				
Fluorescing tumors, no. (%)	13 (15.8)	59 (77.6)		
Avg. ± SD	5.80 ± 5.6	15.5 ± 15.1		
Nonfluorescing tumors, no. (%)	69 (83.2)	17 (22.4)		
Avg. ± SD	5.02 ± 5.4	8.08 ± 6.3		
	NS	*P* = .02 (*t* test)		
MGMT promoter methylation (subset), no. (%)				
No	5 (29.4)	24 (39.3)	8 (100)	NS, (χ^2 test); grade. 4: number too small for meaningful analysis
Yes	12 (70.6)	37 (60.7)	0 (0)	
1p19q status (only determined if OA or OD)				
Wild type, no. (%)	0 (0)	2 (11.8)		NS (χ^2 test)
Co-deletion, no. (%)	19 (100)	15 (88.2)	ND	
IDH1 status, no. (%)				
Wild type	23 (34.8)	25 (51.0)	ND	.02 (χ^2 test)
Mutation	43 (65.2)	24 (49.0)		
Fluorescence, no. %				
None	69, 84.1[b] vs III°, IV°	17, 22.4	0, 0	.00 (χ^2 test)
Weak, patchy, no. (%)	5 (61.0)	9 (11.8)	1 (12.5)	
Weak, homogeneous, no. (%)	4 (4.9)	34 (44.7)[b] vs II°	1 (12.5)	
Strong, no. (%)	4 (4.9)	16 (21.1)[b] vs II°	6 (75.0)[b] vs II°, III°	

[a] °, grade; WHO, World Health Organization; avg., average; ANOVA, analysis of variance; MGMT, O^6-methylguanine DNA methyltransferase; NS, not significant; OA, oligoastrocytoma; OD, oligodendroglioma; ND, not determined.
[b] $P < .05$ (after Bonferroni correction).

Histological and Molecular Findings

The MIB index closely mirrored pathological grade (5% for grade II, 13% for grade III, and 27% for WHO grade IV tumors) (Table 2). All of the tumors in our selected GBM cohort failed to reveal MGMT promoter methylation. Of 61 patients with grade III gliomas, 37 (60.7%) showed MGMT promoter methylation. A total of 65% of grade II tumors showed IDH1 (R132H) mutations compared with only 49% of grade III tumors and none of the GBMs. The presence of 1p/19q co-deletions was assessed in all patients with light microscopy findings suggestive of oligodendro- or oligoastrocytoma (n = 19 grade II and 17 grade III gliomas). All grade II tumors with these findings were found to have this genetic aberration compared with 88% of WHO grade III tumors. The presence of 1p/19q co-deletions was not determined in our subset of GBMs.

Ex Ante Prediction of Fluorescence

For predicting intraoperative tumor fluorescence, all preoperative imaging and available biological patient characteristics were considered. These included age, sex, Karnofsky Performance Status, tumor size and enhancement (including type of enhancement, ie, weak, patchy, strong), edema on MRI, and the peak uptake ratio on ^{18}F-FET PET.

CRT analysis (Figure 3) revealed *any* preoperative contrast-enhancement to be the strongest discriminator between visibly fluorescing and nonfluorescing tumors, ie, 78.0% of enhancing tumors showed fluorescence in contrast to 16% of nonenhancing tumors. The type of enhancement (strong, weak, or patchy) was not found to be predictive of fluorescence per se.

However, we did find a relationship between the type of enhancement and the type of fluorescence. Strongly enhancing tumors tended to fluoresce strongly (Table 4).

For the subgroup of contrast-enhancing tumors, age (>44 years) and volume were further discriminators, eg, 96.7% of all enhancing tumors of patients older than 44 years of age and a volume greater than >6.7 cm would show fluorescence. For nonenhancing tumors (n = 83), 12 (16%) were found to fluoresce. Of these, the likelihood for fluorescence for tumors

TABLE 3. Intraoperative Fluorescence Findings and Preoperative Enhancement on Magnetic Resonance Imaging in Grade III Tumors

Grade III Tumors Only	No Enhancement	Enhancement	*P* Value
No.	10	66	
No fluorescence, no. (%)	3 (30)	11 (16.7)	.31 (χ^2 test)
Fluorescence, no. (%)	7 (70)	55 (83.3)	

with an uptake ratio greater than 1.85 and a size larger than 10.6 cm for showing fluorescence was 46.2% of cases with these characteristics. No fluorescence was observed with an uptake ratio of less than 1.85 (n = 23).

