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5-Aminolevulinic acid for enhanced surgical visualization of high-grade gliomas: a prospective, multicenter study

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OBJECTIVE Greater extent of resection (EOR) is associated with longer overall survival in patients with high-grade gliomas (HGGs). 5-Aminolevulinic acid (5-ALA) can increase EOR by improving intraoperative visualization of contrast-enhancing tumor during fluorescence-guided surgery (FGS). When administered orally, 5-ALA is converted by glioma cells into protoporphyrin IX (PPIX), which fluoresces under blue 400-nm light. 5-ALA has been available for use in Europe since 2010, but only recently gained FDA approval as an intraoperative imaging agent for HGG tissue. In this first-ever, to the authors' knowledge, multicenter 5-ALA FGS study conducted in the United States, the primary objectives were the following: 1) assess the diagnostic accuracy of 5-ALA–induced PPIX fluorescence for HGG histopathology across diverse centers and surgeons; and 2) assess the safety profile of 5-ALA FGS, with particular attention to neurological morbidity.

METHODS This single-arm, multicenter, prospective study included adults aged 18–80 years with Karnofsky Performance Status (KPS) score > 60 and an MRI diagnosis of suspected new or recurrent resectable HGG. Intraoperatively, 3–5 samples per tumor were taken and their fluorescence status was recorded by the surgeon. Specimens were submitted for histopathological analysis. Patients were followed for 6 weeks postoperatively for adverse events, changes in the neurological exam, and KPS score. Multivariate analyses were performed of the outcomes of KPS decline, EOR, and residual enhancing tumor volume to identify predictive patient and intraoperative variables.

RESULTS Sixty-nine patients underwent 5-ALA FGS, providing 275 tumor samples for analysis. PPIX fluorescence had a sensitivity of 96.5%, specificity of 29.4%, positive predictive value (PPV) for HGG histopathology of 95.4%, and diagnostic accuracy of 92.4%. Drug-related adverse events occurred at a rate of 22%. Serious adverse events due to intraoperative neurological injury, which may have resulted from FGS, occurred at a rate of 4.3%. There were 2 deaths unre-

ABBREVIATIONS 5-ALA = 5-aminolevulinic acid; AE = adverse event; AUC = area under the receiver operating characteristic curve; CRET = complete resection of enhancing tumor; CTCAE = Common Terminology Criteria for Adverse Events; EOR = extent of resection; FGS = fluorescence-guided surgery; GBM = glioblastoma; HGG = high-grade glioma; iMRI = intraoperative MRI; KPS = Karnofsky Performance Scale; OS = overall survival; PFS = progression-free survival; PPIX = protoporphyrin IX; PPV = positive predictive value; PTV = preoperative tumor volume; RTV = residual contrast-enhancing tumor volume; SAE = serious adverse event.

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lated to FGS. Compared to preoperative KPS scores, postoperative KPS scores were significantly lower at 48 hours and 2 weeks but were not different at 6 weeks postoperatively. Complete resection of enhancing tumor occurred in 51.9% of patients. Smaller preoperative tumor volume and use of intraoperative MRI predicted lower residual tumor volume.

CONCLUSIONS PPIX fluorescence, as judged by the surgeon, has a high sensitivity and PPV for HGG. 5-ALA was well tolerated in terms of drug-related adverse events, and its application by trained surgeons in FGS for HGGs was not associated with any excess neurological morbidity.

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KEYWORDS 5-aminolevulinic acid; high-grade glioma; glioblastoma; extent of resection; fluorescence-guided surgery; diagnostic accuracy; oncology

Maximal safe resection in the initial management of newly diagnosed or recurrent high-grade gliomas (HGGs) is associated with greater overall and progression-free survival (PFS) compared to surgical treatment with biopsy or chemoradiation alone.[1–7] However, due to the infiltrative nature of HGG, its similarity in gross appearance at its margins to surrounding brain parenchyma, and the frequent involvement of eloquent or critical structures, defining and achieving a maximal safe resection for any individual patient can be challenging.[8] To address this concern, fluorescence-guided surgery (FGS) has emerged as a technique that may be used as an alternative, or in addition to other intraoperative adjuncts such as neuronavigation, cortical/subcortical stimulation mapping, and intraoperative imaging.

Multiple fluorescence agents have been evaluated for their use in HGG FGS. Compared to intravenous agents such as fluorescein sodium and indocyanine green, which leak through a disrupted blood-brain barrier into the extracellular compartment of a tumor,[9,10] the specific properties of 5-aminolevulinic acid (5-ALA) confer greater sensitivity and specificity for HGG tissue. As a naturally occurring metabolite in the heme biosynthesis pathway, 5-ALA when exogenously administered enters cells, where it is enzymatically converted into the fluorescent molecule protoporphyrin IX (PPIX). Due to a deficiency in ferrochelatase, which converts PPIX to heme, PPIX accumulates in a variety of neoplastic cells at high levels, becoming visible as a violet-red color when excited by blue light in the 400-nm range.[11] Levels of PPIX peak in glioma cells 6–8 hours following 5-ALA ingestion.[12–14] The most common side effects of oral 5-ALA are mild and include transient hypotension, liver enzyme elevations, nausea, and skin photosensitivity.[15,16]

