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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.[1–3] 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.[4] 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.[5] 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 deceased 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.[6] A recent study by Mischkulnig et al. found significantly decreased ABCG2 mRNA and protein expression in fluorescing tumor specimens compared to

Table 1 Landmark Clinical 5-ALA Papers—Glioma

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	Age predicts EOR and RTV; tumor surface area, preop tumor volume, and SAVR predict RTV; MGMT status predicts PFS, RTV, SAVR, and MGMT predict OS
5/1/16	Lau et al.	26544781	Case series	59	Primary: correlation of 5-ALA fluorescence with tumor cellularity	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.
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 has high correlation with tumor and is more useful for indicating residual tumor compared to contrast-enhanced MRI and spectrometry.
2/1/14	Coburger et al.	24484256	Case series	45 (33 HGG 11 Mets)	Primary: correlation of pathological samples and 5-ALA fluorescence	Mets: no benefit of 5-ALA for visualization; border zone: 5-ALA has higher sensitivity and lower specificity for tumor than contrast MRI; infiltrating tumor: 5-ALA better in both sensitivity and specificity.
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

Table 1 Continued

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.

nonfluorescing samples from adult patients with diffuse gliomas (WHO grade II–IV) after 5-ALA administration.[7] 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).[8] Dosing of oral 5-ALA at 20 mg/kg 2–4 h before induction of anesthesia was performed with the 2006 randomized, controlled trial.[5] Rodent preclinical studies revealed peak fluorescence occurred 6 h after 5-ALA administration[6] 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.[8] 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.[9] 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.[10] 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.[11] 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.[12] 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

Table 2 Landmark Clinical 5-ALA Papers—Meningiomas

Date Published	Paper	PMID	Study Type	Number Pts	Outcomes Measured	Result of Study
6/1/07	Kajimoto et al	17564181	Case series	24	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
6/10/10	Coluccia et al.	20535506	Case series	33	Intraop fluorescence	94% fluorescence. Did not correlate with grade, edema, or steroid use
12/1/14	Cornelius et al.	25117928	Case series	31	Qualitative and quantitative intraop fluorescence, histopathologic correlation, and degree of resection	94% fluorescence, correlation with grade, improved extent of resection especially in grades 2 and 3
2/18/16	Puppa et al	24410157	Case series	12	Intraop fluorescence of both tumor and bone invasion	Tumor and bone fluoresced 100%. Good tool to identify bone invasion but hyperostotic bone may limit absorption.
3/25/16	Millesi et al.	27015401	Case series	190	Intraop fluorescence in the body of tumor, dural tail, and adjacent tissues	Fluorescence 91% of lesions as well as satellite lesions and infiltrated bone flaps; however, not in dural tail. No long-term outcome data.
9/1/16	Foster & Eljamel	27235278	Meta-analysis	206	Intraop fluorescence	95% of meningiomas light up. No correlation to grade.
5/2/20	Kaneko et al.	32361907	Cohort	12	Fluorescence intensity and PpIX 5–6 h after administration. Also compared to 229 glioblastomas	Meningiomas had higher-fluorescence intensity and PpIX concentration. PpIX cleared faster in meningiomas.
7/1/20	Wadiura et al.	32608510	Case series	191	Fluorescence of banked tissue samples from tumor, bone, arachnoidea, and dura	Fluorescence of tumor was 100%, bone flap 92%, arachnoidea 83%, dura 75% but absent from nearly all adjacent cortical samples

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	Fluorescence presence, quality, homogeneity, and histopathology	66% fluoresced (34% strong, 32% weak), most were heterogeneous. Melanomas showed least. Ductal breast cancer the most.
2/3/21	Mercea et al.	33546427	Case series	88	Peritumoral tissue samples looking at fluorescence, infiltration, and angiogenesis. Recurrence and 1 y survival.	69% of samples fluoresced, associated with angiogenesis and poor survival
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.