ROC analysis incorporates all possible thresholds that can be defined if a diagnostic test yields values on a continuous numerical basis. The overall diagnostic accuracy for ^{18}F-FET PET as a predictor of fluorescence was calculated by ROC analysis as 69% (*P* = .00) (Figure 4). For nonenhancing tumors, a similar value was obtained, albeit with marginal significance (70% accuracy, *P* = .06) (Figure 4).

The diagnostic accuracy calculated for the contrast enhancement feature for predicting fluorescence was higher, ie, 81.3% (sensitivity, 86.6%, specificity, 76.2%).

In these analyses, accuracy is defined as (TP + TN)/(P + N), sensitivity as TP/(TP + FN), and specificity as TN/(TN + FP), where TP is a true positive, FP is a false positive, FN is a false negative, TN is a true negative, P is all positives, and N is all negatives.

Histological Findings Stratified by Intraoperative Fluorescence

We wanted to examine the significance of visible fluorescence regarding associated histological findings, as summarized in Table 5. If any fluorescence was found, the Ki-67/MIB-1 index was significantly higher than in nonfluorescing tumors (14.5% vs 5.8%). Furthermore, a high-grade (grade III or IV) pathology was significantly more frequently observed if fluorescence was

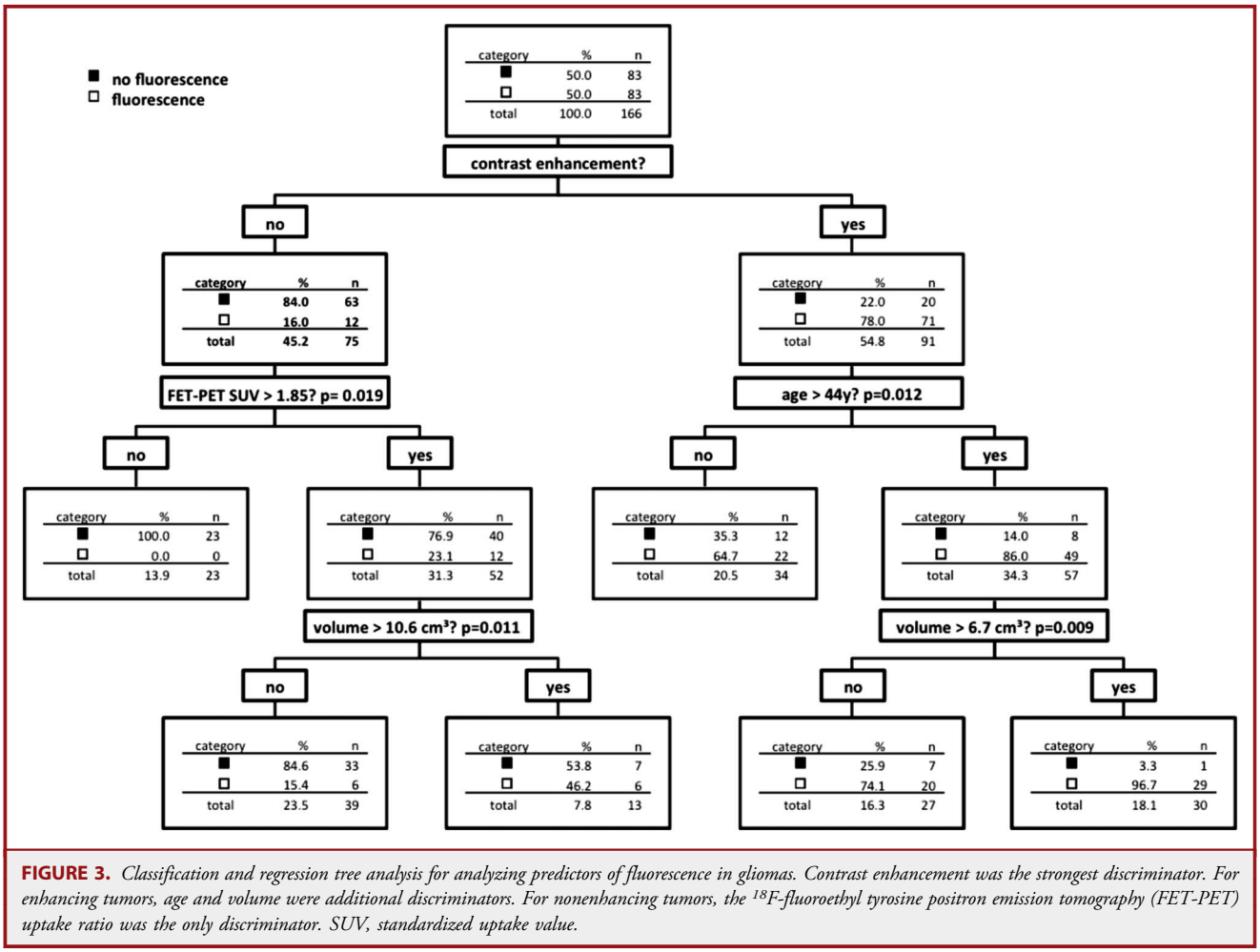

FIGURE 3. *Classification and regression tree analysis for analyzing predictors of fluorescence in gliomas. Contrast enhancement was the strongest discriminator. For enhancing tumors, age and volume were additional discriminators. For nonenhancing tumors, the ^{18}F-fluoroethyl tyrosine positron emission tomography (FET-PET) uptake ratio was the only discriminator. SUV, standardized uptake value.*