In a phase III randomized controlled multicenter German trial published in 2006, 5-ALA FGS improved the probability of complete resection and led to a doubling in 6-month PFS compared to conventional white-light resection without other intraoperative adjuncts.[17] Based on these data and additional diagnostic accuracy and safety data from four other multicenter German trials, 5-ALA was approved by the US Food and Drug Administration (FDA) in 2017 as an intraoperative imaging agent in HGG surgery.[18] However, given the lack of any more recent phase III trial data demonstrating an improvement in HGG surgery outcomes, there are lingering questions regarding the utility of 5-ALA as implemented across different centers in the current technological environment, and the safety of its application in FGS.[19] Specifically, the lack of safety adjuncts in previous trials, which are commonplace today, raises concerns that the extent of resection (EOR) benefits from FGS are achieved at the expense of more frequent neurological complications, particularly in patients with eloquent tumors.[20]

Here, we undertook what is to our knowledge the first multicenter, prospective trial of 5-ALA FGS in the United States, which enrolled patients from both academic and community hospitals offering HGG surgery. Our primary goals were the following: 1) assess the diagnostic accuracy of 5-ALA–induced PPIX fluorescence, as judged by the operating surgeon, for HGG histopathology in tumors with a presumptive diagnosis of HGG based on preoperative MRI; and 2) evaluate the safety of 5-ALA FGS, with particular attention to neurological morbidity during the 6-week postoperative period. Although not an endpoint of this study, EOR outcomes were examined in a secondary analysis to facilitate contextualization of our results with previous studies.

Methods

Study Design and Patient Selection

We enrolled patients from 15 sites across the United States between 2016 and 2019 under the FDA investigational new drug application (no. 112246) held by coauthor C.G.H. (ClinicalTrials.gov ID: NCT02632370). Patients had the option of simultaneously enrolling in a correlative biomarker study examining circulating microvesicles. Approval was obtained from the institutional review boards at the data coordination center (Mount Sinai Hospital) and all participating sites. To be included, patients were required to have a suspected or established diagnosis of new or recurrent WHO grade 3 or 4 HGG for which an open resection was indicated and planned. Other inclusion criteria were: age 18–80 years, Karnofsky Performance Scale (KPS) score > 60, and laboratory data showing normal blood counts, liver function, and renal function. Patients with tumors predominantly involving nonresectable midline brain regions, such as the basal ganglia, thalamus, or brainstem, were excluded. Patients were also excluded if they had a personal or family history of porphyria or allergic reaction to compounds similar to 5-ALA. Further exclusion criteria included pregnancy, history of gastrointestinal perforation and/or peptic ulcer disease, and uncontrolled infectious, cardiac, or psychiatric illness. Race and ethnicity of patients was self-reported.

Interventions

All investigators were trained on 5-ALA FGS by coau-

thor C.G.H. and NXDC (NX Development Corporation). A 20-mg/kg dose of 5-ALA (Gleolan, NXDC and Photonamic GmbH & Co. KG) was administered in an inpatient setting 3–5 hours prior to skin incision. Resection proceeded with a standard operating microscope fitted with an FL400 filter set to generate fluorescent excitation in the 390–440 nm (blue) range and allow visualization in the 600–700 nm (bright red) range. The filters transmit tumor-associated red PPIX fluorescence and a portion of backscattered blue excitation light from nonfluorescing surrounding tissue. Additional surgical adjuncts such as neuronavigation, electrophysiological monitoring, stimulation mapping, and intraoperative imaging (MRI or ultrasound) were employed per the surgeon's usual practice. Once the tumor was exposed, 3–5 biopsies were sampled from separate violet-red fluorescing areas during blue light microscopy, and then sent for permanent histopathology as individually labeled specimens (Fig. 1). If, after adequate surgical exposure of the lesion, no red fluorescence was identified, surgeons were instructed to take at least 3 biopsies of any areas of nonfluorescing pathology. Surgeons were instructed to judge the fluorescence status of each study specimen as "fluorescent" or "not fluorescent." For the remainder of the tumor resection, surgeons were permitted to switch between the white and blue light modes on the microscope at their own discretion.

Outcome Measures

A board-certified neuropathologist examined each biopsy specimen to determine the presence of malignant glioma tumor cells and tumor grading according to WHO 2016 criteria. Neuropathologists were blinded to the fluorescence status of the specimens. Molecular testing, performed according to the standard of care at each institution, was used as needed to confirm the diagnosis and included detection of *IDH1/2* mutation, *EGFR* amplification, *ATRX* mutation, *MGMT* promoter methylation, p53 mutation, and 1p/19q codeletion.

Patients were evaluated at the time of study enrollment (baseline), before surgery at the time of 5-ALA ingestion (day 0), within 48 hours postoperatively, and at 2 and 6 weeks postoperatively for KPS score, adverse events (AEs), and serious adverse events (SAEs). AEs and SAEs were coded for intensity and relationship to 5-ALA in accordance with the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE), version 4.0.

