# Adjuncts for Maximizing Resection: 5-Aminolevuinic Acid

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The rationale for maximizing resection in surgery for malignant glioma has become broadly accepted in the neuro-oncology community, and technological adjuncts to facilitate achievement of this goal have incrementally assisted the neurosurgeon. One such adjunct of considerable interest today is 5-aminolevuinic acid (5-ALA)–induced protoporphyrin IX (PpIX) fluorescence. Preoperative oral administration of 5-ALA leads to preferential accumulation of the fluorophore PpIX within tumor cells, and under the violet-blue light illumination of the surgical field with an adapted operating microscope, tumor tissue exhibits visible red and near-infrared fluorescence. A German multi-institutional trial in 2006 demonstrated the potential utility of this technique,[1] and although Food and Drug Administration approval in the United States is still pending, Investigational New Drug (IND)-supported experience in this country has furthered our understanding of this technology. This article presents the background for this methodology, the early clinical experience, its practical utility in intraoperative tumor boundary appreciation, its role near the end of resection, the development of quantitative fluorescence techniques, the experience in low-grade gliomas, and the implications of using this technique with respect to the objectives of surgery.

## BACKGROUND

In the prospective, randomized clinical trial reported by Stummer et al,[1] 270 patients with malignant glioma were randomized between fluorescence-guided resection and conventional white light resection. The primary end points had been reached at interim analysis, with significantly more patients having complete resection of contrast-enhancing tumor on postoperative magnetic resonance imaging (MRI; (65% vs 36%) and near-doubling of progression-free survival at 6 months (41% vs 21.1%) in the fluorescence-guided group. The study was underpowered to show a difference in survival. Since this seminal report, a number of smaller, largely single-institution experiences have been published.[2-10] Dartmouth was particularly interested in understanding the specific imaging and pathological features associated with the 5-ALA–induced PpIX fluorescence phenomenon and undertook a study in which multiple specimens obtained during the course of tumor resection were characterized with respect to intraoperative fluorescent properties and then correlated with preoperative MRI and with subsequent histopathological analysis. We were able to demonstrate in a study of 11 malignant gliomas a very high correlation between the degree of intraoperative visual fluorescence in tissue and the degree of gadolinium enhancement of that specific tissue on preoperative MRI. Furthermore, the degree of fluorescence correlated highly with the degree of histological anaplasia and with the degree of tumor infiltration in the tissue.[9] Statistical measures summarizing the relationship between PpIX fluorescence and tissue histopathology demonstrated a sensitivity for neoplastic tissue of 0.75 (95% confidence interval, 0.65-82), a specificity of 0.71 (95% confidence interval, 0.42-0.90), a positive predictive value of 0.95 (95% confidence interval, 0.88-0.98), and a negative predictive value of 0.26 (95% confidence interval, 0.14-0.43). The low negative predictive value reflected, in part, the nonfluorescent quality of necrotic tumor.[9] Subsequent developments with improved fluorescence detection (discussed in the Quantitative Fluorescence section) have improved on this.


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## UTILITY IN MALIGNANT GLIOMA RESECTION

The roles of conventional image guidance and fluorescence guidance in tumor resection are largely complementary. In the planning and initial exposure, the former is often extremely helpful, whereas the latter is not yet applicable. Over the course of surgery, however, progressive deterioration in the accuracy of coregistration between preoperative imaging and the surgical field renders image guidance less reliable,[11] and the direct visualization of tumor tissue identified by its fluorescent signature becomes more useful. The 2 aspects of resection in which fluorescence appears to be most useful are boundary determination during resection and assessment of completeness of resection near the end of resection (including potential detection of tumor that may not give rise to discernible MRI contrast).