TABLE 4. Correlation Fluorescence vs Enhancement

	No Fluorescence	Weak Fluorescence[a]	Strong Fluorescence[a]
No enhancement	62	12	0
Weak/patchy enhancement	19	28	14
Strong enhancement	4	14	12

Overall $P = .00$.
[a]$P < .05$ vs no fluorescence.

encountered. No relationship was noted between fluorescence and IDH mutations, 1p19q co-deletions, or MGMT promotor methylation.

We looked specifically at the Ki-67/MIB-1 index, comparing grade II and grade III gliomas with or without fluorescence (Table 2). The Ki-67/MIB-1 index for nonfluorescing grade II tumors was 5.80% and 5.02% for fluorescing grade II tumors. Thus, the Ki-67/MIB-1 index did not provide an explanation for the presence of fluorescence in some low-grade gliomas in our cohort. For grade III tumors, on the other hand, the Ki-67/MIB-1 index of fluorescing grade III gliomas was almost double (15.5%) the value compared with nonfluorescing grade III gliomas (8.1%).

We analyzed whether oligodendroglial or oligoastrocytic differentiation was associated with fluorescence findings in grade II and III gliomas. We compared these with tumors diagnosed as diffuse astrocytomas (Table 6). No major differences were found between these subtypes regarding fluorescence accumulation. Fluorescence accumulation depended most strongly on WHO grade rather than subtype.

When considering fluorescence as a diagnostic test for high-grade histology using the data given in Tables 2 and 5, the sensitivity can be calculated as 83.3%, with a specificity of 84.1% (accuracy, 81.8%). This was very similar when the presence of any enhancement on MRI (patchy, weak, or strong) was used as a diagnostic test for high-grade histology. The respective values were 86.9%, 76.8%, and 81.9%.

However, the positive predictive value of fluorescence for predicting grades III or IV pathology was 84.3%. This value was slightly lower for the contrast enhancement feature (79.8%).

DISCUSSION

This analysis was performed to determine which preoperative imaging factors might serve to predict fluorescence accumulation in gliomas that do not show the typical radiological characteristics of glioblastoma, ie, marginal nodular contrast enhancement with central necrosis on MRI. To date, the use 5-ALA for fluorescence-guided resections is well established for malignant gliomas, mainly GBMs. In the seminal study by Stummer et al,[2] the largest study to date on 5-ALA and malignant gliomas, 98% of patients had GBMs, and most studies on fluorescence so far are focused on this most common of glial tumors.[6,24-28]

On the other hand, even though being numerically less frequent, patients with low-grade gliomas have been described as having tumors with visible porphyrin fluorescence in 10% to 20% of cases[10,11] when using the microscope to visualize fluorescence. When more sensitive technology is used for detecting tissue protoporphyrin IX, such as confocal microscopy[29]