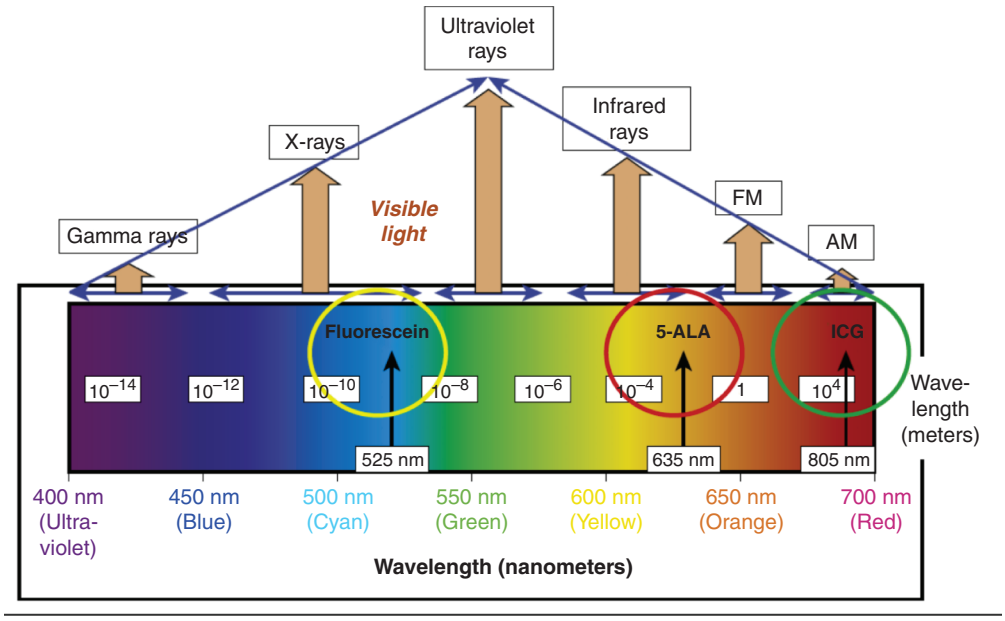

Fig. 1 Fluorescence emission wavelengths of the most commonly used fluorophores used in glioma surgery. AM, amplitude modulation; FM, frequency modulation; ICG, indocyanine green. (Permission from Hadjipanayis CG, Stummer W. *Fluorescence Guided Neurosurgery*. New York, NY: Thieme Medical Publishers;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

