Glioblastoma Multiforme Treatment with Clinical Trials for Surgical Resection (Aminolevulinic Acid)

David W. Roberts, MD[a,b,c,*], Pablo A. Valdés, BS[a,b,d], Brent T. Harris, MD, PhD[e], Alexander Hartov, PhD[c,d], Xiaoyao Fan, BE[c,d], Songbai Ji, DSc[c,d], Frederic Leblond, PhD[c,d], Tor D. Tosteson, ScD[a,c], Brian C. Wilson, PhD[f], Keith D. Paulsen, PhD[c,d,g]

KEYWORDS

• 5-ALA • Fluorescence • Glioma • Surgery

KEY POINTS

- 5-aminolevulinic acid (5-ALA)-induced fluorescence is a user-friendly, technologically efficient, and clinically safe surgical adjunct for the identification of malignant glial tumor tissue.
- A multi-institutional clinical trial comparing fluorescence-guided versus white light tumor resection reported significant improvement in completeness of resection and 6-month progression-free survival; the trial was underpowered to show improvement in overall survival.
- The degree of 5-ALA-induced fluorescence correlates with histopathologic grade of tumor, degree of tumor cell infiltration, and proliferation indices.
- Quantitative methodologies for assessment of tissue fluorescence have significantly improved the ability to detect tumor tissue and intraoperative diagnostic performance, as assessed by receiver operating characteristic curve analysis.
- These developments extend the applicability of this technology to additional tumor histologies and provide the rationale for further instrumentation development.

Over the past 2 decades, increasing evidence has accumulated correlating more complete surgical resection of malignant glioma with improved survival. Numerous surgical technologies have been developed to facilitate optimal resection, many of which function to guide the surgeon during resection. This article focuses on the use of 5-aminolevulinic acid (5-ALA)-induced fluorescence and its present role in the surgical resection of high-grade gliomas.

Disclosure of funding: National Institutes of Health Grant Nos. R01NS052274-01A2 and K25CA138578. Carl Zeiss Surgical GmbH (Oberkochen, Germany) for operating microscope equipment. Medtronic Navigation (Louisville, CO) for the StealthStation Treon navigation system. DUSA Pharmaceuticals (Tarrytown, NY) for supplying the ALA.

[a] Dartmouth Medical School, Hanover, NH, USA; [b] Section of Neurosurgery, Dartmouth-Hitchcock Medical Center, Lebanon, NH, USA; [c] Norris Cotton Cancer Center, Lebanon, NH, USA; [d] Thayer School of Engineering, Dartmouth College, Hanover, NH, USA; [e] Department of Pathology and Neurology, Georgetown University Medical Center, Washington, DC, USA; [f] Department of Medical Biophysics, Ontario Cancer Institute, University of Toronto, Toronto, Ontario, Canada; [g] Dartmouth-Hitchcock Medical Center, Lebanon, NH, USA

* Corresponding author. Section of Neurosurgery, Dartmouth-Hitchcock Medical Center, Lebanon, NH 03756.
E-mail address: david.w.roberts@dartmouth.edu

Neurosurg Clin N Am 23 (2012) 371–377
doi:10.1016/j.nec.2012.04.001

5-ALA is a precursor in the hemoglobin synthesis pathway, and exogenous, oral administration of this molecule several hours before surgery leads to the preferential accumulation of the molecule protoporphyrin IX (PpIX) within tumor cells. Under blue-violet light conditions, the fluorophore PpIX emits light in the red region of the visible spectrum, enabling identification of tumor tissue that might otherwise be difficult to distinguish from normal brain. Several commercial operating microscope systems have been adapted to use this phenomenon, providing the illumination and optical apparatus for reliable and efficient fluorescence guidance during surgery.

The greatest impediment to wider clinical application of this technology has been the limited access to 5-ALA for use in intracranial tumor resection. Approved for intracranial use in Europe, Canada, and Japan, 5-ALA in the United States has not yet received such approval by the US Food and Drug Administration (FDA). Clinical investigation in the United States at the time of this writing requires an Investigational New Drug (IND) exemption, and all clinical studies in the United States have been performed under this exemption.