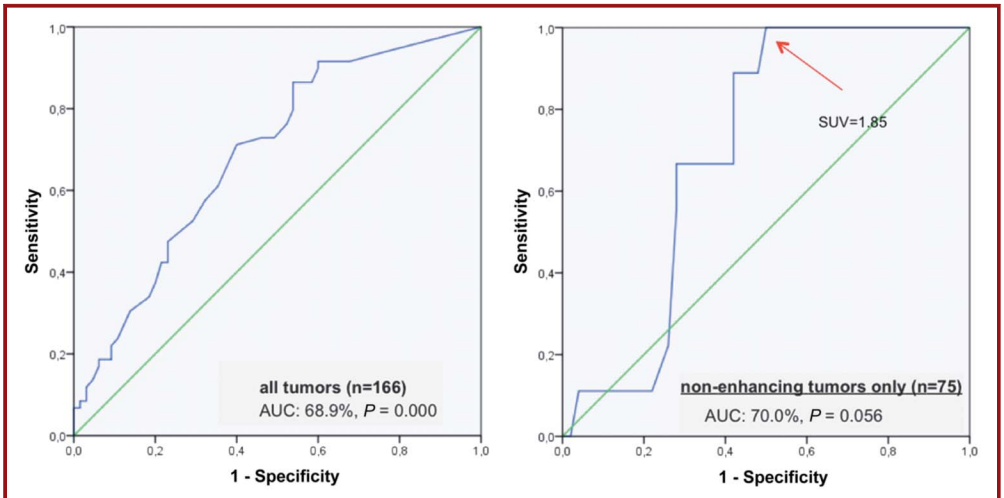

FIGURE 4. *Receiver-operating characteristic curves demonstrating the relationship of the ^{18}F-fluoroethyl tyrosine positron emission tomography uptake ratio as a diagnostic indicator of intraoperative fluorescence. The accuracy of positron emission tomography for predicting fluorescence was 69% (left) and 70% for nonenhancing tumors (right). Note the threshold of 1.85 for nonenhancing tumors. Fluorescence was only observed above this threshold. AUC, area under the curve. Color version available online only.*

TABLE 5. Histological and Molecular Findings of Tumor Tissue Biopsies Stratified by Intraoperative Fluorescence Findings[a]

	Fluorescence	No Fluorescence	*P* Value
No.	83	83	
MIB index, avg. ± SD	14.5 ± 14.6	5.77 ± 5.99	.00 (2-sided *t* test)
Median	10	4	
Range	5-60	1-30	
MGMT promoter methylation (subset), no. (%)			
No	26 (41.9)	11 (45.8)	.11 (χ^2 test)
Yes	36 (58.1)	13 (54.2)	
1p19q status, no. (%)			
Wild type	1 (5.9)	1 (5.3)	.94 (χ^2 test)
Co-deletion	16 (94.1)	18 (994.7)	
IDH1 status, no. (%)			
Wild type	26 (52.0)	26 (37.7)	.12 (χ^2 test)
Mutation	24 (48.0)	43 (62.3)	
WHO grade, no. (%)			
II	13 (15.7)[b]	69 (83.1)	.00 (χ^2 test)
III	62 (74.7)[b]	14 (16.9)	
IV	8 (9.6)[b]	0 (0)	

[a]avg., average; MGMT, O^6-methylguanine DNA methyltransferase; WHO, World Health Organization.
[b]$P < .05$ vs no fluorescence after Bonferroni correction.

and spectrography,[30,31] almost all low-grade gliomas appear to display some degree of porphyrin accumulation.

The broad category of grade III gliomas would be expected to show intermediate behavior regarding fluorescence. This is the first series to study a large cohort of gliomas without imaging features typical of GBMs to determine the propensity of 5-ALA for intraoperative marking of tumor tissue. With the exception of 8 tumors that histologically were found to be GBMs but did not show typical imaging features on preoperative MRI, all our tumors were grade II (n = 82) or III (n = 76). With our study, we now verify earlier observations regarding ^{18}F-FET PET, fluorescence, and histology in a large cohort of patients. To this end, several studies[10,15,16] have demonstrated that tumors with fluorescence are more likely to have a high Ki-67/MIB-1 index as an indicator for proliferation and have found a relationship between ^{18}F-FET and MET-PET, and fluorescence. These authors uniformly regard this phenomenon as being useful for finding anaplastic foci in diffuse low-grade gliomas to avoid undergrading these tumors. Our observations support this view and further expand existing knowledge because ours is the largest collective of truly non-contrast-enhancing tumors. Others[10] have investigated a subgroup of patients with nonsignificant enhancement that included tumors of patients with patchy/faint and small regional enhancement, but did not explicitly discern tumors *without* any enhancement. In our series, we found a meaningful number of patients with grade III tumors without fluorescence (n = 10). Despite their lack of enhancement, 70% of these grade III gliomas revealed intraoperative fluorescence, demonstrating that fluorescence is more sensitive than enhancement in detecting malignant pathology.

TABLE 6. Grades II and III Glioma Subtypes Stratified by Intraoperative Fluorescence Findings[a]

Histology	No.	Fluorescence	No Fluorescence	*P* Value
DA, no. (%)				
II	64	9 (14.1)	55 (85.9)	.00 (χ^2 test)
III	51	43 (84.3)	8 (15.7)	
OD, no. (%)				
II	2	1 (50.0)	1 (50.0)	.33 (χ^2 test)
III	11	9 (81.8)	2 (18.2)	
OA, no. (%)				
II	16	3 (18.8)	13 (81.2)	.004 (χ^2 test)
III	14	10 (71.4)	4 (28.6)	
OA or OD, no. (%)				
II	18	4 (22.2)	14 (77.8)	.00 (χ^2 test)
II	25	19 (76.0)	6 (24.0)	

[a]DA, diffuse astrocytoma; OD, oligodendroglioma; OA, oligoastrocytoma.