### Boundary Determination

Whether one is dissecting along a planned resection plane early in surgery (before debulking more centrally and progressive misregistration) or later in the procedure (after substantial tumor volume has been resected), visible fluorescence

corresponding to the enhancing pseudocapsule of a tumor can be very useful. The steepness of the fluorescent gradient varies considerably within and between tumors—much as the histology does—and the correlation between fluorescence and histology renders the enhanced visualization helpful; under white light conditions, it is often impossible to discern such a boundary. In instances when the fluorescent gradient is steep and a demarcated border is easy to follow, the variable and irregular error of a conventional image guidance heads-up display contour is evident. Such an instance is illustrated in Figure 1. Independently of such registration error, image guidance along a contour can be difficult when such a plane is oblique and not orthogonal to the line of sight because the optical depth of field potentially confounds accurate localization of the contour.

### End of Resection

It is perhaps near the end of resection that the utility of fluorescence guidance is most evident. Tissue displacement and distortion at this point in the procedure will have maximally degraded registration of conventional image guidance, and observation of the resection cavity under violet-blue light conditions often enables appreciation of residual tumor tissue (see Figure 2). Resection of this tissue can proceed under either violet-blue light (often easiest and most efficient) or white light illumination until any visualized fluorescence is absent. It is essential that the surgeon remain aware that only exposed tissue will be visualized in this manner and that a heterogeneous malignant glioma may have intervening nonfluorescent (eg, necrotic) tissue obscuring additional tumor at greater depth. In this context, the synergy between fluorescence and image guidance can be invaluable.

A second area of concern is that residual tumor may be difficult to appreciate in less easily visualized portions of the tumor bed such as the underside of any overhanging rim. With awareness of this risk and with experience, we hope that this will be minimized.

## QUANTITATIVE FLUORESCENCE

Nearly all reports to date of tumor resection with 5-ALA have used visible fluorescence as appreciated by the surgeon through the operating microscope. Technically, it is possible to extract more information from the fluorescent signal with advanced optical detection methods that allow the spectrum of PpIX to be resolved. Simply using the net fluorescent light intensity signal, eg, heights or area under various peaks of the emission spectrum, is confounded by the optical properties of particular tissue—specifically, its absorption and its scattering of light at different wavelengths—and this markedly limits the utility of such an approach. With the use of a fiberoptic probe that can be placed directly on the tissue, a spectroscopic method has been developed to first interrogate the same tissue with white light to collect the remitted white light reflectance and to compute the wave-length–dependent absorption and scattering values of the tissue and then interrogate with violet-blue light to collect the spectrally resolved fluorescence emissions. Using the obtained values (ie, optical properties and fluorescence) to determine an absorption- and scattering-independent value for the fluorescence and finally fitting the known spectra of the major fluorophores in the tissue, we can derive absolute concentrations for PpIX. The entire process takes a couple seconds.

Initial experience with quantification of fluorescence in clinical cases has revealed considerable benefit. Tumor tissue across a spectrum of histologies, including malignant glioma, low-grade glioma, meningioma, and metastatic tumor, that has not appeared to be fluorescent to the surgeon's eye frequently has had PpIX levels well in excess of, and distinguishable from, normal, noninvolved tissue. A common descriptor of the performance of a diagnostic test is the receiver-operating characteristic curve, and 1 measure of that performance is the area under the receiver-operating characteristic curve. If one analyzes the diagnostic performance of visible fluorescence (the subjective perception of fluorescence by the surgeon, as in the present conventional practice) for high-grade gliomas, the area under the curve is 0.78; the area under the curve assessed by quantitative fluorescence is improved to 0.96. A finding with perhaps greater implications was a similar substantial improvement in low-grade gliomas in which visible fluorescence area under the curve had been a disappointing 0.54 (ie, not significantly better than chance) but quantitative assessment achieved an area under the curve of 0.75.[10,12] Although still poorer than what is seen in high-grade gliomas, it is worth noting that that performance is similar to what is achieved today for high-grade gliomas assessed by conventional fluorescence technique. The implications for potential wider application of