EARLY WORK

One of the earliest reports of the use of 5-ALA-induced fluorescence for tumor resection was that published by Stummer in 2000.[1] In this study, 52 patients with subsequently confirmed glioblastoma multiforme (GBM) underwent fluorescence-guided resection in which all fluorescent tissue considered safe to resect was removed. Absence of contrast-enhancing tumor on postoperative magnetic resonance imaging (MRI) was documented in 33 patients (63%), with 18 of the 19 patients with residual enhancement having had residual fluorescence intraoperatively, the location of which was considered to preclude safe resection. Intraoperatively, 2 types of fluorescence were noted: solid fluorescence, which corresponded with coalescent tumor on histology, and vague fluorescence, representing infiltrative tumor. Independent factors significantly associated with survival in multivariate analysis were patient age, residual intraoperative fluorescence, and residual contrast enhancement on postoperative MRI. There was no perioperative mortality, 1 case of new, permanent morbidity (severe hemiparesis), and 3 patients with transient worsening of preexisting symptoms. No serious adverse events related to the ingestion of 5-ALA were observed.[1] This positive and encouraging experience showed the safety and feasibility of using 5-ALA-induced fluorescence as a surgical guide.

THE GERMAN MULTI-INSTITUTIONAL TRIAL

The publication in 2006 of a randomized multi-institutional study comparing 5-ALA-induced fluorescence-guided surgery with conventional white light surgery for malignant glioma[2] represented the first randomized surgical study for malignant glioma and was a landmark in fluorescence-guided surgical resection. Seventeen centers enrolled 322 patients in this study, the primary end points of which were residual contrast-enhancing tumor on early postoperative MRI and 6-month progression-free survival assessed by MRI. The study was terminated at interim analysis, with 270 patients randomized between the 5-ALA (139 patients) and white-light (131 patients) groups.[2]

Complete resection of contrast-enhancing tumor on MRI was achieved in 90 (65%) of 5-ALA patients and in 47 (36%) of white-light patients, a difference significant to $P<.0001$. Six-month progression-free survival was observed in 41% versus 21.1% of patients in the respective treatment groups, a difference that again was highly significant ($P<.0003$). There was no difference in severe adverse events at 7 days. The study was not powered to show a difference in overall survival, although restratification based on early postoperative MRI showed that patients without residual contrast-enhancing tumor of postoperative MRI had a survival advantage. Subgroup analysis also showed a longer time to reintervention for older patients (age >55 years: 10.2 months [5-ALA group] versus 7.1 months [white-light group]).[2]

The large number of well-documented patients undergoing GBM resection in the German ALA Glioma Study Group afforded an opportunity to look at the effect of completeness of resection on postoperative MRI with overall survival in patients stratified with respect to the recursive partitioning analysis (RPA) of the Radiation Therapy Oncology Group (RTOG).[3] Historically, the confounding variable of patient selection bias has rendered difficult the interpretation of any intervention effect in malignant glioma, and this partitioning strategy recognizes the important prognostic variables of age, Karnofsky Performance Scale (KPS), neurologic condition, and mental status.

The 243 patients with newly diagnosed GBM in the multicenter German study, independent of their assignment to 5-ALA or white-light groups, were looked at with respect to their RTOG-RPA class. Median overall survival times for classes III, IV, and V were 17.8, 14.7, and 10.7 months, respectively; 2-year survival in these classes were 26%, 12%, and 7%. Stratification for completeness of resection within RPA class revealed clear differences: for class IV, survival with complete resection

was 17.7 months versus 12.9 months with incomplete resection, and for class V, survival of 13.7 months versus 10.4 months. Two-year survival within class IV was 21.0% versus 4.4%, and within class V, 11.1% versus 2.6%. The number of patients within class III was too small to detect significant differences. Overall, the predictive capability of the RTOG-RPA classes was confirmed by the ALA Glioma Study Group, and differences in survival seen in class IV and class V support a relationship between maximum cytoreduction and survival.[4]