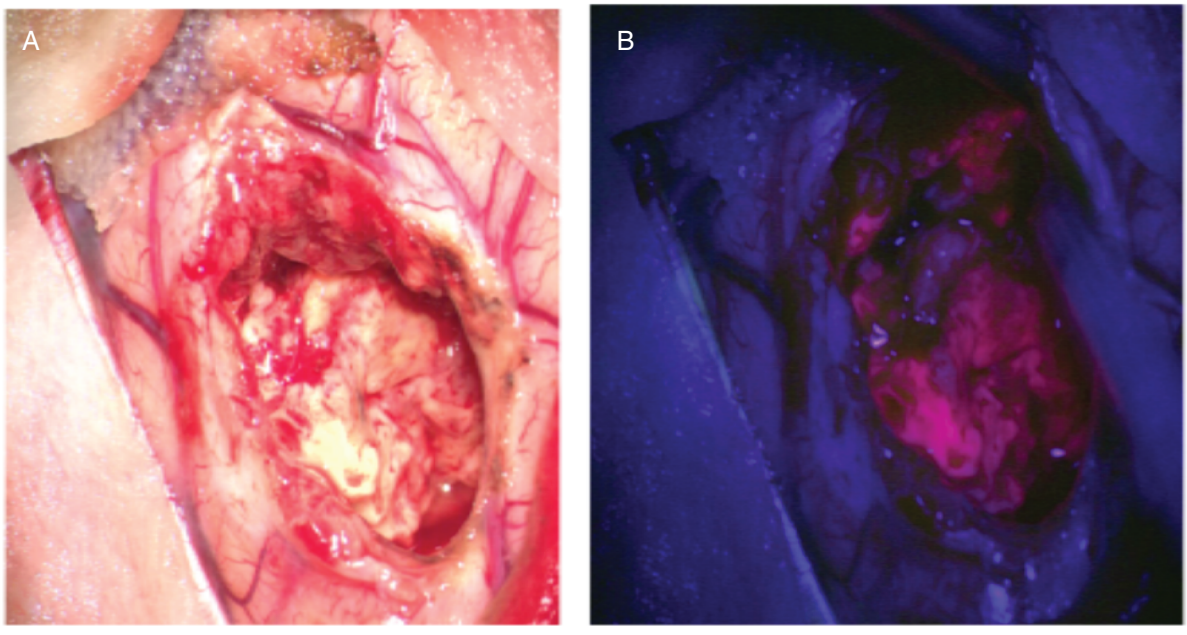

Fig. 2 Fluorescence-guided surgery of a HGG using 5-ALA. (**A**) demonstrates white light visualization of the surgical resection cavity and (**B**) shows the surgical field under blue light (635 nm), with the red and pink fluorescence representing tumor cell density, compared to the normal brain appearing 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.[13] 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,[14–16] US neurosurgeons sought FDA approval of 5-ALA, specifically for use as intraoperative optical agent in suspected HGG surgery.[4] 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.[14] 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.[17]

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.[18–21] 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%.[17] 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).[22,23] 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.[24,25] 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.[26,27]

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 postoperative MRI had significantly improved overall survival.[27] 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%).[5] 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.[28–30] 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.[29,30]

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.[5,17,31]

Neurologic deficits may transiently increase after 5-ALA FGS. With greater extent of tumor resection, there may be an increase in patient morbidity, particularly in the case of tumors near eloquent regions of the brain. In the 2006 randomized controlled trial as well as the US multicenter trial, postoperative functional declines were seen in both cohorts undergoing 5-ALA FGS 48 h after surgery. However, in both studies, this decline improved and the patients were back to baseline functional status 6 weeks following surgery.[5,17] These findings suggest that 5-ALA FGS permitted surgeons to resect up to eloquent areas, but not disrupt these regions, and therefore avoid permanent neurological deficits.

5-ALA has been shown to be safe and effective in recurrent HGG resection as well as newly diagnosed tumors. Similar to new HGG cases, 5-ALA fluorescence is sensitive for detection of recurrent tumor tissue, making it an effective surgical adjunct in these tumors that have undergone adjuvant therapies (radiation and chemotherapy).[25,32–34] Despite its affinity for HGG tissue, 5-ALA false positive fluorescence has been found in gliotic tissues and other postradiation changes.[35–38]

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.[39,40] 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.[41] 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.[42–44] 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.[41] 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.[45] 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.[46] Several other studies have assessed 5-ALA fluorescence in meningiomas, and have found fluorescence rates between 83% and 94%.[47,48] 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.[49]

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.[50] Gross total resection remains the gold standard for large, symptomatic metastatic tumors, particularly in cases where there is cerebral compression, and mass effect.[51] Complete resections are necessary, as subtotal resection can lead to a significant local tumor recurrence.[52]To maximize the rate of CRET, fluorescence may be useful in these cases (Table 3). 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.[7] 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.[53] 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.[54] 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.[55,56]

5-ALA and Radiotherapy

For malignant gliomas, local recurrence most commonly occurs within 2 cm of the primary tumor location.[57] 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.[58,59] 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.[57] 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.[60] 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.[61] 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,[62] 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.[63] 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.[63] Future uses of this technology may revolve around incorporation of Raman spectroscopy within intraoperative handheld tools.[64] 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.[65] 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.[66] 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.[67] 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.[12] 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 PpIX 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 PpIX fluorescence will further advance our detection of brain tumors in the operating room.