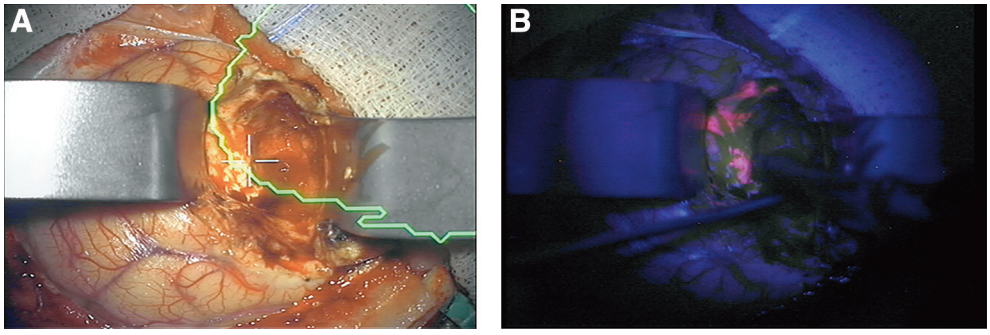


**FIGURE 1.** A, during resection along the lateral aspect of this glioblastoma multiforme, the overlaid contour representing the preoperatively segmented contrast-enhancement on MRI provides a general guide to the proposed extent of resection. B, under violet-blue light conditions, fluorescence in the tissue corresponding to that MRI contrast enhancement facilitates resection of that tissue.

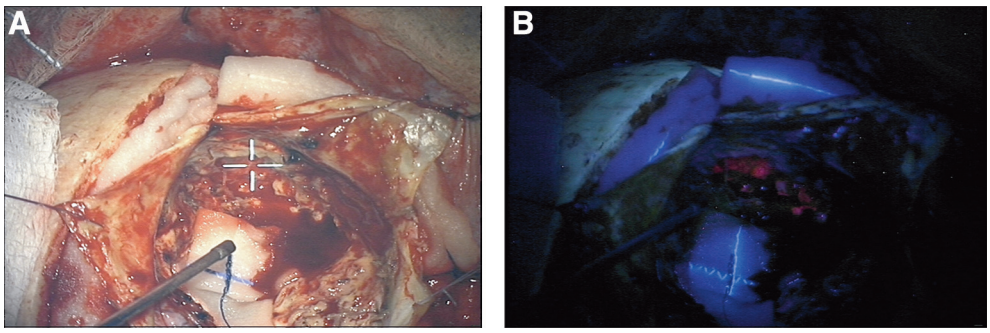


**FIGURE 2.** A, near the end of resection of this glioblastoma multiforme, residual tumor is not evident by either image guidance or white light appearance of the tissue. B, under blue light conditions, remaining tumor, corresponding to a portion of the gadolinium-enhancing tumor on MRI, is readily appreciated.

fluorescent technologies and for instrumentation development warrant attention.

## LOW-GRADE GLIOMAS

Because early experience demonstrated relatively little visible fluorescence in low-grade gliomas, the technology was infrequently used in these tumors. Using the same study design as in the investigation of the correlation of fluorescent tissue with gadolinium contrast enhancement, we also studied the fluorescent properties of a small number of low-grade gliomas. Of the first 6 tumors, only 1 tumor (a mixed oligoastrocytoma) demonstrated visible fluorescence.[13] With quantitative fluorescence, however, elevated PpIX concentrations were regularly detected, as reflected in the receiver-operating characteristic performance noted above.

Widhalm and colleagues reported[8] the correlation of PpIX concentration with markers of potentially more aggressive behavior, including both $^{11}$C-methionine positron emission tomography and MIB-1 labeling indexes. In a report by Valdés et al,[13] significant statistical correlations were found between quantitative PpIX levels and MIB-1 proliferation indexes ($r = 0.70$, $P < .001$), PpIX levels and total number of cells ($r = 0.41$, $P < .001$), and PpIX levels and total number of proliferating cells ($r = 0.71$, $P < .001$). These findings suggest a value in intraoperative fluorescence that helps to ensure resection of more aggressive portions of a tumor.

