Turning on the light for brain tumor surgery: A 5-aminolevulinic acid story

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Abstract
To aid surgeons in more complete and safe resection of brain tumors, adjuvant technologies have been developed to improve visualization of target tissue. Fluorescence-guided surgery relies on the use of fluorophores and specific light wavelengths to better delineate tumor tissue, inflammation, and areas of blood–brain barrier breakdown. 5-aminolevulinic acid (5-ALA), the first fluorophore developed specifically for brain tumors, accumulates within tumor cells, improving visualization of tumors both at the core, and infiltrative margin. Here, we describe the background of how 5-ALA integrated into the modern neurosurgery practice, clinical evidence for the current use of 5-ALA, and future directions for its role in neurosurgical oncology.

Maximal safe resection remains the standard of care for most brain tumors. Gross total resection of high-grade gliomas (HGGs) is associated with greater overall survival and progression-free survival (PFS) in comparison to subtotal resection or adjuvant treatment therapies alone. A major challenge neurosurgeons encounter when resecting infiltrative gliomas is identification of the glioma tumor margin to perform a radical resection while avoiding and preserving eloquent regions of the brain. 5-aminolevulinic acid (5-ALA) remains the only optical-imaging agent approved by the FDA for use in glioma surgery and identification of tumor tissue. A multicenter randomized, controlled trial revealed that 5-ALA fluorescence-guided surgery (FGS) almost doubled the extent of tumor resection and also improved 6-month PFS. In this review, we will highlight the current evidence for use of 5-ALA FGS in brain tumor surgery, as well as discuss the future directions for its use.

Keywords
fluorescence-guided surgery | 5-aminolevulinic acid, 5-ALA | extent of resection | glioma | metastasis

5-ALA Background and Clinical Information

5-aminolevulinic acid is a metabolic precursor agent that is taken up by tumor cells and enters the mitochondrion heme biosynthesis pathway, where it is metabolized to protoporphyrin IX (PpIX). Due to decreased ferrochelatase enzyme activity which converts PpIX to heme, PpIX accumulation occurs in tumor cells. A lack of efflux of PpIX out of tumor cells through the transporter ATP-binding Cassette Subfamily B Member 2 (ABCG2) is also responsible for PpIX intratumoral accumulation. 5-ALA uptake and metabolism to PpIX readily occurs in glioma cells, particularly HGGs. A recent study by Mischkulnig et al. found significantly decreased ABCG2 mRNA and protein expression in fluorescing tumor specimens compared to