We did not find an influence of oligodendroglial or oligoastrocytic differentiation on fluorescence findings in grade II and III gliomas and demonstrate in a large cohort of patients that these subtypes do not behave differently to diffuse astrocytomas regarding fluorescence.

On the other hand, we also define novel relationships to other factors (age, tumor size, contrast enhancement) to predict in which tumors intraoperative fluorescence can be found, which would in turn serve to specifically interrogate such areas of anaplasia for histology, even in the absence of ^{18}F-FET PET. Under certain circumstances we also found contrast-enhancing tumors not to accumulate visible fluorescence, eg, in some younger patients.

Overall, we consider these observations of value for the concept of fluorescence-guided resections, not only for identifying patients with tumors but with the potential to show intraoperative fluorescence if 5-ALA is administered preoperatively. Our observations indicate that not only should areas that fluoresce be sampled specifically, they should also be resected if safely possible because fluorescence often indicates high-grade pathology, and the removal of fluorescing tissues may be relevant for prognosis.

Ex Ante Factors for Predicting Intraoperative Fluorescence

In our study, the best discriminator for predicting whether a tumor would show fluorescence was any form of contrast enhancement on preoperative MRI. We also observed a relationship between the degree of enhancement and the degree of fluorescence. Interestingly, for contrast-enhancing tumors, both age and volume could further discriminate tumors with fluorescence from nonfluorescing tumors. The reasons for this

observation can only be speculated on. Older patients tend to harbor more aggressive (ie, higher grade tumors[32]), and size in low-grade gliomas has been associated with a worse prognosis in a large European Organization for Research and Treatment of Cancer series.[33] Overall, however, only 16.0% of nonenhancing tumors showed significant fluorescence in our study, which included subsets of grade II and III tumors. Conversely, 16% of low-grade gliomas in our cohort revealed fluorescence detectable with the surgical microscope.

In contrast to our results, Stockhammer et al[15] found a higher threshold of 2.32 of the ^{18}F-FET PET uptake ratio for predicting fluorescence than the currently encountered value of 1.85. However, their series of 13 patients included only 1 patient with a grade II tumor, 1 patient with a grade III tumor, and 11 GBMs. In our 8 GBMs, the peak uptake ratio was also lower than found by Stockhammer et al.[15] This is best explained by our particular, highly preselected subgroup of GBMs that did not show typical imaging features of GBMs on preoperative imaging, such as central necrosis or nodular, marginal contrast enhancement (cf. Figure 2).

When Would Administration of 5-ALA Be of Potential Value?

From a practical point of view, when faced with the decision of whether to administer 5-ALA in patients with gliomas without features of GBMs for finding anaplastic foci and for performing fluorescence-guided resections, our CRT analysis shows that any form of contrast enhancement in a glioma will result in a high likelihood of finding useful fluorescence (78%). In addition, if patients are older than 44 years of age, this likelihood increases to 86%. If tumors are further larger than approximately 7 cm (a diameter of ~2.2 cm), the likelihood increases to 97%.

Still, 5-ALA may be of value for nonenhancing tumors in approximately 20% of cases. If surgeons have access to ^{18}F-FET PET, our CRT analysis showed that this can be used for predicting visible fluorescence. Our data show that an SUV ratio greater than 1.85 is the strongest factor predicting such fluorescence. No tumor with a ratio of less than 1.85 revealed fluorescence. If tumors additionally have a volume exceeding 10.6 cm (corresponding to a diameter of ~2.8 cm), the likelihood of fluorescence is 46%, making patients with such tumors the best candidates for 5-ALA.

The degree of contrast enhancement will be prognostic of which type of visible fluorescence to expect, ie, strong enhancement will predict strong fluorescence in many cases and patchy or weak enhancement mostly weak fluorescence.

What Does Fluorescence Signify If Encountered?

From the ex post perspective, if fluorescence was found intraoperatively, the likelihood for a tumor with high-grade pathology was 85%, which reproduces the positive predictive value calculated by Widhalm et al[10] for forecasting high-grade tumors from fluorescence alone. Their series also included patients with patchy, weak, or focal fluorescence termed tumors with "nonsignificant" enhancement. In that study, a number of morphological factors apart from the Ki-67/MIB-1 index were found to indicate a high-grade glioma phenotype if fluorescence was observed.