In the 2006 report of the ALA Glioma Study Group, only marginal differences were found between the 5-ALA and white-light groups. In a 2011 study, the investigators looked more closely at the issue of neurologic morbidity using the final intent-to-treat patient populations of their study (176 patients in the 5-ALA group and 173 in the white-light group). This analysis again confirmed the earlier findings of more complete tumor resection and improved progression-free survival. Using the National Institutes of Health Stroke Scale (NIH-SS) as a measure of neurologic status, the analysis also showed a higher incidence of deterioration in the NIH-SS by 1 or more points at the 48-hour postoperative time point in the 5-ALA group (26.2% vs 14.5%; $P = .02$). This measure was no longer statistically significant at 7 days, and at 3 months, the percentages with neurologic deterioration of 1 point or greater were 19.6% versus 18.6%, respectively. KPS scores showed no statistically significant differences between the 2 groups (deterioration in the KPS score at 6 weeks postoperatively seen in 32.9% for the ALA group vs 28.8% for the white-light group, $P = 1.0$, and deterioration at 6 months seen in 35.7% and 49.1%, respectively, $P = .12$). Patients at risk of neurologic deterioration were those with preexisting neurologic deficits unresponsive to steroids, consistent with these deficits more likely related to tumor infiltration than surrounding edema.[5]

Further analysis in this same study attempted to assess the trade-off between a higher incidence of temporary and generally mild neurologic deterioration with 5-ALA-guided resection and improved likelihood of complete resection. In long-term