Postoperative brain MRIs, including a volumetric contrast-enhanced T1-weighted sequence, were performed within 48 hours following surgery to verify that tumor resections had been completed as intended. Nonenhanced T1-weighted sequences were used to identify blood products. Tumor volumes were quantified with Brainlab SmartBrush version 2.5 by coauthors R.B.B., W.C., and A.J.S., then verified by senior author R.L.Y. and a board-certified neuroradiologist. Preoperative tumor volume (PTV) and residual contrast-enhancing tumor volume (RTV) were used to calculate the EOR percentage, calculated as (PTV – RTV)/PTV × 100%. Complete resection of enhancing tumor (CRET) was defined, similarly to previous studies,[17,21] as RTV less than 0.1 cm^3.

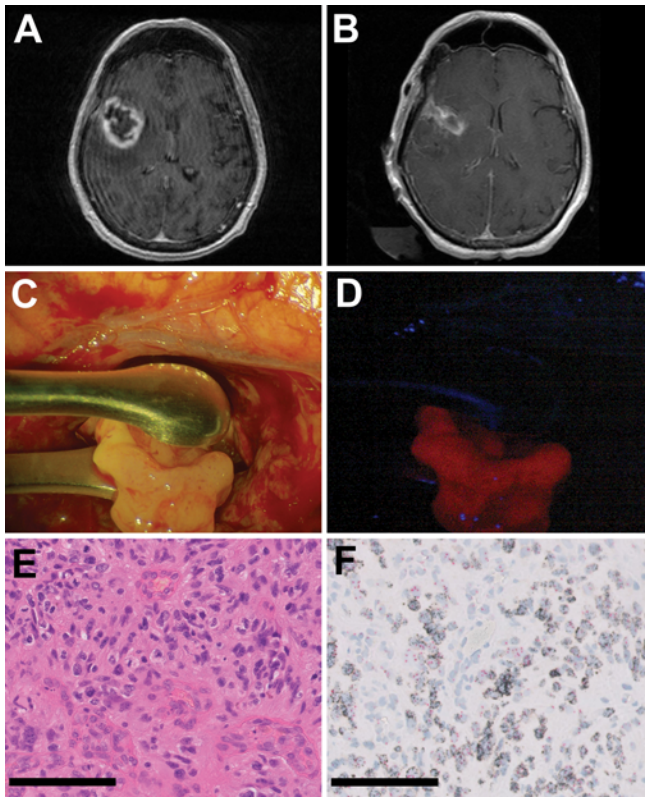

FIG. 1. A: Preoperative T1-weighted postcontrast MR image demonstrating a right frontal lesion with peripheral contrast enhancement and areas of central necrosis, suspicious for GBM. **B:** Postoperative T1-weighted postcontrast MR image demonstrating resection of the contrast-enhancing tumor seen in panel A. **C:** Intraoperative photograph of the lesion seen in panel A under white light. **D:** Intraoperative photograph of the lesion seen in panel A under blue light following 5-ALA administration. **E:** H&E stain of a representative fluorescing biopsy specimen demonstrating microvascular proliferation and mitoses. **F:** Detection of *EGFR* gene amplification in the specimen shown in panel E by in situ hybridization. Bars = 100 μm.

Statistics

Mean KPS scores at assessment time points were evaluated with one-way ANOVA using a mixed-effects model for random missing values, followed by Tukey's multiple comparisons test. Multiple linear regression was performed using a least squares method with a Gaussian distribution of residuals assumed. Nested models, using only variables with $p < 0.5$, were compared to initial multivariate models for goodness of fit using the extra sum-of-squares F test. Only the best-fitting models are reported. All statistical analyses were performed on Statistical Analysis Software version 9.4 (SAS Institute Inc.) or GraphPad Prism version 9. The study sponsor had no access to raw data and no role in data analysis or interpretation.

Results

Patient Demographics and Tumor Diagnoses

Eighty patients from 15 centers (range 1–17 patients per center) were consented and enrolled in the trial between June 9, 2016 and December 24, 2018, with 69 pa-

TABLE 1. Demographic characteristics of enrollment population

	Values
Age, yrs	60.5 ± 12.4
Sex	
Male	61.4 (43/70)
Female	38.6 (27/70)
Ethnicity	
Hispanic or Latino	15.8 (9/57)
Not Hispanic or Latino	84.2 (48/57)
Race	
Asian	1.5 (1/67)
White	92.5 (62/67)
Black	4.5 (3/67)
Other	1.5 (1/67)
Tumor diagnosis (%)	
Anaplastic astrocytoma	4.3 (3/69)
Anaplastic oligodendroglioma	5.8 (4/69)
Glioblastoma	81.2 (56/69)
Gliosarcoma	4.3 (3/69)
Macrophage infiltration/gliosis	1.4 (1/69)
Primary CNS lymphoma	1.4 (1/69)
Metastatic carcinoma	1.4 (1/69)
Recurrent HGG	43.5 (30/69)
Lt hemisphere tumors	46.4 (32/69)
Baseline KPS score	88.6 ± 8.3

Values are shown as percentage (no. of patients/n) or mean ± SD.