Interestingly, we were able to identify 2 subgroups of patients with tumors histologically diagnosed as grade II that did not differ in terms of the Ki-67/MIB-1 index. Thus, other factors apart from proliferation were driving protoporphyrin IX fluorescence accumulation in these tumors. We do not know the significance of this finding, but are following these patients closely to determine whether fluorescence plays a prognostic role even though the Ki-67/MIB-1 index was the same. In the broad category of grade III tumors, in contrast, fluorescing tumors revealed a higher Ki-67/MIB-1 index than nonfluorescing tumors, as expected.

No relationship was established for fluorescence and the other molecular markers IDH1, 1p/19q, and MGMT promoter methylation. Although a correlation between fluorescence and 1p19q co-deletion or MGMT promoter methylation would appear unexpected, a correlation between fluorescence and IDH mutations could be hypothetically be possible.

IDH1 is involved in the Krebs cycle, which generates succinyl coenzyme A from α-ketoglutarate, which is the product of dehydrogenation of D-isocitrate by IDH. Succinyl coenzyme A, together with glycine, forms ALA under physiological conditions. Thus, tumors with IDH mutations might have demonstrated differences regarding protoporphyrin IX synthesis, possibly upregulation of this important pathway in response to a lower physiological availability of 5-ALA. Such differences were not observed, however, making a significant influence unlikely.

Limitations

Albeit derived from data from a prospectively collected database, our analysis lacked source information on whether intraoperatively observed areas of fluorescence corresponded to preoperatively defined areas of hypermetabolism on the ^{18}F-FET PET or contrast enhancement on MRI. However, we[16] and others[10] previously noted a strong spatial relationship between ^{18}F-FET-PET hypermetabolism and porphyrin fluorescence.

Also, determining radiographic tumor volume was not uniformly possible when regarding the wide range of tumor subtypes and their respective imaging features, especially in the grade III category. Although FLAIR volume is considered a good indicator of tumor volume in low-grade gliomas,[34] a subgroup of grade III tumors showed edema that would be difficult to distinguish from significant tumor on the FLAIR images. Thus, for these tumors with significant enhancing tumor volumes and edema, we instead chose the contrast-enhancing tumor as depicted on T1-weighted images with contrast for our volume determination. However, volume was only found to be of predictive value in our subgroup of nonenhancing tumors in which uniformly the FLAIR volume was measured and edema was not an issue.

Our analysis in nonenhancing tumors was based on ^{18}F-FET PET imaging, which is not available to many neurosurgeons on a routine basis. In the absence of ^{18}F-FET PET, however, the

MET PET and chemical shift imaging have been used to detect hot spots and to correlate these with intraoperative fluorescence findings.[10] To our knowledge, a specific study on the correlation between chemical shift imaging and intraoperative fluorescence is missing, but certainly warranted, to avoid the necessity of amino acid PET for those surgeons who do not have access to this technology.

Finally, our categorization of enhancement on MRI as none, patchy, weak, strong, and homogeneous was simple but not strictly validated, although a similar system was used in an earlier study with comparable aims.[10]

Also, the classification of fluorescence as strong, weak, and/or patchy was not determined by objective criteria, such as spectrometry, as previously described.[30,31] To this end, a recent study from our group demonstrated a good correlation between visually and spectrographically observed fluorescence,[30] and we feel confident, at least for the sake of this analysis and its practical implementation, that these assessments are acceptable.

For the future, we are planning independent validation of our findings in an independent cohort of patients. It is well accepted that CRTs or similar analyses might depend on known or unknown factors, which are center or cohort specific.

CONCLUSION

We present a simple system for predicting fluorescence after 5-ALA administration in patients with low-grade and high-grade gliomas that do not fulfill imaging characteristics of GBMs. These data can be used for selecting patients that might benefit from 5-ALA fluorescence-guided resection. We find that if fluorescence is encountered, this fluorescence mostly highlights tissue areas with malignant glioma morphology. Biopsies of such areas should be performed specifically and, if safely possible, resected. We do not provide an explanation for why some low-grade gliomas show fluorescence because this finding was not related to any of the molecular factors that were analyzed, including the Ki-67/MIB-1 index.

Disclosures

Walter Stummer has received consultant fees from medac and speakers fees from Zeiss. The other authors have no personal, financial, or institutional interest in any of the drugs, materials, or devices described in this article.