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| Date Published | Paper | PMID | Study Type | Number Pts | Outcomes Measured | Result of Study |
|----------------|-------|------|------------|------------|-------------------|-----------------|
| 10/8/21        | Schupper et al. | 34624862 | Case series | 69 | Primary: KPS decline, EOR, and residual enhancing tumor volume. | 5-ALA fluorescence has high sensitivity and positive predictive value for HGG; 5-ALA is well-tolerated; there was no excess neurological morbidity. |
| 10/8/21        | Black et al. | 34625597 | Case series | 128 | Primary: correlating PpIX concentration with fluorescence intraoperatively. | PpIX concentration correlates with proliferation indices, WHO grade, and fluorescence visibility. |
| 5/21/21        | Hosmann et al. | 34064222 | Case series | 59 | Primary: PFS, OS, 5-ALA fluorescence, EOR in WHO II glioma. | 5-ALA fluorescence presence is correlated with poor PFS and OS; 5-ALA correlated to IDH-wildtype tumors; higher EOR correlates to increased OS. |
| 10/1/19        | Kaneko et al. | 31058995 | Case series | 68 | Primary: quantification of fluorescence intensities and correlation with tumor PpIX concentrations. | 5-ALA fluorescence is present longer than 4–5 h after administration. |
| 6/18/16        | Cordova et al. | 26463215 | Case series | 30 | Primary: EOR and RTV; Secondary: PFS and OS | Bright 5-ALA fluorescence is highly predictive of tumor; negative-predictive value is low; 5-ALA is predictive of tumor cellularity when fluorescence is present; reactive changes in the brain may lead to 5-ALA fluorescence. |
| 5/1/16         | Lau et al. | 26544781 | Case series | 59 | Primary: correlation of 5-ALA fluorescence with tumor cellularity | 5-ALA has high correlation with tumor and is more useful for indicating residual tumor compared to contrast-enhanced MRI and spectrometry. |
| 2/17/16        | Teixidor et al. | 26885645 | Case series | 85 | Primary: safety data; Secondary: EOR, PFS, and OS | 5-ALA has good safety profile; GTR in 54%; PFS at 6 months 58%; OS was 14.2 months. |
| 4/1/14         | Diez Valle et al. | 23870657 | Cohort | 251 | Primary: EOR and PFS. | 5-ALA use was associated with increased EOR and PFS in HGG, compared to non-use. |
| 3/1/14         | Stummer et al. | 24335821 | Case series | 33 | Primary: correlation of pathological samples and 5-ALA fluorescence and comparison to contrast-enhanced MRI. | 5-ALA fluorescence has high sensitivity and lower specificity for tumor than contrast MRI; infiltrating tumor: 5-ALA better in both specificity and specificity. |
| 2/1/14         | Coburger et al. | 24484256 | Case series | 45 (33 HGG 11 Mets) | Primary: correlation of pathological samples and 5-ALA fluorescence | 5-ALA has high correlation with tumor and is more useful for indicating residual tumor compared to contrast-enhanced MRI and spectrometry. |
| 5/28/13        | Zhao et al. | 23723993 | Review and Meta-analysis | 10 studies systematic review; 5 studies meta-analysis | Primary: OR of diagnosis of HGG, Secondary: EOR, PFS, and OS | Level 2 evidence that 5-ALA is more effective than WL surgery; increased diagnostic accuracy, increased PFS, increased OS |
| 3/11/11        | Stummer et al. | 20397896 | Randomized Control Trial | 349 | Long-term follow-up of phase III trial of 5-ALA. | Higher residual tumor volume in WL group; 5-ALA group more neuro def at 48 h–pts at risk were unresponsive to steroids; less cumulative incidence of repeat surgery in 5-ALA group; incomplete resections had quicker neuro deterioration |
nonfluorescing samples from adult patients with diffuse gliomas (WHO grade II–IV) after 5-ALA administration. They also found up-regulation of heme biosynthesis enzyme protein expression in fluorescing tumor specimens suggesting heme biosynthesis pathway activity in general is enhanced in fluorescing gliomas with up-regulation of PpIX generating enzymes and decreased ABCG2 mediated PpIX efflux.

PpIX emits violet-red (∼635 nm) fluorescence after excitation with blue light in the absorption spectrum of 375–440 nm, (Figure 1). Dosing of oral 5-ALA at 20 mg/kg 2–4 h before induction of anesthesia was performed with the 2006 randomized, controlled trial. Rodent preclinical studies revealed peak fluorescence occurred 6 h after 5-ALA administration and therefore the 2–4 h window was established to allow time for anesthesia induction and the craniotomy completion so that the neurosurgeon would have access to peak fluorescence during the tumor resection portion of the surgery. More recent studies, however, have found that 5-ALA PpIX fluorescence may have a longer window with a delayed peak response than previously seen in early rodent studies. Kaneko et al. found in a cohort of 201 tumor samples a maximal intensity at 7–8 h postadministration, with a weaker peak signal at 8–9 h. Maragkos et al. found in a retrospective study of 16 patients with HGG who received 5-ALA over 4 h before induction of anesthesia, which all patients' tumors displayed adequate fluorescence even up to 28 h. This extended time window appears true for not only HGGs, as Kaneko et al. recently showed that low-grade gliomas (LGGs) also demonstrated peak fluorescence 7–8 h after administration. By broadening the administration window, the prodrug may become easier to administer, as it may be useful in the operating room even with unforeseen delays in surgical start times.