Quantitative technique, as in its current implementation with a handheld probe, may help resection in lower-grade gliomas in a manner similar to its usefulness in higher-grade tumor. Both boundary determination and end-of-resection inspection are points in resection in which tissue characterization has been useful. Figure 3 illustrates its role along an obliquely sloping margin of a mixed oligoastrocytoma. Analogous to the already described value vis-à-vis the superposed contour of conventional image-guidance, in this instance, a handheld probe acquiring quantitative PpIX-level information both directly characterizes tissue within the surgical field and refines localization relative to the preoperative MRI.

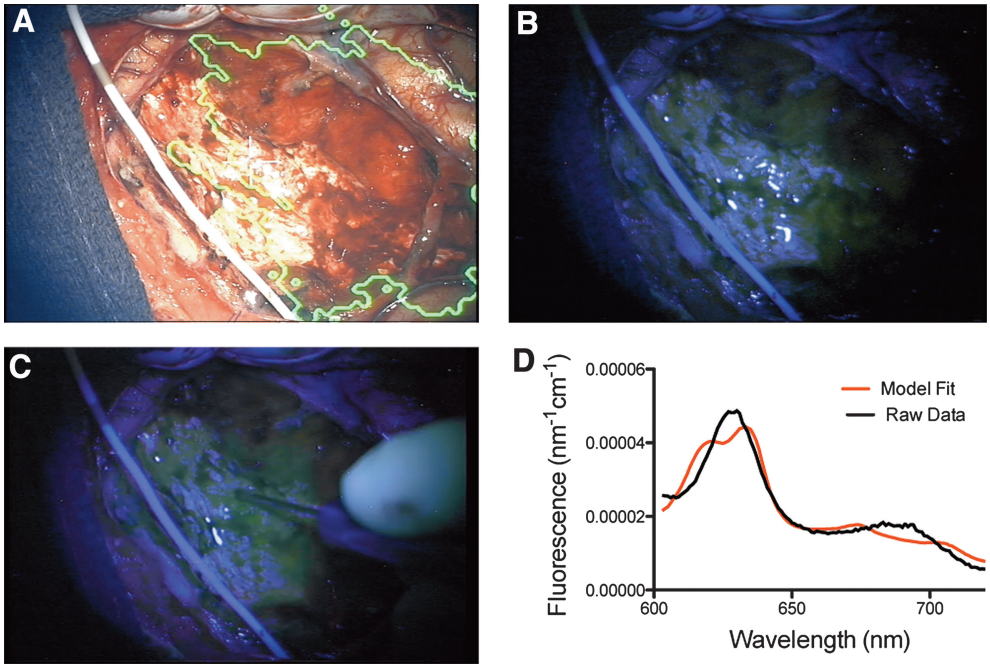


**FIGURE 3.** A, during resection along the lateral aspect of this mixed oligoastrocytoma, the overlaid contour indicates the planned margin of resection on preoperative MRI. B, under violet-blue light conditions, no visible fluorescence is evident. C, under violet-blue light conditions, the handheld probe can be seen at the same presumed tumor margin. D, quantitative protoporphyrin IX (PpIX) data obtained from the handheld probe shows the characteristic spectral signal of PpIX with a major peak at 635 nm and a concentration of PpIX of 0.21 μg/mL, a value consistent with tumor tissue.

## BALANCING AGGRESSIVE RESECTION AND PRESERVATION OF FUNCTION

With the assistance of any new technology guiding the extent of resection, the level of uncertainty that prohibits resection of potentially functional tissue is presumably reduced, and more accurate, more extensive resection is consequently achieved. With assisting technology and enhanced resections, the balance between extent of resection and preservation of normal function becomes more explicit.