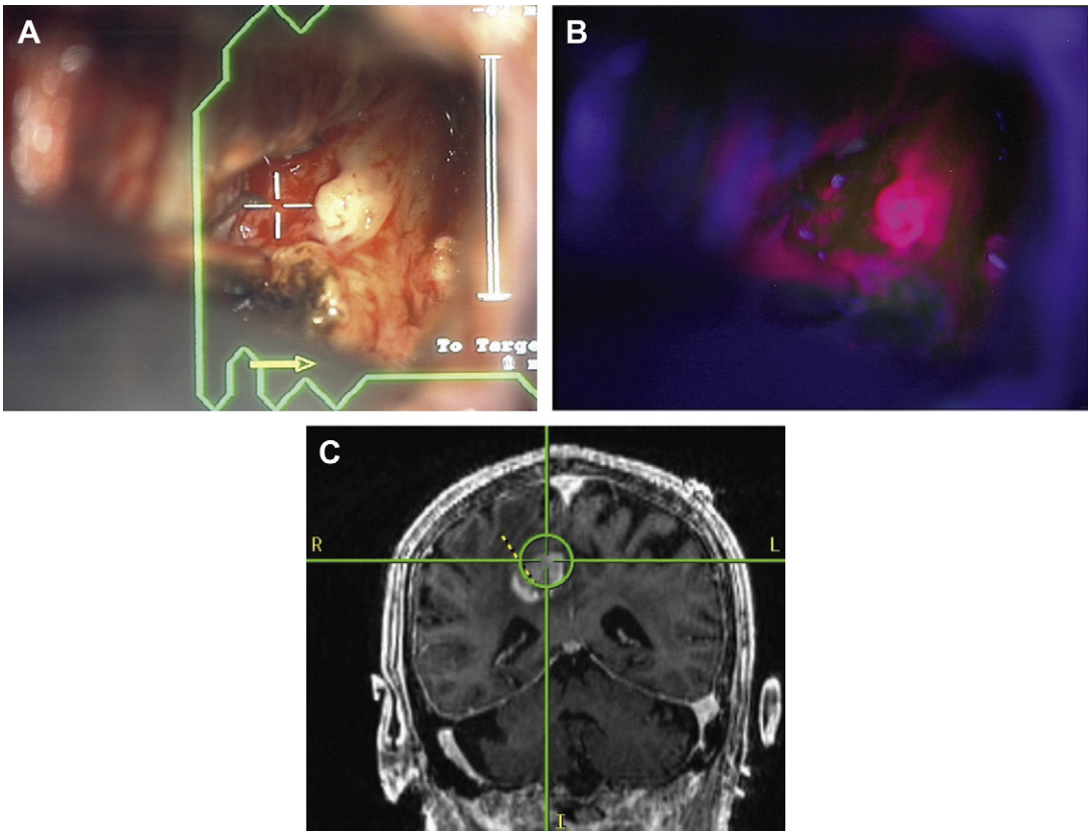

Fig. 1. (*A*) White-light operating microscope image at partial resection of this left parietal GBM tumor in a 79-year-old man. (*B*) Blue-violet light image of the same operative field. (*C*) Image guidance monitor image showing location of the focal point of the operating microscope at corresponding time during the procedure.

follow-up, a higher incidence of repeat surgery was found in the white-light group (39% vs 30%, $P = .0311$). Stratification by completeness of resection showed quicker time to progression and neurologic deterioration in those patients with incomplete resection ($P = .0036$).[5]

DARTMOUTH STUDIES

The aim of an initial investigative study at our institution using 5-ALA-induced fluorescence guidance in 11 patients with newly diagnosed GBM was to study the correlation between intraoperative visual fluorescence and preoperative MRI features as well as between fluorescence and histopathology (**Figs. 1** and **2**). Using a study design in which multiple locations of fluorescence assessment were spatially coregistered with preoperative MRI using image guidance, highly significant differences were found between fluorescent and nonfluorescent tissue in 2 measures of MRI contrast enhancement (gadolinium-enhanced signal intensity, $P = .018$; and normalized contrast ratio, $P<.001$).[6]

Biopsy specimens were obtained at each of 124 spatially coregistered sites in these 11 patients, 86 of which (69.4%) reported intraoperative fluorescence. Eighty-two of these 86 specimens (95.3%) were positive for tumor cells; 3 specimens (3.5%) showed either necrosis or abnormal, prominent vasculature, and 1 specimen (1.2%) showed no abnormality. Of the 38 nonfluorescent specimens, 28 (73.7%) were positive for tumor cells, 8 (21.1%) showed either necrosis or abnormal, prominent vasculature or reactive gliosis, and 2 (5.3%) showed no abnormalities. The sensitivity of visual fluorescence to identify tumor in this study was 0.75 (95% confidence interval [CI], 0.65–0.82); the specificity, 0.71 (0.42–0.90); the positive predictive value, 0.95 (0.88–0.98); and the negative predictive value, 0.26