Beyond identifying tumor fluorescence, the next level of information for tumor surgeons is quantifying fluorescence. Quantifying fluorescence levels may be useful in cases where there are lower levels of PpIX accumulation that are visible to the eye, such as in LGG tissue. Valdés et al. found that 45% of LGG tumors that visibly demonstrated no fluorescence accumulated significant levels of PpIX, and were able to be quantitatively detected. Confirming these results, Widhalm et al. found that 50% of LGG samples showed no visible fluorescence yet significant PpIX concentration, and that there was no difference

| Date Published | Paper | PMID   | Study Type | Number Pts | Outcomes Measured | Result of Study                                      |
|---------------|-------|--------|------------|------------|-------------------|-----------------------------------------------------|
| 3/1/11        | Roberts et al. | 20380535 | Case series | 11         | Primary: difference in tissue fluorescence | Significant relationship between preop contrast enhancement on MRI and intraoperative fluorescence; tumor aggressiveness and fluorescence correlates. |
| 3/1/11        | Diez Valle et al. | 20607351 | Cohort     | 36         | Primary: correlation of pathological samples and 5-ALA fluorescence; Secondary: immediate neurological and mortality outcomes | 5-ALA has 100% positive-predictive value for histological tumor; 1-month postop: no mortality, 8.2% neurological morbidity. |
| 12/1/09       | Nabavi et al. | 19934966 | Case series | 36         | Primary: correlation of pathological samples and 5-ALA fluorescence | 5-ALA has high-predictive value for histological tumor; prior radiation and chemotherapies do not interfere with 5-ALA use. |
| 1/14/09       | Hadjipanayis et al. | 30644008 | Review     | NA         | NA                | Discussion of FDA approval of 5-ALA                  |
| 10/1/08       | Eljamel et al. | 17926079 | Randomized Control Trial | 27         | Primary: 5-ALA and photodynamic therapy for GBM, OS, KPS, and KPS. | 5-ALA and photodynamic therapy were associated with increased OS, PFS, and KPS compared with controls. |
| 3/22/08       | Hefti et al. | 18363116 | Case series | 74         | Primary: correlation of pathological samples and 5-ALA fluorescence | 5-ALA has high-predictive value for histological tumor; prior radiation and chemotherapies do not interfere with 5-ALA use. |
| 5/7/06        | Stummer et al. | 16648043 | Randomized Control Trial | 322        | Primary: contrast enhancement on postop MRI, 6-month PFS. Secondary: volume postop MRI, OS, neuro def, and toxic effects | 65% versus 36% GTR contrast tumor; 41% versus 21.1% 6 month PFS. No difference in adverse events. |

PMID, PubMed identification number.
### Table 3 Landmark Clinical 5-ALA Papers—Metastases

| Date Published | Paper | PMID | Study Type | Number Pts | Outcomes Measured | Result of Study |
|----------------|-------|------|------------|------------|-------------------|-----------------|
| 9/27/19        | Marhold et al. | 31561223 | Case series | 154 | Intraop fluorescence of nearby structures after resection | 83% fluoresced. Venous, dural, and skull edges each demonstrated strong fluorescence, suggesting utility in invasive lesions for initial resection |
| 2/3/21         | Mercea et al. | 33546427 | Case series | 88 | Peritumoral tissue samples looking at fluorescence, infiltration, and angiogenesis. Recurrence and 1 y survival. | 66% fluoresced (34% strong, 32% weak), most were heterogeneous. Melanomas showed least. Ductal breast cancer the most. |
| 11/28/07       | Utsuki et al. | 18095131 | Case series | 11 | PpIX fluorescence in tumor and peritumoral tissue | Fluorescence present in peritumoral tissue free of tumor likely due to leak |
| 12/10/18       | Kamp et al. | 30535595 | Case series | 218 | Degree of resection, local progression, overall survival | Degree of resection did not correlate with fluorescence. Higher local progression in nonfluorescent mets. When fluorescence present, local progression and overall survival were significantly better. |
| 8/11/20        | Hussein et al. | 32850007 | Cohort | 175 | Comparing regular white light versus 5-ALA for in-brain recurrence and mortality | 5-ALA had lower recurrence and mortality but neither achieved significance |

PMID, PubMed identification number.
Fig. 1  Fluorescence emission wavelengths of the most commonly used fluorophores used in glioma surgery. AM, amplitude modulation; FM, frequency modulation; ICG, indocyanine green. (Permission from Hadjipanayis CG, Stummer W. Fluorescence Guided Neurosurgery. New York, NY: Thieme Medical Publishers; 2018).