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References

1. Lacroix M, Abi-Said D, Fourney DR, et al. A multivariate analysis of 416 patients with glioblastoma multiforme: prognosis, extent of resection, and survival. *J Neurosurg.* 2001;95(2):190–198.
2. Sanai N, Berger MS. Glioma extent of resection and its impact on patient outcome. *Neurosurgery.*2008;62(4):753–764.
3. Stummer W, Reulen HJ, Meinel T, et al. Extent of resection and survival in glioblastoma multiforme: identification of and adjustment for bias. *Neurosurgery.* 2008;62(3):564–576.
4. Hadjipanayis CG, Stummer W. 5-ALA and FDA approval for glioma surgery. *J Neurooncol.* 2019;141(3):479–486.
5. 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.
6. Stummer W, Stocker S, Novotny A, et al. In vitro and in vivo porphyrin accumulation by C6 glioma cells after exposure to 5-aminolevulinic acid. *J Photochem Photobiol B.* 1998;45(2–3):160–169.
7. Mischkulnig M, Roetzer-Pejrimovsky T, Lötsch-Gojo D, et al. Heme biosynthesis factors and 5-ALA induced fluorescence: analysis of mRNA and protein expression in fluorescing and non-fluorescing gliomas. *Front Med (Lausanne).* 2022;9:907442. doi:10.3389/fmed.2022.907442. PMID: 35665365.
8. Hadjipanayis C, Stummer W. *Fluorescence-Guided Neurosurgery.* 1st ed. Switzerland: Thieme; 2018.
9. Kaneko S, Suero Molina E, Ewelt C, Warneke N, Stummer W. Fluorescence-based measurement of real-time kinetics of Protoporphyrin IX after 5-aminolevulinic acid administration in human in situ malignant gliomas. *Neurosurgery.* 2019;85(4):E739–E746. doi:10.1093/neuros/nyz129. PMID: 31058995.
10. Maragkos GA, Schüpper AJ, Lakomkin N, et al. Fluorescence-guided high-grade glioma surgery more than four hours after 5-aminolevulinic acid administration. *Front Neurol.* 2021;12:644804. doi:10.3389/fneur.2021.644804. PMID: 33767664.
11. Kaneko S, Suero Molina E, Sporns P, et al. Fluorescence real-time kinetics of protoporphyrin IX after 5-ALA administration in low-grade glioma. *J Neurosurg.* 2021;136(1):9–15. doi: 10.3171/2020.10.JNS202881. PMID: 34144512.
12. Valdés PA, Jacobs V, Harris BT, et al. Quantitative fluorescence using 5-aminolevulinic acid-induced protoporphyrin IX biomarker as a surgical adjunct in low-grade glioma surgery. *J Neurosurg.* 2015;123(3):771–780.