REFERENCES

1. Roberts DW, Valdés PA, Harris BT, et al. Coregistered fluorescence-enhanced tumor resection of malignant glioma: relationships between δ-aminolevulinic acid-induced protoporphyrin IX fluorescence, magnetic resonance imaging enhancement, and neuropathological parameters. Clinical article. *J Neurosurg.* 2011;114(3):595-603.
2. Stummer W, Pichlmeier U, Meinel T, Wiestler OD, Zanella F, Reulen HJ; ALA Glioma Study Group. Fluorescence-guided surgery with 5-aminolevulinic acid for resection of malignant glioma: a randomised controlled multicentre phase III trial. *Lancet Oncol.* 2006;7(5):392-401.
3. Stummer W, Novotny A, Stepp H, Goetz C, Bise K, Reulen HJ. Fluorescence-guided resection of glioblastoma multiforme by using 5-aminolevulinic acid-induced porphyrins: a prospective study in 52 consecutive patients. *J Neurosurg.* 2000;93(6):1003-1013.
4. Stummer W, Stocker S, Wagner S, et al. Intraoperative detection of malignant gliomas by 5-aminolevulinic acid-induced porphyrin fluorescence. *Neurosurgery.* 1998;42(3):518-525.
5. Diez Valle R, Tejada Solis S, Idoate Gastearena MA, García de Eulate R, Domínguez Echávarri P, Aristu Mendiroz J. Surgery guided by 5-aminolevulinic fluorescence in glioblastoma: volumetric analysis of extent of resection in single-center experience. *J Neurooncol.* 2011;102(1):105-113.
6. Aldave G, Tejada S, Pay E, et al. Prognostic value of residual fluorescent tissue in glioblastoma patients after gross total resection in 5-aminolevulinic acid-guided surgery. *Neurosurgery.* 2013;72:915-920.
7. Schucht P, Beck J, Abu-Isa J, et al. Gross total resection rates in contemporary glioblastoma surgery: results of an institutional protocol combining 5-ALA intraoperative fluorescence imaging and brain mapping. *Neurosurgery.* 2012;71(5):927-935.
8. Nabavi A, Thurm H, Zountsas B, et al; 5-ALA Recurrent Glioma Study Group. Five-aminolevulinic acid for fluorescence-guided resection of recurrent malignant gliomas: a phase ii study. *Neurosurgery.* 2009;65(6):1070-1076.
9. Floeth FW, Pauleit D, Wittsack HJ, et al. Multimodal metabolic imaging of cerebral gliomas: positron emission tomography with [18F]fluoroethyl-L-tyrosine and magnetic resonance spectroscopy. *J Neurosurg.* 2005;102(2):318-327.
10. Widhalm G, Kiesel B, Woehrer A, et al. 5-Aminolevulinic acid induced fluorescence is a powerful intraoperative marker for precise histopathological grading of gliomas with non-significant contrast-enhancement. *PLos One.* 2013;8(10):e76988.
11. Nishikawa R. Fluorescence illuminates the way. . .. *Neuro Oncol.* 2011;13(8):805.
12. Stockhammer F, Plotkin M, Amthauer H, van Landeghem FK, Woiciechowsky C. Correlation of F-18-fluoro-ethyl-tyrosin uptake with vascular and cell density in non-contrast-enhancing gliomas. *J Neurooncol.* 2008;88(2):205-210.
13. Kunz M, Thon N, Eigenbrod S, et al. Hot spots in dynamic (18)FET-PET delineate malignant tumor parts within suspected WHO grade II gliomas. *Neuro Oncol.* 2011;13(3):307-316.
14. Floeth FW, Sabel M, Ewelt C, et al. Comparison of (18)F-FET PET and 5-ALA fluorescence in cerebral gliomas. *Eur J Nucl Med Mol Imaging.* 2011;38(4):731-741.
15. Stockhammer F, Misch M, Horn P, Koch A, Fonyuy N, Plotkin M. Association of F18-fluoro-ethyl-tyrosin uptake and 5-aminolevulinic acid-induced fluorescence in gliomas. *Acta Neurochir (Wien).* 2009;151(11):1377-1383.
16. Ewelt C, Floeth FW, Felsberg J, et al. Finding the anaplastic focus in diffuse gliomas: the value of Gd-DTPA enhanced MRI, FET-PET, and intraoperative, ALA-derived tissue fluorescence. *Clin Neurol Neurosurg.* 2011;113(7):541-547.
17. Pallud J, Capelle L, Taillandier L, et al. Prognostic significance of imaging contrast enhancement for WHO grade II gliomas. *Neuro Oncol.* 2009;11(2):176-182.
18. Pichlmeier U, Bink A, Schackert G, Stummer W; the ALA Glioma Study Group. Resection and survival in glioblastoma multiforme: an RTOG recursive partitioning analysis of ALA study patients. *Neuro Oncol.* 2008;10(6):1025-1034.
19. Weckesser M, Langen KJ, Rickert CH, et al. O-(2-[18F]fluorethyl)-L-tyrosine PET in the clinical evaluation of primary brain tumours. *Eur J Nucl Med Mol Imaging.* 2005;32(4):422-429.
20. Louis DN, Ohgaki H, Wiestler OD, et al. The 2007 WHO classification of tumours of the central nervous system. *Acta Neuropathol.* 2007;114(2):97-109.
21. Capper D, Zentgraf H, Balss J, Hartmann C, von Deimling A. Monoclonal antibody specific for IDH1 R132H mutation. *Acta Neuropathol.* 2009;118(5):599-601.
22. Felsberg J, Rapp M, Loeser S, et al. Prognostic significance of molecular markers and extent of resection in primary glioblastoma patients. *Clin Cancer Res.* 2009;15(21):6683-6693.
23. Nigro JM, Takahashi MA, Ginzinger DG, et al. Detection of 1p and 19q loss in oligodendroglioma by quantitative microsatellite analysis, a real-time quantitative polymerase chain reaction assay. *Am J Pathol.* 2001;158(4):1253-1262.
24. Kamp MA, Felsberg J, Sadat H, et al. 5-ALA-induced fluorescence behavior of reactive tissue changes following glioblastoma treatment with radiation and chemotherapy. *Acta Neurochir (Wien).* 2015;157(2):207-213.
25. Schucht P, Knittel S, Slotboom J, et al. 5-ALA complete resections go beyond MR contrast enhancement: shift corrected volumetric analysis of the extent of resection in surgery for glioblastoma. *Acta Neurochir (Wien).* 2014;156(2):305-312.
26. Tejada-Solís S, Aldave-Orzaiz G, Pay-Valverde E, Marigil-Sánchez M, Idoate-Gastearena MA, Díez-Valle R. Prognostic value of ventricular wall fluorescence during 5-aminolevulinic-guided surgery for glioblastoma. *Acta Neurochir (Wien).* 2012;154(11):1997-2002.