tients undergoing 5-ALA FGS (see study flow diagram, Supplemental Fig. 1). The study was closed to enrollment by the sponsor in 2019 when Gleolan became commercially available. Thirty-nine (56.5%) subjects had a newly diagnosed brain tumor while the remainder had recurrent disease. The average age was 60.5 years, with 61% male (Table 1). The majority (92.5%) of patients were White. Of the 69 patients enrolled in the study, 66 had tumors confirmed to be WHO grade 3 or 4 glioma on histopathology. Of these, 84.8% were glioblastoma (GBM), and 10.6% were anaplastic astrocytoma or anaplastic oligodendroglioma. One patient was diagnosed with lymphoma, one patient was found to have metastatic carcinoma, and a third patient was found to have reactive gliosis only. Thirty-two of the 69 tumors (46.4%) were localized in the left cerebral hemisphere. Volumetric analysis of the 54 patients with complete imaging data demonstrated a mean preoperative tumor volume of 27.3 cm^3.

Patient Safety Data

Fifteen patients (22%) experienced an adverse event that was deemed related or possibly related to 5-ALA administration (Table 2). Three patients experienced skin photosensitivity, all within 48 hours of 5-ALA administration. Twelve patients experienced abnormalities in liver function tests 48 hours to 6 weeks following 5-ALA administration. One patient experienced photophobia immediately after 5-ALA ingestion. At 48 hours, one patient experienced headache and another experienced fatigue. Seventeen protocol deviations occurred in which 5-ALA was ingested outside the 3–5 hour window prior to skin incision; however, in all these cases, fluorescent tumor was visualized.

There were 20 SAEs in 17 patients in the FGS population (overall rate 24.6%), including 2 deaths (Table 3). All were determined to be unrelated to any known pharmacological effect of 5-ALA. One patient died from hydrocephalus secondary to shunt failure 6 weeks after FGS, and the second patient died from a temporal intracerebral hematoma that developed spontaneously 2 weeks after FGS. Three patients (4.3%) experienced neurological worsening in the postoperative period due to intraoperative neurological injury, which may have been related to the application of 5-ALA in FGS: 1 patient was found to have a postoperative ischemic middle cerebral artery territory stroke, 1 patient developed new hemiplegia, and 1 patient developed worsened aphasia from a peritumoral hemorrhage within 48 hours of surgery. The remaining 8 postoperative SAEs, including 2 infections, a pulmonary embolism, hydrocephalus, hyponatremia, and glioma progression, were not thought to be related to the application of 5-ALA in FGS.

Diagnostic Accuracy of PPIX Fluorescence for HGG Pathology

Sixty-five patients had a total of 275 tissue samples processed for histopathology (Supplemental Data). Sixty-four patients had 3–5 samples taken, while 1 patient had only 2 samples taken. Of the 275 biopsies, 261 (94.9%) from 62 patients were deemed fluorescent by the operating surgeon, with 249 of these from 59 patients positive for HGG tissue (Table 4). The positive predictive value (PPV) for HGG tissue was therefore 95.4% (95% CI 93.9%–96.6%) (Table 5). Pathologies that were judged by the surgeon to fluoresce but were not HGG came from 3 patients and included lymphoma (5 samples from 1 patient), atypical glial cells (3 samples from 1 patient), and gliosis (4 samples from 2 patients). Of the 14 samples from 4 patients that were deemed negative for fluorescence by the operating surgeon, 9 samples (64.3%) from 3 patients were HGG tissue, and 5 samples (35.7%) from 1 patient were poorly differentiated carcinoma. The negative predictive value for non-HGG tissue was therefore 35.7% (95% CI 17.3%–59.6%).

Of 61 HGGs, 59 provided at least 1 fluorescent sample and 2 did not exhibit fluorescence at all. The 4 patients in whom HGG tissue was not diagnosed included 1 patient with lymphoma that fluoresced and 1 patient with poorly differentiated carcinoma that did not. The remaining 2 patients had both received adjuvant treatment within 12 months of FGS and their samples exhibited fluorescence intraoperatively despite the presence of only reactive or atypical glia seen in 7 of 8 biopsies combined. Overall, PPIX fluorescence had a sensitivity of 96.5% (95% CI 93.5%–98.3%) and specificity of 29.4% (95% CI 10.3%–56.0%) for HGG tissue, with an accuracy of 92.4% and a diagnostic OR of 11.5 (95% CI 3.16–46.5). The area under the receiver operating characteristic curve (AUC) for