Fig. 2  Fluorescence-guided surgery of a HGG using 5-ALA. (A) demonstrates white light visualization of the surgical resection cavity and (B) shows the surgical field under blue light (635 nm), with the red and pink fluorescence representing tumor cell density, compared to the normal brain appearing blue (devoid of fluorescence). (Permission from Hadjipanayis CG, et al. Fluorescence Guided Brain Tumor Surgery. Youmans & Winn Neurological Surgery, 8th ed. Chapter 157B. New York, NY: Elsevier; 2021).
in mean PpIX concentration between HGG and LGG tumors. Despite the reliability of visible fluorescence with 5-ALA, using tools to quantify PpIX concentration in tumor cells may permit greater detection in tumors such as LGGs.

FDA Approval

While 5-ALA has been widely used throughout Europe since 2007, it has more recently become a commonly used surgical adjunct in the US. Following the 2006 randomized controlled trial by Stummer et al. as well as multiple other multicenter European studies, US neurosurgeons sought FDA approval of 5-ALA, specifically for use as intraoperative optical agent in suspected HGG surgery. Other primary brain tumors, such as LGGs, metastases, and benign tumors, such as meningiomas, are not included in the current FDA approval. After a multiyear effort, 5-ALA gained FDA approval in 2017, making it the first intraoperative imaging agent approved by the Food and Drug Administration for use in glioma surgery. Since FDA approval, 5-ALA FGS has been rapidly used throughout the US as an intraoperative adjunct for HGG surgery, and the results of the first US multicenter clinical trial were recently published, affirming safety, and efficacy of 5-ALA in HGG tumors.

5-ALA and High-Grade Gliomas

To date, many studies have affirmed that 5-ALA is able to reliably differentiate between normal brain and glioma tissue in HGGs (Table 1). Recent analyses have shown a sensitivity and specificity ranging from 83%–87% to 89%–100%, respectively. In the recent US multicenter study, 5-ALA was found to have a sensitivity of 96.5%, with a positive predictive value of 95.4% and accuracy of 92.4%. In addition, 5-ALA can help indicate the tumor cell density by its fluorescence intensity, with a violet-red fluorescence often demonstrating the tumor core and a less robust pink emission in regions of infiltration near the tumor margin (Figure 2). Prior studies have shown 5-ALA fluorescence discrimination in cell density as low as 10%–20%, allowing for resection beyond the contrast-enhancing border identified with MRI. Beyond improved detection of low-tumor cell density, 5-ALA intensity has also been shown to correlate with histological grading and malignant characteristics such as Ki-67 and MIB-1 index. By improving the rate of complete resection of enhancing tumor (CRET), 5-ALA FGS has been assessed for not only improvement tumor extent of resection, but patient outcomes as well. In a study of 52 glioblastoma patients undergoing 5-ALA FGS, patients with no residual contrast on postradiation MRI had significantly improved overall survival. To date, 5-ALA is the only fluorescent agent that has been studied in a large phase III randomized controlled trial. In 2006, Stummer et al. found that 322 patients (half undergoing 5-ALA FGS) had significantly higher rate of CRET, and improved 6-month PFS (41% vs 21.1%). As part of this trial, patients had an average CRET rate of 65%, which has continued to improve over the past decade with the use of 5-ALA intraoperative fluorescence. To further improve the rate of CRET in eloquent-region tumors, multiple studies have combined brain mapping with 5-ALA FGS, and found that combining mapping with FGS improves the rate of GTR compared to literature controls, with a favorable safety profile in terms of long-term postoperative morbidity.

Beyond its evidence for improve surgical and patient outcomes, 5-ALA has shown to be safe for use. Administered orally 3–4 h before induction of anesthesia (per FDA guidelines), Gleolan® (NXDC Corporation) is dosed at 20 mg/kg. To date, since first approved by the European Medicines Agency in 2007, thousands of patients have safely ingested 5-ALA as part of their presurgical preparation. Numerous clinical trials have confirmed patient safety, and have found only mild, reversible adverse effects, including transient liver enzyme elevations, skin photosensitivity, and nausea.