A later analysis of the German multi-institutional trial shed light on this issue. Secondary outcome measures in that trial included the National Institutes of Health Stroke Scale score and the Karnofsky Performance Scale score. Although the 5-ALA group had higher percentages of complete resection and 6-month progression-free survival, 5-ALA was also associated with a higher frequency of deterioration in the National Institutes of Health Stroke Scale score at 48 hours (deterioration by $\geq 1$ point in 26.2% vs 14.5%; $P = .02$). The difference in change in the National Institutes of Health Stroke Scale scores between the 2 groups did not reach statistical significance at 7 days, 6 weeks, or 3 months. No statistical difference in change in Karnofsky Performance Scale scores was demonstrated, and the only difference in neurological serious adverse events that reached statistical significance was that for intracranial hypertension, which was higher in the conventional surgery group (2.3% vs 0%; $P = .04$). Of note, stratification by completeness of resection revealed earlier deterioration in patients with incomplete resection, and patients in the ALA group had a lower cumulative incidence of repeat surgery.[14] For a perhaps mildly increased risk of a usually transient neurological adverse event, a greater long-term benefit appears to be achieved, a tradeoff recognized and largely accepted by both the patient and the neuro-oncology community. Individualization of that decision making by patient and neurosurgeon together, of course, is mandated.

Use of fluorescence-guidance technology can enable more accurate and complete tumor resection, and it is not mutually exclusive with other surgical adjuncts. Image guidance, enhanced structural and functional imaging, intraoperative imaging, intraoperative tissue characterization, and intraoperative mapping and monitoring all provide the resecting neurosurgeon with valuable, patient-specific information. How these tools, singly or in combination, work to optimize surgical resection in malignant glioma deserves and requires thoughtful investigation and analysis.

## Disclosure

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## REFERENCES

1. Stummer W, Pichlmeier U, Meinel T, Wiestler OD, Zanella F, Reulen HJ. 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.
2. Pichlmeier U, Bink A, Schackert G, Stummer W. Resection and survival in glioblastoma multiforme: an RTOG recursive partitioning analysis of ALA study patients. *Neuro Oncol.* 2008;10(6):1025-1034.
3. Stummer W, Nestler U, Stockhammer F, et al. Favorable outcome in the elderly cohort treated by concomitant temozolomide radiochemotherapy in a multicentric phase II safety study of 5-ALA. *J Neurooncol.* 2011;103 (2):361-370.
4. Hayashi Y, Nakada M, Tanaka S, et al. Implication of 5-aminolevulinic acid fluorescence of the ventricular wall for postoperative communicating hydrocephalus associated with cerebrospinal fluid dissemination in patients with glioblastoma multiforme: a report of 7 cases. *J Neurosurg.* 2010;112(5):1015-1019.
5. Hefti M, von Campe G, Moschopulos M, Siegner A, Looser H, Landolt H. 5-Aminolevulinic acid induced protoporphyrin IX fluorescence in high-grade glioma surgery: a one-year experience at a single institution. *Swiss Med Wkly.* 2008;138(11-12):180-185.
6. Ritz R, Feigl GC, Schuhmann MU, et al. Use of 5-ALA fluorescence guided endoscopic biopsy of a deep-seated primary malignant brain tumor. *J Neurosurg.* 2011;114(5):1410-1413.
7. Tamura Y, Kuroiwa T, Kajimoto Y, Miki Y, Miyatake S, Tsuji M. Endoscopic identification and biopsy sampling of an intraventricular malignant glioma using a 5-aminolevulinic acid-induced protoporphyrin IX fluorescence imaging system: technical note. *J Neurosurg.* 2007;106 (3):507-510.
8. Widhalm G, Wolfsberger S, Minchev G, et al. 5-Aminolevulinic acid is a promising marker for detection of anaplastic foci in diffusely infiltrating gliomas with nonsignificant contrast enhancement. *Cancer*. 2010;116(6): 1545-1552.
9. 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: clinical article. *J Neurosurg.* 2011;114(3):595-603.
10. Valdés 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):11-17.
11. Valdés PA, Fan X, Ji S, Harris BT, Paulsen KD, Roberts DW. Estimation of brain deformation for volumetric image updating in protoporphyrin IX fluorescence-guided resection. *Stereotact Funct Neurosurg.* 2010;88(1): 1-10.
12. 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.
13. Valdés 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):846-856.
14. 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: clinical article. *J Neurosurg.* 2011;114(3):613-623.

 