TABLE 2. 5-ALA drug–related adverse events

Pt No.	Time Point	Diagnosis	Grade	SAE	Relation to Study Drug	Treatment	Outcome
1	2 wks	Elevated LFTs	3	No	Related	None	Recovered w/o sequelae
2	48 hrs	Rash	2	No	Related	None	Recovered w/o sequelae
2	2 wks	Elevated LFTs	3	No	Related	None	Recovered w/o sequelae
4	48 hrs	Rash	1	No	Related	None	Recovered w/o sequelae
4	2 wks	Elevated LFTs	2	No	Related	None	Recovered w/o sequelae
5	2 wks	Elevated LFTs	2	No	Related	None	Recovered w/o sequelae
6	6 wks	Elevated LFTs	2	No	Related	None	Unknown
17	2 wks	Elevated LFTs	2	No	Related	None	Recovered w/o sequelae
21	48 hrs	Fatigue	1	No	Possibly related	None	Recovered w/o sequelae
29	2 wks	Elevated LFTs	2	No	Related	None	Recovered w/o sequelae
33	2 wks	Elevated LFTs	2	No	Related	None	Recovered w/o sequelae
45	0 hrs	Photophobia	1	No	Possibly related	None	Recovered w/o sequelae
53	48 hrs	Headache	1	No	Possibly related	None	Recovered w/o sequelae
53	48 hrs	Elevated LFTs	1	No	Related	None	Recovered w/o sequelae
55	48 hrs	Elevated LFTs	1	No	Related	None	Recovered w/o sequelae
56	2 wks	Elevated LFTs	1	No	Related	None	Recovered w/o sequelae
60	2 wks	Elevated LFTs	1	No	Related	None	Recovered w/o sequelae
64	48 hrs	Rash	1	No	Related	None	Recovered w/o sequelae

LFT = liver function test; pt = patient.

TABLE 3. Serious adverse events

Pt No.	Time Point	Diagnosis	Grade	Relation to FGS	Treatment	Outcome
5	6 wks	CSF leak	3	Unlikely related	None	Recovered w/o sequelae
9	2 wks	Pulmonary embolism	3	Unrelated	Anticoagulation, IVC filter	Recovered w/o sequelae
9	6 wks	Subdural hematoma	3	Unrelated	Reversal of anticoagulation	Not yet recovered
10	48 hrs	Ischemic stroke	3	Possibly related	None	Recovered w/ sequelae
13	2 wks	Death (temporal intracerebral hematoma)	5	Unlikely related	None	Mortality
23	0	Fever	1	Unrelated	None	Recovered w/o sequelae
27	B	Progression of disease	3	Unrelated	Tumor resection	Recovered w/o sequelae
30	B	Delirium	2	Unrelated	Resumption of steroids	Recovered w/o sequelae
34	2 wks	Adenocarcinoma	2	Unrelated	None	Not yet recovered
35	2 wks	Prolonged hospitalization waiting for rehabilitation	1	Unrelated	None	Recovered w/o sequelae
36	2 wks	Fall	3	Unrelated	None	Recovered w/o sequelae
37	2 wks	Hyponatremia	3	Unrelated	Fluid restriction	Recovered w/o sequelae
37	6 wks	Intracranial infection	4	Unrelated	Wound revision, hardware removal	Recovered w/o sequelae
38	2 wks	Intracranial infection	4	Unrelated	Antibiotics for ventriculitis, wound washout	Recovered w/o sequelae
43	2 wks	Communicating hydrocephalus	3	Unlikely related	Shunt placement	Recovered w/o sequelae
43	6 wks	Death (shunt failure)	5	Unrelated	None	Mortality
49	48 hrs	Rt hemiplegia	2	Possibly related	None	Not yet recovered
53	48 hrs	Peritumoral hemorrhage	3	Possibly related	None	Not yet recovered
63	B	Seizures	2	Unrelated	None	Recovered w/o sequelae
67	6 wks	Disease progression	3	Unrelated	None	Not yet recovered

B = baseline; IVC = inferior vena cava.

TABLE 4. Diagnostic performance of PPIX fluorescence for HGG tissue

Tumor Present	Fluorescence		
	Yes	No	Total
Yes	249	9	258
No	12	5	17
Total	261	14	275

intraoperative fluorescence was 0.63 (95% CI: 0.47–0.79). No single study site had more than 1 case in which a false-positive or false-negative result occurred.

Functional Status Following FGS

There was significant variation in KPS score by study time point (one-way ANOVA, $p < 0.0001$). The mean ± SD KPS score at baseline was 88.6 ± 8.3 (Fig. 2). This did not differ from the mean KPS score just before 5-ALA ingestion on day 0 ($p = 0.2576$, Tukey's test). The mean KPS scores at 48 hours (80.0 ± 14.3) and 2 weeks (83.1 ± 12.2) postoperatively were significantly lower compared to those for day 0 (both $p < 0.0001$, Tukey's test). At the end of the study period at 6 weeks postoperatively, however, the mean KPS score (85.8 ± 12.4) was not significantly different from the day 0 KPS score ($p = 0.7879$, Tukey's test). Multiple linear regression analysis demonstrated that older female patients with recurrent tumors and higher baseline KPS tended to have greater declines in KPS at 2 weeks (Supplemental Table 1).