13. Widhalm G, Olson J, Weller J, et al. The value of visible 5-ALA fluorescence and quantitative protoporphyrin IX analysis for improved surgery of suspected low-grade gliomas. *J Neurosurg.* 2019;10:1–10. doi:10.3171/2019.1.JNS182614. Epub ahead of print. PMID: 31075771; PMCID: PMC7184556.
14. Nabavi A, Thurm H, Zountsas B, et al. Five-aminolevulinic acid for fluorescence-guided resection of recurrent malignant gliomas: a phase ii study. *Neurosurgery.* 2009;65(6):1070–1076.
15. Senders JT, Muskens IS, Schnoor R, et al. Agents for fluorescence-guided glioma surgery: a systematic review of preclinical and clinical results. *Acta Neurochir (Wien).* 2017;159(1):151–167.
16. Stummer W, Tonn JC, Goetz C, et al. 5-Aminolevulinic acid-derived tumor fluorescence: the diagnostic accuracy of visible fluorescence qualities as corroborated by spectrometry and histology and postoperative imaging. *Neurosurgery.* 2014;74(3):310–319.
17. Schupper AJ, Baron RB, Cheung W, et al. 5-Aminolevulinic acid for enhanced surgical visualization of high-grade gliomas: a prospective, multicenter study. *J Neurosurg.* 2021;8:1–10. doi:10.3171/2021.5.JNS21310. Epub ahead of print. PMID: 34624862.
18. Eljamel S. 5-ALA fluorescence image guided resection of glioblastoma multiforme: a meta-analysis of the literature. *Int J Mol Sci.* 2015;16(5):10443–10456.
19. Stummer W, Stocker S, Wagner S, et al. Intraoperative detection of malignant gliomas by 5-aminolevulinic acid-induced porphyrin fluorescence. *Neurosurgery.* 1998;42(3):518–525.
20. Su X, Huang QF, Chen HL, Chen J. Fluorescence-guided resection of high-grade gliomas: a systematic review and meta-analysis. *Photodiagnosis Photodyn Ther.* 2014;11(4):451–458.
21. Zhao S, Wu J, Wang C, et al. Intraoperative fluorescence-guided resection of high-grade malignant gliomas using 5-aminolevulinic acid-induced porphyrins: a systematic review and meta-analysis of prospective studies. *PLoS One.* 2013;8(5):e63682.
22. Schipmann S, Schwake M, Suero Molina E, Stummer W. Markers for identifying and targeting glioblastoma cells during surgery. *J Neurol Surg A Cent Eur Neurosurg.* 2019;80(6):475–487.
23. Stummer W, Tonn JC, Goetz C, et al. 5-Aminolevulinic acid-derived tumor fluorescence: the diagnostic accuracy of visible fluorescence qualities as corroborated by spectrometry and histology and postoperative imaging. *Neurosurgery.* 2014;74(3):310–319.
24. Diez Valle R, Tejada Solis S, Idoate Gastearena MA, et al. Surgery guided by 5-aminolevulinic fluorescence in glioblastoma: volumetric analysis of extent of resection in single-center experience. *J Neurooncol.* 2011;102(1):105–113.
25. Schucht P, Knittel S, Slotboom J, et al. 5-ALA complete resections go beyond MR contrast enhancement: shift corrected volumetric analysis of the extent of resection in surgery for glioblastoma. *Acta Neurochir (Wien).* 2014;156(2):305–312.
26. Aldave G, Tejada S, Pay E, et al. Prognostic value of residual fluorescent tissue in glioblastoma patients after gross total resection in 5-aminolevulinic acid-guided surgery. *Neurosurgery.* 2013;72(6):915–920.
27. Idoate MA, Diez Valle R, Echeveste J, Tejada S. Pathological characterization of the glioblastoma border as shown during surgery using 5-aminolevulinic acid-induced fluorescence. *Neuropathology.* 2011;31(6):575–582.
28. Coburger J, Hagel V, Wirtz CR, Konig R. Surgery for glioblastoma: impact of the combined use of 5-aminolevulinic acid and intraoperative MRI on extent of resection and survival. *PLoS One.* 2015;10(6):e0131872.
29. Della Puppa A, De Pellegrin S, d'Avella E, et al. 5-aminolevulinic acid (5-ALA) fluorescence guided surgery of high-grade gliomas in eloquent areas assisted by functional mapping. Our experience and review of the literature. *Acta Neurochir (Wien).* 2013;155(6):965–972.