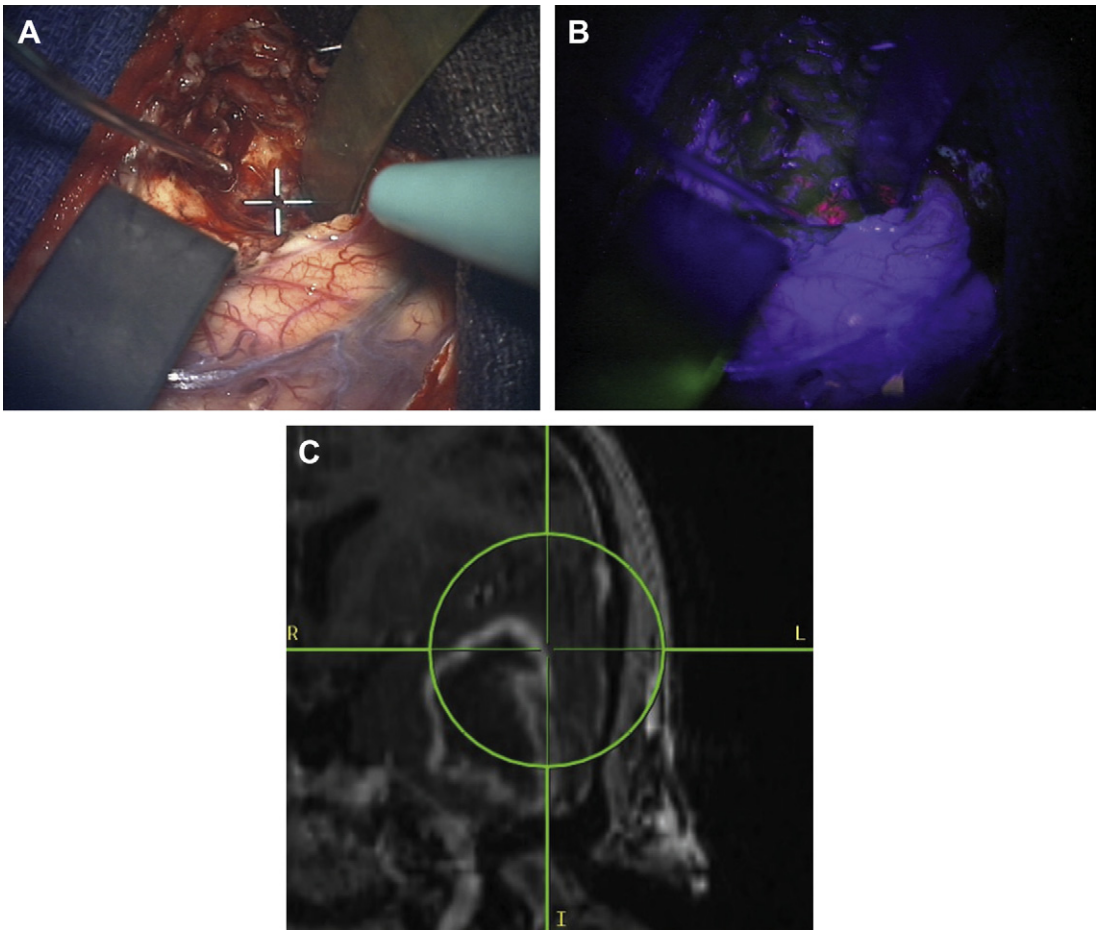

Fig. 2. (*A*) White-light operating microscope image at partial resection of this right temporal GBM tumor in a 68-year-old man. (*B*) Blue-violet light image of the same operative field. (*C*) Image guidance monitor image showing location of the focal point of the operating microscope at corresponding time during the procedure.

(0.14–0.43). As an indicator of abnormal tissue consistent with tumor, fluorescence had a positive predictive value of 0.99 (95% CI, 0.93–1.00). A low negative predictive value reflected the nonfluorescent property of fully necrotic tissue.[6]

Biopsy specimens in this study were also graded by World Health Organization (WHO) criteria for glial tumors (0–IV), independent of the overall WHO grade, which was IV for all patients. A Spearman rank correlation analysis was performed between this histologic grade and the level of visual fluorescence (0–3), yielding a correlation coefficient of 0.51 ($P<.001$). A similar analysis was performed between the degree of tumor infiltration in each biopsy specimen and the level of visual fluorescence, and again a highly significant correlation coefficient of 0.49 was found ($P<.001$). When a measure of the extent of necrosis was analyzed with respect to visual fluorescence, no statistical correlation was found (correlation coefficient –0.02; $P = .79$).[6]

Quantitative ex vivo tissue measurements of PpIX and Ki-67 immunohistochemistry to assess tissue proliferation were the focus of another study involving 23 patients undergoing fluorescence-guided resection of either low-grade or high-grade glial tumor (**Fig. 3**). Biopsy specimens from sites showing visual fluorescence had significantly higher levels of PpIX and tissue proliferation. Quantitative PpIX concentration (C_{PpIx}) levels correlated strongly with proliferation index ($r = 0.70$, $P<.0001$). Increasing levels of C_{PpIx} indicated regions of increasing malignancy. Approximately 40% of biopsies that were positive for tumor histologically but nonfluorescent intraoperatively had C_{PpIx} levels greater than 0.1 μg/mL; only 2 specimens with levels greater than this threshold were negative for tumor. Showing a quantitative relationship between C_{PpIx} and increasing malignancy in both low-grade and high-grade gliomas, this study suggests an opportunity for greater use of this fluorescence technology through improved PpIX fluorescence detection.[7]