5-ALA and Low-Grade Gliomas

While the evidence for 5-ALA FGS of HGGs is firmly established by a number of studies, the use of 5-ALA for LGG has been less robust. LGG tumors are well known to be difficult to delineate from surrounding tumor tissue due to their lack of more defining features HGG tumors possess (low vascularity, low cellularity, and more infiltration). While 5-ALA FGS has the potential to really help neurosurgeons identify these tumors during surgery, detection of fluorescence in LGGs occurs in <20% of these tumors with currently used visualization devices. A reliable method of identifying which LGGs will fluoresce has not been established. A new study by Müther et al. has found that preoperative MRI contrast enhancement of intermediate grade gliomas (WHO grade II and III gliomas) strongly correlates with visible fluorescence during surgery. They also found observed fluorescence with MIB-1 index but not with isocitrate dehydrogenase (IDH) status, 1p19q codeletion, or methylguanine DNA methyltransferase promoter (MGMT) methylation.

Recent studies have confirmed that LGG tumors with no significant contrast enhancement actually have anaplastic
foci present characterizing malignant transformation. 5-ALA fluorescence can detect these areas of higher proliferation and malignancy for proper characterization of tumors. Furthermore, Hosmann et al. determined that patients with LGG that demonstrated fluorescence had significantly shorter PFS and OS compared to nonfluorescing LGG tumors, and that intraoperative fluorescence may be a predictor of outcome for these patients. As discussed earlier, PpIX fluorescence can be detected in LGG tumors by novel new visualization technologies. Our current microscope technologies, however, may not be useful in detecting fluorescence in LGGs which do not contrast-enhance.

5-ALA and Meningiomas

Meningiomas are a new tumor type rapidly gaining interest for 5-ALA FGS (Table 2). In the largest single study to date, Millesi et al. identified 5-ALA fluorescence in 91% of cases in 204 lesions in 190 patients undergoing meningioma resection. In that study, they also found fluorescence and tumor presence with meningioma dural tails and overlying hyperostotic bone. In a series of 12 patients with bone-invading meningiomas, Della Puppa et al. found 100% fluorescence in both tumor and bone invasion, with a sensitivity of 89% and 100% specificity for bony invasion. Several other studies have assessed 5-ALA fluorescence in meningiomas, and have found fluorescence rates between 83% and 94%. Interestingly, PpIX fluorescence kinetics appear to differ between meningioma and glioblastoma, with meningiomas demonstrating a higher intensity and rate of clearance, with no differences between samples from 2 atypical to 10 benign meningiomas.

5-ALA and Brain Metastases

Cerebral metastases are the most common malignant brain tumor in adults with hundreds of thousands of cases a year. In many patients, resection of symptomatic brain metastases in combination with adjuvant stereotactic radiosurgery can improve patient outcomes. Gross total resection remains the gold standard for large, symptomatic metastatic tumors, particularly in cases where there is cerebral compression, and mass effect. Complete resections are necessary, as subtotal resection can lead to a significant local tumor recurrence. To maximize the rate of CRET, fluorescence may be useful in these cases. Due to the heterogeneity of metastatic lesions originating from many different primary cancers, 5-ALA does not fluoresce in all cases. To date, primary cancers with cerebral metastases known to fluoresce with 5-ALA are epithelial in origin and include lung, breast, colon, bladder, and melanoma. However, in a study of 154 patients with 157 metastatic tumors, Marhold et al. found a visible fluorescence rate of 66%, with 84% of cases showing heterogeneous fluorescence. Mercea et al. saw visible fluorescence in 69% of metastatic tumor samples, and found that 5-ALA fluorescence was associated with angiogenesis and subsequent poorer patient survival. 5-ALA-induced fluorescence has also been shown in areas of edema surrounding metastatic tumors and therefore must be used with caution in these cases.