30. Schucht P, Beck J, Abu-Isa J, et al. Gross total resection rates in contemporary glioblastoma surgery: results of an institutional protocol combining 5-aminolevulinic acid intraoperative fluorescence imaging and brain mapping. *Neurosurgery.* 2012;71(5):927–935.
31. Teixidor P, Arraez MA, Villalba G, et al. Safety and efficacy of 5-aminolevulinic acid for high grade glioma in usual clinical practice: a prospective cohort study. *PLoS One.* 2016;11(2):e0149244.
32. Hickmann AK, Nadji-Ohl M, Hopf NJ. Feasibility of fluorescence-guided resection of recurrent gliomas using five-aminolevulinic acid: retrospective analysis of surgical and neurological outcome in 58 patients. *J Neurooncol.* 2015;122(1):151–160.
33. Ringel F, Pape H, Sabel M, et al. Clinical benefit from resection of recurrent glioblastomas: results of a multicenter study including 503 patients with recurrent glioblastomas undergoing surgical resection. *Neuro Oncol.* 2016;18(1):96–104.
34. Suchorska B, Weller M, Tabatabai G, et al. Complete resection of contrast-enhancing tumor volume is associated with improved survival in recurrent glioblastoma-results from the DIRECTOR trial. *Neuro Oncol.* 2016;18(4):549–556.
35. Brandes AA, Tosoni A, Spagnolli F, et al. Disease progression or pseudoprogression after concomitant radiochemotherapy treatment: pitfalls in neurooncology. *Neuro Oncol.* 2008;10(3):361–367.
36. de Wit MC, de Bruin HG, Eijkenboom W, Sillevis Smitt PA, van den Bent MJ. Immediate post-radiotherapy changes in malignant glioma can mimic tumor progression. *Neurology.* 2004;63(3):535–537.
37. Taal W, Brandsma D, de Bruin HG, et al. Incidence of early pseudoprogression in a cohort of malignant glioma patients treated with chemoirradiation with temozolomide. *Cancer.* 2008;113(2):405–410.
38. Kamp MA, Felsberg J, Sadat H, et al. 5-ALA-induced fluorescence behavior of reactive tissue changes following glioblastoma treatment with radiation and chemotherapy. *Acta Neurochir (Wien).* 2015;157(2):207–213.
39. Paulus W, Peiffer J. Intratumoral histologic heterogeneity of gliomas. A quantitative study. *Cancer.* 1989;64(2):442–447.
40. Hosmann A, Millesi M, Wadiura LI, et al. 5-ALA fluorescence is a powerful prognostic marker during surgery of low-grade gliomas (WHO Grade II)-experience at two specialized centers. *Cancers (Basel).* 2021;13(11):2540.
41. Müther M, Jaber M, Johnson TD, Orringer DA, Stummer W. A data-driven approach to predicting 5-aminolevulinic acid-induced fluorescence and world health organization grade in newly diagnosed diffuse gliomas. *Neurosurgery.* 2022;90(6):800–806.
42. Ewelt C, Floeth FW, Felsberg J, et al. Finding the anaplastic focus in diffuse gliomas: the value of Gd-DTPA enhanced MRI, FET-PET, and intraoperative, ALA-derived tissue fluorescence. *Clin Neurol Neurosurg.* 2011;113(7):541–547.
43. Jaber M, Wolfer J, Ewelt C, et al. The value of 5-aminolevulinic acid in low-grade gliomas and high-grade gliomas lacking glioblastoma imaging features: an analysis based on fluorescence, magnetic resonance imaging, 18F-Fluoroethyl tyrosine positron emission tomography, and tumor molecular factors. *Neurosurgery.* 2016;78(3):401–411.
44. Widhalm G, Kiesel B, Woehrer A, et al. 5-Aminolevulinic acid induced fluorescence is a powerful intraoperative marker for precise histopathological grading of gliomas with non-significant contrast-enhancement. *PLoS One.* 2013;8(10):e76988.
45. Millesi M, Kiesel B, Mischkulnig M, et al. Analysis of the surgical benefits of 5-ALA-induced fluorescence in intracranial meningiomas: experience in 204 meningiomas. *J Neurosurg.* 2016;125(6):1408–1419.
46. Della Puppa A, Rustemi O, Gioffre G, et al. Predictive value of intraoperative 5-aminolevulinic acid-induced fluorescence for detecting bone invasion in meningioma surgery. *J Neurosurg.* 2014;120(4):840–845.