Tumor Resections

Among all tumor resections, 43.5% utilized intraoperative MRI (iMRI), 33.3% utilized intraoperative ultrasound, 53.6% utilized some form of stimulation mapping, and 33.3% were performed in awake patients. With the exception of 3 cases, all surgeries were performed with neuronavigation. The mean EOR percentage and RTV were 85.1% and 5.3 cm^3, respectively. The rate of CRET was 51.9%. We performed a multiple linear regression with EOR percentage as the dependent variable and age, sex, ethnicity, race, baseline KPS, KPS decline, tumor volume, tumor laterality, new or recurrent diagnosis, tumor grade, use of other intraoperative adjuncts, make of operating microscope, and surgeon experience with FGS as independent variables (Table 6). Only right-sided tumor laterality predicted a greater EOR percentage with statistical significance. In performing the same analysis with RTV as the dependent variable, both smaller preoperative tumor volume and use of iMRI were predictive of lower RTV (Table 7).

TABLE 5. Summary statistics of diagnostic performance of PPIX fluorescence for HGG tissue

	Value (95% CI)
Sensitivity	96.51% (93.48–98.39%)
Specificity	29.41% (10.31–55.96%)
PPV	95.40% (93.85–96.58%)
NPV	35.71% (17.30–59.61%)
Positive LR	1.37 (1.01–1.86)
Negative LR	0.12 (0.04–0.32)
Accuracy	92.36% (88.56–95.21%)
AUC	0.6298 (0.4720–0.7876)
OR	11.53 (3.16–46.5)

LR = likelihood ratio.

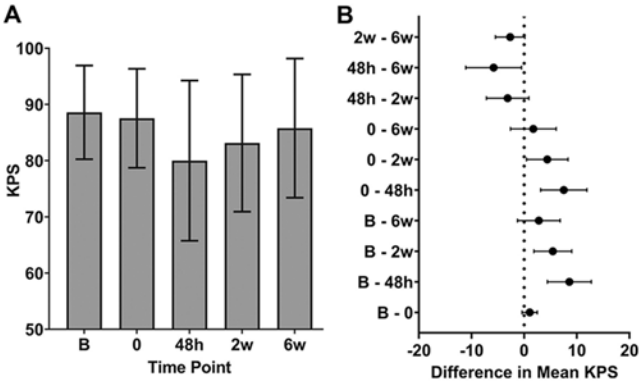

FIG. 2. A: Mean KPS scores of the study cohort through time. B = baseline at study enrollment; 0 = preoperatively on day of surgery; 48h = within 48 hours postoperatively; 2w = at 2 weeks postoperatively; 6w = at 6 weeks postoperatively. Bars = SD. **B:** Difference in mean KPS score (95% CIs) at the time points shown in panel A. *Dotted line* indicates no significant difference.

Discussion

In the pivotal trial by Stummer et al. on 5-ALA FGS, published 15 years ago,[17] patients in the experimental arm saw nearly double the rate of CRET (65% vs 36%) and 6-month PFS (41% vs 21%), compared to the control white-light resection arm. Neither arm was permitted the use of other intraoperative adjuncts such as neuronavigation, iMRI, electrophysiological monitoring, or stimulation mapping. Moreover, temozolomide was not yet a standard component of adjuvant therapy. Given the lack of a demonstrated overall survival (OS) benefit and the significant changes that have occurred in practice patterns since 2006, FDA approval on the basis of improved HGG patient survival could not be obtained without new level 1 evidence.[18] However, as a tool for intraoperative visualization of HGG tissue, the accumulated diagnostic performance data from several European clinical trials were deemed strong enough to justify US regulatory approval in 2017.[17,22–24]

In this trial, we sought to generate confirmatory evidence of the high diagnostic accuracy of PPIX fluorescence for HGG tissue under contemporary, real-world operating room conditions. Although advances have been made in quantitative detection methods such as high-resolution optical-sectioning microscopy,[25] PPIX fluorescence is still commonly assessed subjectively and in a binary fashion by the neurosurgeon. The surgeon's perception of fluorescence was therefore used as the diagnostic test "re-

TABLE 6. Multiple linear regression analysis of percent EOR.

Variable	Estimate	SE	95% CI	|t|	p Value
Age	−0.4031	0.3223	−1.059 to 0.2527	1.251	0.2198
Ethnicity (Hispanic = 1, non-Hispanic = 0)	15.79	11.12	−6.833 to 38.42	1.420	0.1650
Race (non-White = 1, White = 0)	19.33	16.75	−14.75 to 53.41	1.154	0.2568
KPS score					
Day 0	−0.8535	0.6071	−2.089 to 0.3817	1.406	0.1691
Decline at 2 days postop	−0.3568	0.5062	−1.387 to 0.6730	0.7049	0.4858
Decline at 2 wks postop	0.4934	0.5092	−0.5427 to 1.529	0.9689	0.3397
Preop tumor vol	−0.1158	0.1560	−0.4332 to 0.2016	0.7422	0.4632
Hemisphere (lt = 1, rt = 0)	−23.36	8.689	−41.04 to −5.688	2.689	**0.0111**
Recurrent = 1, newly diagnosed = 0	4.964	9.262	−13.88 to 23.81	0.5359	0.5956
iMRI used	16.30	9.457	−2.944 to 35.53	1.723	0.0942
Intraop ultrasound used	−18.06	11.21	−40.86 to 4.744	1.611	0.1167
Stimulation mapping used	10.89	11.31	−12.12 to 33.90	0.9628	0.3426
Microscope make (Zeiss = 1, Leica = 0)	12.50	11.70	−11.32 to 36.31	1.068	0.2935
Surgeon FGS experience	−1.844	1.172	−4.228 to 0.5392	1.574	0.1250