Toward this aim, a handheld fiber-optic probe for in vivo quantitative fluorescence measurement has been developed and used clinically. Connected to a spectrometer, this probe interrogates tissue with emission of a blue-violet light (405-nm wavelength)

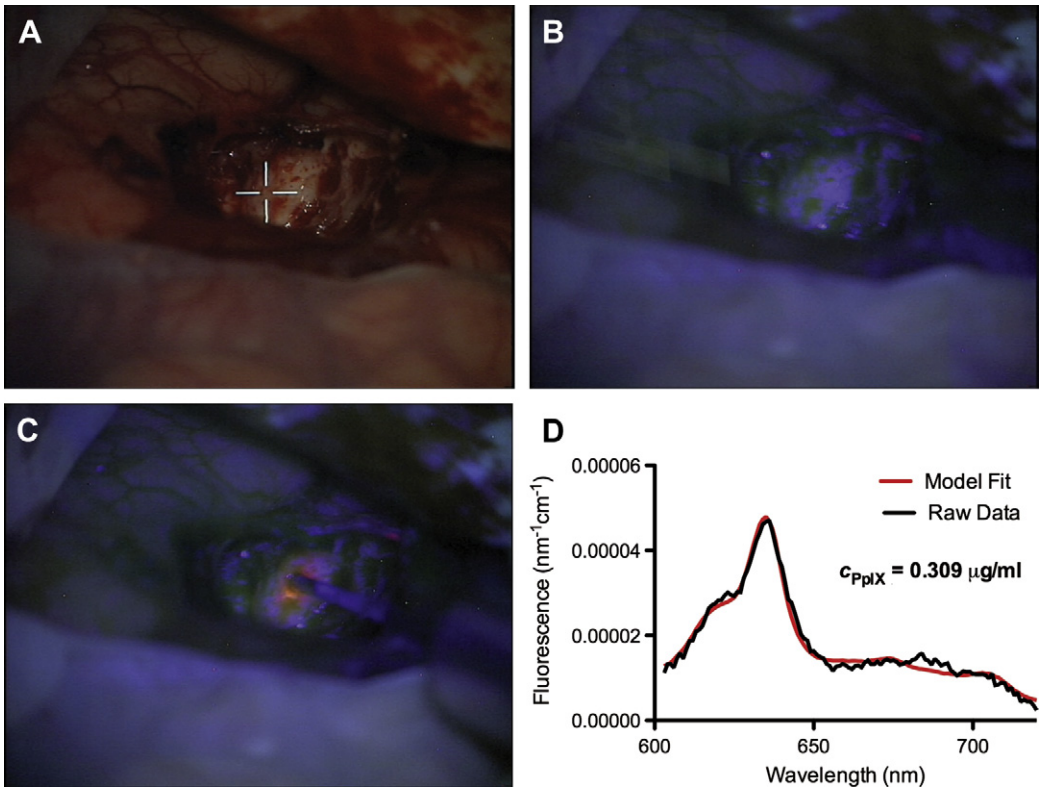

Fig. 3. (*A*) White-light operating microscope image at late stage of resection of a left temporal lobe GBM in a 73-year-old man. (*B*) Blue-violet light image corresponding to (A). (*C*) Handheld quantitative fluorescence probe acquiring data. (*D*) Raw and modeled data from probe acquisition, showing a quantitative level of the fluorophore PpIX that indicates tumor.

and with white light (450-nm to 720-nm wavelength), the latter enabling the accounting for tissue light absorption and scattering, and correction of the fluorescence spectrum. Spectral decomposition then allows determination of PpIX concentration. This device was used in a series of 14 patients harboring a variety of tumors including low-grade and high-grade gliomas as well as metastatic tumors and meningiomas, operated on using 5-ALA-induced fluorescence guidance.[8]