27. Piccirillo SG, Dietz S, Madhu B, et al. Fluorescence-guided surgical sampling of glioblastoma identifies phenotypically distinct tumour-initiating cell populations in the tumor mass and margin. *Br J Cancer.* 2012;107(3):462-468.
28. Roessler K, Becherer A, Donat M, Cejna M, Zachenhofer I. Intraoperative tissue fluorescence using 5-aminolevolinic acid (5-ALA) is more sensitive than contrast MRI or amino acid positron emission tomography ((18)F-FET PET) in glioblastoma surgery. *Neurol Res.* 2012;34(3):314-317.
29. Sanai N, Snyder LA, Honea NJ, et al. Intraoperative confocal microscopy in the visualization of 5-aminolevulinic acid fluorescence in low-grade gliomas. *J Neurosurg.* 2011;115(4):740-748.
30. Stummer W, Tonn JC, Goetz C, et al. 5-Aminolevulinic acid-derived tumor fluorescence: the diagnostic accuracy of visible fluorescence qualities as corroborated by spectrometry and histology and postoperative imaging. *Neurosurgery.* 2014;74(3):310-319.
31. Valdés PA, Kim A, Leblond F, et al. Combined fluorescence and reflectance spectroscopy for in vivo quantification of cancer biomarkers in low- and high-grade glioma surgery. *J Biomed Opt.* 2011;16(11):116007.
32. Ostrom QT, Gittleman H, Farah P, et al. CBTRUS statistical report: primary brain and central nervous system tumors diagnosed in the United States in 2006-2010. *Neuro Oncol.* 2013;15(suppl 2):ii1-ii56.
33. Pignatti F, van den Bent M, Curran D, et al; European Organization for Research and Treatment of Cancer Brain Tumor Cooperative Group; European Organization for Research and Treatment of Cancer Radiotherapy Cooperative Group. Prognostic factors for survival in adult patients with cerebral low-grade glioma. *J Clin Oncol.* 2002;20(8):2076-2084.
34. Smith JS, Chang EF, Lamborn KR, et al. Role of extent of resection in the long-term outcome of low-grade hemispheric gliomas. *J Clin Oncol.* 2008;26(8):1338-1345.

COMMENT

As fluorescence-guided surgery is increasingly incorporated into routine neurosurgical oncology practices worldwide, its utility is naturally expanding beyond conventional WHO grade IV gliomas. In this report, the authors demonstrate the utility of 5-ALA for certain low-grade and nonenhancing high-grade gliomas. Patient parameters such as age, tumor volume, and metabolic imaging results can be used to identify nonenhancing gliomas that will likely exhibit regions of macroscopic tumor fluorescence. Intraoperatively, this cannot only improve the extent of resection, but increase the likelihood that the highest grade component of the tumor will be sampled for pathology.

Nader Sanai
Phoenix, Arizona