Boldface type indicates statistical significance.

sult" and the biopsy histology as the true disease state. Using this methodology, our observed measures of diagnostic performance, including high sensitivity and PPV, but low specificity and NPV, were consistent with what has previously been reported.[11,16,26,27]

We considered all non-HGG histologies, even if pathological, to be true disease negatives. Numerous non-HGG neoplasms, however, are well recognized to exhibit PPIX fluorescence after 5-ALA administration, including meningioma,[28] many metastatic tumors,[29] primary CNS lymphoma,[30] hemangioblastoma,[31] and pediatric brain tumors such as medulloblastoma and atypical teratoid rhabdoid tumor (ATRT).[32] Reactive gliosis associated with glioma pseudoprogression is also a well-recognized nonneoplastic pathology that fluoresces.[33,34] Most of the non-HGG biopsies that showed fluorescence in this study were either lymphoma or reactive gliosis in the setting of recent chemoradiation. One specimen from a patient who had received radiation, temozolomide (TMZ), and lomustine was read as type 1 hippocampal sclerosis, but there was no history of seizures. From a practical standpoint, this category of false positive does not necessarily pose a problem for the neurosurgeon, since pathological tissue is still being visualized for resection. Indeed, expanded use of 5-ALA as a visualization aid in a wide variety of non-HGG tumor and nontumor pathologies is actively being explored (ClinicalTrials.gov IDs: NCT04305470, NCT04055688, NCT02191488, NCT04559685).[35]

Fluorescence of nonpathological tissue, on the other hand, as reported in a study examining ventricular surfaces, could pose safety concerns.[36] However, we observed no instances of solely normal brain tissue being identified in any of the fluorescing biopsy samples obtained in this study, making the rate of false-positive normal tissue sampling less than 0.4%. Finally, nonfluorescence of HGG tissue (false negatives) occurred at a rate of 3.5%, and only 2 patients with histopathology-confirmed HGG tissue samples did not show fluorescence. One patient

TABLE 7. Multiple linear regression analysis of residual contrast-enhancing tumor volume.

Variable	Estimate	SE	95% CI	|t|	p Value
Age	0.3016	0.1536	−0.009708 to 0.6129	1.963	0.0572
Sex (F = 1, M = 0)	5.616	4.462	−3.426 to 14.66	1.258	0.2161
Race (non-White = 1, White = 0)	−11.03	8.498	−28.25 to 6.190	1.298	0.2024
KPS score decline at 2 wks postop	−0.2117	0.2100	−0.6373 to 0.2139	1.008	0.3200
Preop tumor vol	0.2807	0.07543	0.1278 to 0.4335	3.721	**0.0007**
Hemisphere (lt = 1, rt = 0)	7.788	4.278	−0.8794 to 16.46	1.821	0.0768
Intraop MRI used	−9.187	4.489	−18.28 to −0.08998	2.046	**0.0479**
Intraop ultrasound used	2.619	4.871	−7.251 to 12.49	0.5376	0.5941
Stimulation mapping used	−4.309	5.009	−14.46 to 5.840	0.8602	0.3952
Microscope make (Zeiss = 1, Leica = 0)	−6.651	5.491	−17.78 to 4.474	1.211	0.2335

Boldface type indicates statistical significance.

had an anaplastic oligodendroglioma, and the other had a GBM. In both cases, no protocol deviations occurred to reveal any clear cause of the false-negative results. However, suboptimal timing of administration of 5-ALA, variability in uptake of 5-ALA by glioma cells, and low penetrance of blue light into deep operative corridors have all been suggested as explanations for false negatives in other studies.[11,26,37,38]

The second main objective of our study was to reassess the safety profile of 5-ALA. AEs simply from 5-ALA administration, such as transaminitis and skin photosensitivity, are well documented in multiple studies and may occur in up to 70% of patients.[11,17,26] The most common AE we observed was transient transaminitis, which occurred in 17% of patients who underwent FGS, and resolved without sequelae in all cases; we did not observe any drug reactions that have not previously been reported. On the other hand, the significance of serious neurological complications following 5-ALA FGS for HGG has been unclear. In a multicenter phase II clinical trial, neurological SAEs occurred in 5 of 36 (13.9%) patients who received 5-ALA, without time of onset, whether deficits were recovered, or attribution reported.[24] In another multicenter phase II trial in recurrent gliomas, 3 of 36 patients (8.3%) experienced neurological SAEs, all of which were deemed unlikely to be related to 5-ALA.[22] These SAEs were accompanied by a decrease in the median KPS score by 10 points at 6 weeks and an increase in the National Institutes of Health Stroke Scale (NIHSS) score by 3 points at 1 week postoperatively. Both KPS and NIHSS returned to baseline at 6 months, but measures of statistical significance were not reported. In the phase III randomized trial, the overall rate of neurological SAEs at 1 week postoperatively in the FGS arm was 7.9% compared to 3% in the white light arm (reported as not statistically different).[17] In both treatment arms, median KPS did not differ at baseline, 6 weeks, or 6 months postoperatively; however, in the FGS arm, significantly more patients showed deterioration, and significantly fewer showed improvement, in NIHSS score compared to the white light arm at 48 hours. There were no differences at 1 or 6 weeks postoperatively.