47. Kajimoto Y, Kuroiwa T, Miyatake S, et al. Use of 5-aminolevulinic acid in fluorescence-guided resection of meningioma with high risk of recurrence. Case report. *J Neurosurg.* 2007;106(6):1070–1074.
48. Coluccia D, Fandino J, Fujioka M, et al. Intraoperative 5-aminolevulinic-acid-induced fluorescence in meningiomas. *Acta Neurochir (Wien).* 2010;152(10):1711–1719.
49. Kaneko S, Brokinkel B, Suero Molina E, et al. Real-time in vivo kinetics of protoporphyrin IX after administration of 5-aminolevulinic acid in meningiomas and comparative analyses with glioblastomas. *Acta Neurochir (Wien).* 2020;162(9):2197–2202.
50. Schupper AJ, Price G, Hadjipanayis CG. Robotic-assisted digital exoscope for resection of cerebral metastases: a case series. *Oper Neurosurg (Hagerstown).* 2021;21(6):436–444.
51. Al-Shamy G, Sawaya R. Management of brain metastases: the indispensable role of surgery. *J Neurooncol.* 2009;92(3):275–282.
52. Patchell RA, Tibbs PA, Walsh JW, et al. A randomized trial of surgery in the treatment of single metastases to the brain. *N Engl J Med.* 1990;322(8):494–500.
53. Marhold F, Mercea PA, Scheichel F, et al. Detailed analysis of 5-aminolevulinic acid induced fluorescence in different brain metastases at two specialized neurosurgical centers: experience in 157 cases. *J Neurosurg.* 2019;27:1–12. doi:10.3171/2019.6.JNS1997. Epub ahead of print. PMID: 31561223.
54. Mercea PA, Mischkulnig M, Kiesel B, et al. Prognostic value of 5-ALA fluorescence, tumor cell infiltration and angiogenesis in the peritumoral brain tissue of brain metastases. *Cancers (Basel).* 2021;13(4):603.
55. Utsuki S, Miyoshi N, Oka H, et al. Fluorescence-guided resection of metastatic brain tumors using a 5-aminolevulinic acid-induced protoporphyrin IX: pathological study. *Brain Tumor Pathol.* 2007;24(2):53–55.
56. Kamp MA, Grosser P, Felsberg J, et al. 5-aminolevulinic acid (5-ALA)-induced fluorescence in intracerebral metastases: a retrospective study. *Acta Neurochir (Wien).* 2012;154(2):223–228.
57. Archavlis E, Tselis N, Birn G, Ulrich P, Zamboglou N. Salvage therapy for recurrent glioblastoma multiforme: a multimodal approach combining fluorescence-guided resurgery, interstitial irradiation, and chemotherapy. *Neurol Res.* 2014;36(12):1047–1055.
58. Archavlis E, Tselis N, Birn G, et al. Survival analysis of HDR brachytherapy versus reoperation versus temozolomide alone: a retrospective cohort analysis of recurrent glioblastoma multiforme. *BMJ Open.* 2013;3(3):e002262.
59. Tselis N, Kolotas C, Birn G, et al. CT-guided interstitial HDR brachytherapy for recurrent glioblastoma multiforme. Long-term results. *Strahlenther Onkol.* 2007;183(10):563–570. doi:10.1007/s00066-007-1721-2. PMID: 17896088.
60. Takahashi J, Nagasawa S, Doi M, et al. In vivo study of the efficacy and safety of 5-aminolevulinic radiodynamic therapy for glioblastoma fractionated radiotherapy. *Int J Mol Sci.* 2021;22(18):9762.
61. Nordmann NJ, Michael AP. 5-Aminolevulinic acid radiodynamic therapy for treatment of high-grade gliomas: a systematic review. *Clin Neurol Neurosurg.* 2021;201:106430. doi:10.1016/j.clineuro.2020.106430. Epub 2020 Dec 14. PMID: 33360951.
62. Hadjipanayis CG, Widhalm G, Stummer W. What is the surgical benefit of utilizing 5-ALA for fluorescence-guided surgery of malignant gliomas? *Neurosurgery.* 2015;77(5):663–673.
63. Dadario NB, Khatri D, Reichman N, Nwagwu CD, D'Amico RS. 5-aminolevulinic acid–shedding light on where to focus. *World Neurosurg.* 2021;150:9–16. doi:10.1016/j.wneu.2021.02.118. Epub 2021 Mar 5. PMID: 33684574.
64. Lakomkin N, Hadjipanayis CG. The use of spectroscopy handheld tools in brain tumor surgery: current evidence and techniques. *Front Surg.* 2019;6:30. doi:10.3389/fsurg.2019.00030. PMID: 31192217; PMCID: PMC6548876.
65. Wei L, Fujita Y, Sanai N, Liu JTC. Toward quantitative neurosurgical guidance with high-resolution microscopy of 5-aminolevulinic acid-induced protoporphyrin IX. *Front Oncol.* 2019;9:592. doi:10.3389/fonc.2019.00592. PMID: 31334117; PMCID: PMC6616084.
66. Meza D, Wang D, Wang Y, et al. Comparing high-resolution microscopy techniques for potential intraoperative use in guiding low-grade glioma resections. *Lasers Surg Med.* 2015;47(4):289–295.
67. Belykh E, Miller EJ, Hu D, et al. Scanning fiber endoscope improves detection of 5-aminolevulinic acid–induced protoporphyrin IX fluorescence at the boundary of infiltrative glioma. *World Neurosurg.* 2018;113:e51–e69. doi:10.1016/j.wneu.2018.01.151. Epub 2018 Feb 2. PMID: 29408716; PMCID: PMC5924630.