5-ALA and Radiotherapy

For malignant gliomas, local recurrence most commonly occurs within 2 cm of the primary tumor location. Brachytherapy, a type of internal radiation therapy, attempts to solve this problem, by locally placing focal irradiation in the tumor-treated field. In previous studies, brachytherapy has been shown to have added survival benefits in recurrent glioblastoma. In a prospective study of 17 patients receiving salvage fluorescence-guided re-resection, high-dose brachytherapy, and temozolomide, patients experienced an increased survival benefit of 3 months compared to temozolomide controls. In this study, 5-ALA was thought to improve the efficacy of adjuvant therapies, such as brachytherapy, by improving the possibility of gross total resection.

The role between 5-ALA and radiation therapy has yet to be fully established. PpIX has been shown to increase reactive oxygen species byproducts of ionized radiation, and in a recent in vivo study, mouse models treated with 5-ALA before radiotherapy experienced slower tumor progression and tumor regression compared to radiotherapy alone. In a review of 11 studies on 5-ALA radiodynamic therapy (RDT), primarily consisting of in vitro and in vivo studies with several case reports, Nordmann et al. found that RDT offers a promise for adjuvant therapy in HGG, however, further clinical investigation is warranted. Future clinical studies should assess the role of intraoperative photodynamic therapy with brachytherapy, to assess the utility of this potential synergism that has been shown in preclinical models.

Future Directions

While 5-ALA has undoubtedly transformed the care of patients with HGGs in the setting of traditional microscopic resection, future directions revolve around improving the versatility of this tool, both in terms of precision/quantification of PpIX as well as increasing its compatibility with new surgical tools. Although HGGs often appear pink at the tumor margins under microscopic visualization, current methods lack quantitative specificity that may facilitate decision-making in eloquent areas where metabolite concentrations may be below the visualization threshold. As such, several novel techniques have been proposed to identify PpIX, and may provide a future benefit in challenging cases.

Raman spectroscopy involves the use of monochromatic light to create a molecular identification profile for specific compounds. At present, several pilot studies have explored the ability of this technology to identify PpIX within brain tissue in both in vivo as well as ex vivo settings. Desroches et al. developed an in-human Raman spectroscopy system that was integrated with a brain biopsy tool. The authors examined this for 3 patients undergoing
stereotactic needle biopsy and were able to collect spectra that clearly differentiated tumor from normal brain. Future uses of this technology may revolve around incorporation of Raman spectroscopy within intraoperative handheld tools. Contact of this tool with the tumor-brain interface could offer a mechanism of identifying residual tumor that was not previously visualized, and potentially increase the extent of resection in both HGGs and LGGs. Other technologies have been developed for similar purposes. Optical sectioning microscopy is an adjunct tool that can elicit subcellular foci of fluorescence in areas that are traditionally challenging to detect, such as for LGG and HGG-brain parenchyma interfaces. Meza et al. analyzed samples from 7 patients with LGG using dual-axis confocal microscopy, demonstrating consistent detection of PPIX throughout the samples, with resolution that was comparable to histologic samples. Other authors have explored similar technologies, with potential future translation to human models. Belykh et al. employed a scanning fiber endoscope (SFE) to optimize PPIX detection, and demonstrated that this tool had improved sensitivity when compared to the operating microscope in rats with gliomas. Further investigations of these instruments in patients undergoing resection will be important in clarifying their role within the clinical workflow.

The increased versatility and precision of 5-ALA detection may consequently improve its utility for other tumor types. LGGs are known to accumulate PPIX, and extent of resection remains critically important to maintaining successful outcomes in these patients. Other lesions with previously reported intraoperative fluorescence include hemangioblastomas, CNS lymphomas, meningiomas, metastatic tumors, and subependymomas. Due to the heterogeneous nature of fluorescence among these tumor types, the use of PPIX quantification may be particularly useful in harnessing the full utility of 5-ALA.

**Conclusion**

In the present era, tumor surgeons are held to the high standard of near total resection, when feasible, with minimal associated morbidity. Fluorescence-guided surgery, and particularly 5-ALA PiP IX fluorescence, has been established as a reliable surgical adjunct to delineate tumor tissue from the surrounding brain during resection. 5-ALA has been shown to be safe and effective in multiple tumor types and has been most widely studied in the HGG population. Improving technologies to more precisely detect 5-ALA PiP IX fluorescence will further advance our detection of brain tumors in the operating room.

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