Excess neurological morbidity may result from 5-ALA FGS for HGG if the surgeon does not appropriately use functional anatomical data from other intraoperative adjuncts to avoid resecting critical brain structures that fluoresce due to tumor infiltration. In eloquent and near-eloquent tumors, over-aggressive resection will result in permanent new or worsened postoperative neurological deficits, a decline in functional status, and a reduction in any survival gains achieved from cytoreduction.[39,40] On the other hand, transient declines in neurological function, which recover within weeks of surgery, may suggest that tumor resection was appropriately carried up to, but not beyond, the boundary of an eloquent brain region. To capture these events in the current study, we serially tracked KPS and examined all SAEs for the subset that likely resulted from intraoperative neurological injury, since any of these could have been due to FGS-related over-resection. The 4.3% rate we observed is comparable to, if not less than, rates observed in other series of HGG surgeries performed with or without FGS. Furthermore, given the similarities in the percentages of eloquent tumors (46%–53%), rates of CRET (52%–65%, considering our inclusion of recurrent gliomas where rates of CRET may be lower[22]), and lack of neurological decline at 6 weeks or more postoperatively in this study and other prospective trials,[16,17] the cumulative data suggest that 5-ALA FGS is not associated with over-aggressive resections.

Other commonly used intraoperative adjuncts in HGG surgery may provide EOR and functional outcomes similar to those obtained with FGS. A randomized controlled trial of iMRI and neuronavigation versus neuronavigation alone for glioma surgery demonstrated that an RTV < 0.175 cm^3 was achieved in 96% of patients in the experimental arm compared to 68% of control patients, while the rate of new or worsened postoperative neurological deficits was similar at 10% overall. The proportion of patients with eloquent tumors was not provided.[21] Awake intraoperative stimulation mapping with neuronavigation has also been evaluated against neuronavigation alone under general anesthesia in a prospective, nonrandomized comparative study of patients with eloquent supratentorial lesions, most of which were gliomas.[41] The rate of achieving an RTV < 10 cm^3 was significantly higher in the awake craniotomy group (82%) than in the general anesthesia group (40%), while new neurological deficits were significantly more frequent in the general anesthesia group (13% vs 3.3%). Although the use of intraoperative neuronavigation is ubiquitous, the only randomized trial evaluating its effect on HGG surgical outcomes was underpowered to detect any difference in the rate of CRET or neurological worsening between the study arms.[42]

Due to the lack of comparable control groups among these prospective studies, as well as differences in EOR assessment methodology and tumor characteristics, it is not possible to infer whether 5-ALA FGS provides any EOR or functional outcome benefit over other commonly used adjuncts. There has been one study, however, that addressed whether 5-ALA might provide an added benefit, similar to the finding in our multivariate analysis that FGS combined with iMRI may be superior to FGS alone in minimizing RTV. Coburger et al. compared a prospective cohort of patients undergoing GBM surgery with 5-ALA, iMRI, and neuronavigation to a matched, retrospective cohort of patients who underwent surgery with iMRI and neuronavigation only. EOR > 95%, was achieved in all patients who underwent FGS, compared to 82% of patients who did not, while the rate of new, permanent neurological deficits following surgery was 6% in both arms.[43] These results, however, did not translate into any improvement in PFS or OS. Well-designed, randomized, clinical trials will be needed to confirm if synergies exist between 5-ALA FGS and other intraoperative adjuncts in maximizing EOR or improving survival outcomes in HGGs.

Study Limitations

This study is limited by its exclusion of non-HGG pathologies based on preoperative imaging and history, which reduces the confidence with which the specificity and NPV of PPIX fluorescence can be measured. We did not utilize a validated scale, such as the NIHSS, for detailed quantification of neurological function in the post-

operative period, but instead relied on KPS score and CTCAE, which have lower interrater reliability. The follow up period was also relatively short at 6 weeks, which may have resulted in an underreporting of AEs. Other than during collection of biopsy samples, surgeons were permitted at their own discretion to apply or not apply FGS, with or without employing other adjuncts to achieve their surgical goals, and this may have led to an underestimation of neurological morbidity attributable to FGS. Neurosurgeons are known to vary in how likely they are to pursue CRET, even for the same tumor on imaging.[44] Although all surgeons were trained in FGS to maximize HGG EOR, this study was not designed to address the question of whether FGS was associated with additional EOR benefit compared to other surgical adjuncts in the context of individual surgeon intraoperative decision-making.

Conclusions

PPIX fluorescence after oral administration of 5-ALA, as judged by the surgeon's eye, has a high sensitivity and PPV for HGG tissue and has good diagnostic accuracy overall. 5-ALA was well tolerated in terms of drug-related AEs, and its application by trained surgeons in FGS for HGGs was not associated with any excess neurological morbidity.

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