A statistically significant increase in C_{PpIx} ($P<.05$) was seen across all tumor types. Other spectroscopic variables (A_{615}, A_{660}, P_{635} and P_{710}) did not show similar ability to discriminate between tumor and nontumor tissue. Assessment of the diagnostic performance of this probe was then performed using a receiver operating characteristic (ROC) curve analysis and area under the curve (AUC) as a measure of performance. Comparing visual fluorescence (as used in all of the previously described clinical studies) with quantitative fluorescence by this metric, the AUC for high-grade gliomas improved from 0.78 ± 0.06 to 0.96 ± 0.03. Perhaps more important, for the implications with respect to potential application, the AUC for low-grade gliomas (which historically have not shown significant visible fluorescence) improved from 0.54 ± 0.04 to 0.75 ± 0.12, a level of performance below that of quantitative fluorescence in high-grade gliomas but comparable with that of visible fluorescence in those same high-grade tumors. For all of the tumors in this study, quantitative PpIX concentrations had a sensitivity of 84%, a specificity of 92%, a positive predictive value of 95%, and a negative predictive value of 77% (classification efficiency of 87% vs 66% for visible fluorescence). Of course, these values are dependent on the cutoff, or threshold, values chosen, which in this instance were those points on the ROC curve closest to the upper left corner; different threshold values may be chosen, should one wish to weight sensitivity and specificity differently.[8]

PROPOSED RTOG TRIAL

In an effort both to begin larger deployment of this technology in the United States as well as to acquire data required for FDA approval of 5-ALA for intracranial tumor resection, a phase III randomized, placebo-controlled, multicenter trial clinical trial using 5-ALA fluorescence guidance for resection of newly diagnosed GBM is anticipated to begin later this year. Coordinated by Emory University, this study is currently planned to determine whether fluorescence-guided resection improves extent of resection and whether improved extent of resection is associated with improved overall survival.

SUMMARY

Fluorescence guidance in resection of malignant glioma has been shown to improve extent of resection and 6-month progression-free survival in a prospective, multi-institutional clinical trial in Europe, and preliminary experience in the United States has confirmed the high correlation of this fluorescence with imaging and histologic features of malignant tumor. In this early experience, deployment of more sophisticated methods capable of increasing the diagnostic capability of this technique as well as extending the application of the technology to other tumor types have confirmed the potential of fluorescence guidance. Although the as yet unapproved FDA status of 5-ALA has slowed widespread dissemination of this technology, an increasing number of centers are using the drug under IND approvals; a large multicenter trial oriented toward providing data necessary for FDA approval is about to commence.

REFERENCES

1. Stummer W, Novotny A, Stepp H, et al. 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):1003–13.
2. Stummer W, Pichlmeier U, Meinel T, et al. 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. Curran WJ Jr, Scott CB, Horton J, et al. Recursive partitioning analysis of prognostic factors in three Radiation Therapy Oncology Group malignant glioma trials. J Natl Cancer Inst 1993;85(9):704–10.
4. Pichlmeier U, Bink A, Schackert G, et al. Resection and survival in glioblastoma multiforme: an RTOG recursive partitioning analysis of ALA study patients. Neuro Oncol 2008;10(6):1025–34.
5. Stummer W, Tonn JC, Mehdorn HM, et al. Counterbalancing risks and gains from extended resections in malignant glioma surgery: a supplemental analysis from the randomized 5-aminolevulinic acid glioma resection study. J Neurosurg 2011;114(3):613–23.
6. Roberts DW, Valdes PA, Harris BT, et al. Coregistered fluorescence-enhanced tumor resection of malignant glioma: relationships between delta-aminolevulinic acid-induced protoporphyrin IX fluorescence, magnetic resonance imaging enhancement, and neuropathological parameters. J Neurosurg 2011;114(3):595–603.
7. Valdes PA, Kim A, Brantsch M, et al. Delta-aminolevulinic acid-induced protoporphyrin IX concentration

correlates with histopathologic markers of malignancy in human gliomas: the need for quantitative fluorescence-guided resection to identify regions of increasing malignancy. Neuro Oncol 2011;13(8):846–56.

8. Valdes PA, Leblond F, Kim A, et al. Quantitative fluorescence in intracranial tumor: implications for ALA-induced PpIX as an intraoperative biomarker. J Neurosurg 2011;115(1):11–7.