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Review Article

Fluorescence-Guided Resection of High-Grade Glioma Using 5-Aminolevulinic Acid

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Abstract

Background: High-grade gliomas are treated with maximal safe resection plus radiotherapy and temozolomide. Extent of resection predicts survival, but infiltrative margins resist visual discrimination under white light.

Objective: To review 5-aminolevulinic acid (5-ALA) fluorescence-guided resection of high-grade glioma, covering mechanism, the pivotal trial, comparative modalities, and limitations.

Methods: Narrative review of randomized trials, long-term follow-up, systematic reviews, meta-analyses, and mechanistic and health-economic literature.

Findings: The pivotal randomized trial found complete resection in 65% of 5-ALA-guided patients versus 36% under white light, with higher six-month progression-free survival. Subsequent meta-analyses, registries, and head-to-head trials against intraoperative MRI confirm greater resection extent and a favorable cost profile, alongside recognized limitations at infiltrative margins and uneven global access.

Conclusions: 5-ALA fluorescence guidance is a validated, cost-effective surgical adjunct that measurably improves resection extent in high-grade glioma, though it cannot fully resolve diffusely infiltrative margins and remains inconsistently available worldwide.

Keywords: 5-aminolevulinic acid; Fluorescence-guided surgery; High-grade glioma; Glioblastoma; Extent of resection

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Keywords: 5-aminolevulinic acid; fluorescence-guided surgery; high-grade glioma; glioblastoma; extent of resection

Introduction

High-grade gliomas, principally glioblastoma under the 2021 World Health Organization classification of central nervous system tumors, remain among the most lethal solid neoplasms despite maximal treatment [1]. Standard care combines maximal safe surgical resection with concurrent and adjuvant temozolomide chemoradiotherapy, a regimen that established median survival gains still measured in months rather than years [2]. Within this framework, the extent of surgical resection has repeatedly emerged as one of the few modifiable determinants of outcome, motivating decades of effort to help surgeons see tumor tissue that is otherwise visually indistinguishable from adjacent brain [3].

Conventional white-light microsurgery relies on preoperative imaging, neuronavigation, and the surgeon's visual and tactile judgment, all of which are prone to intraoperative brain shift and to the simple fact that infiltrative glioma cells extend beyond any visible tumor margin. Surgeons operating under white light alone must therefore infer the tumor-brain interface indirectly, often stopping resection prematurely to avoid injuring adjacent functional tissue that appears grossly normal but may already harbor infiltrating tumor cells, or conversely resecting tissue that later proves non-neoplastic. Fluorescence-guided surgery using 5-aminolevulinic acid (5-ALA) addresses this problem directly by inducing tumor cells to accumulate a fluorescent metabolite that surgeons can visualize intraoperatively in real time, without interrupting the operative workflow to obtain additional imaging [4].

This narrative review synthesizes the mechanistic basis of 5-ALA fluorescence, the pivotal randomized trial and its long-term and supplemental extensions, the extent-of-resection and survival literature that motivates the entire field, the comparative evidence against intraoperative MRI and other guidance technologies, and the technique's recognized limitations around infiltrative margins, cost, and regulatory access. Rather than a case report, it draws on the published trial, cohort, meta-analytic, and health-economic literature to summarize where the evidence for 5-ALA-guided glioma surgery currently stands and where it remains incomplete.

Mechanism of Protoporphyrin IX Fluorescence

5-ALA is an orally administered precursor in the heme biosynthesis pathway. Following ingestion, it is

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preferentially taken up and metabolized by malignant glial cells, which accumulate the fluorescent intermediate protoporphyrin IX (PpIX) in excess of the surrounding parenchyma because of altered enzyme activity along the heme synthesis cascade and disrupted transport across a compromised blood-brain barrier [4]. Under violet-blue excitation light delivered through a modified surgical microscope, PpIX emits a distinctive red-pink fluorescence that surgeons can distinguish from the faint blue autofluorescence of normal brain, permitting essentially real-time optical discrimination of tumor from non-tumor tissue during resection.

Fluorescence intensity is not binary. Areas of strong, homogeneous red fluorescence typically correspond to densely cellular, solid tumor, whereas fainter or vague pink fluorescence more often marks lower-density infiltration at the tumor periphery [5]. Histopathological correlation studies confirm that fluorescence intensity tracks tumor cell density and anaplastic features, including regions that lack contrast enhancement on preoperative MRI, making 5-ALA useful for identifying anaplastic foci within otherwise non-enhancing tumor and for grading tissue intraoperatively [6]. This graded relationship between fluorescence intensity and tissue biology is the basis for the surgical strategy of resecting to the limit of visible fluorescence rather than to the limit of preoperative imaging alone, and it also explains why the technique performs unevenly across the tumor: strong fluorescence in the tumor core is rarely ambiguous, while the faint, vague fluorescence at the infiltrative rim requires surgeon judgment and can be missed entirely under suboptimal lighting or microscope calibration.

The Pivotal Randomized Trial and Long-Term Outcomes

The evidentiary foundation for clinical adoption is a multicentre, randomized, controlled phase III trial that assigned patients with suspected malignant glioma to resection under 5-ALA-induced fluorescence guidance or under conventional white light [7]. Complete resection of contrast-enhancing tumor, verified by early postoperative MRI, was achieved in 65% of patients in the 5-ALA group compared with 36% in the white-light group, and six-month progression-free survival was likewise higher with fluorescence guidance. Because complete resection is achievable more often without a corresponding rise in new severe neurological deficits, the trial established 5-ALA as an effective adjunct rather than a technique that trades extent for safety.

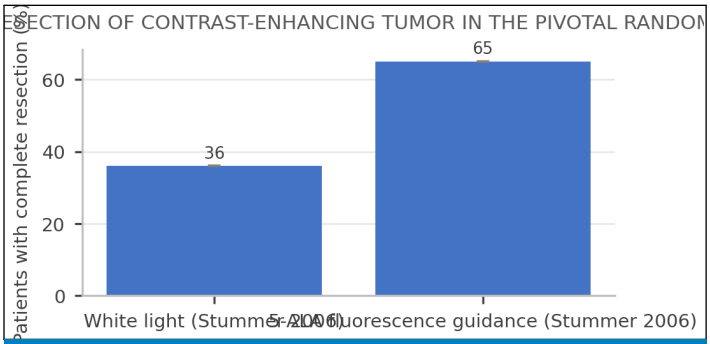

Figure 1: Complete resection of contrast-enhancing tumor in the pivotal randomized trial: 36% under white light versus 65% with 5-ALA fluorescence guidance [7].

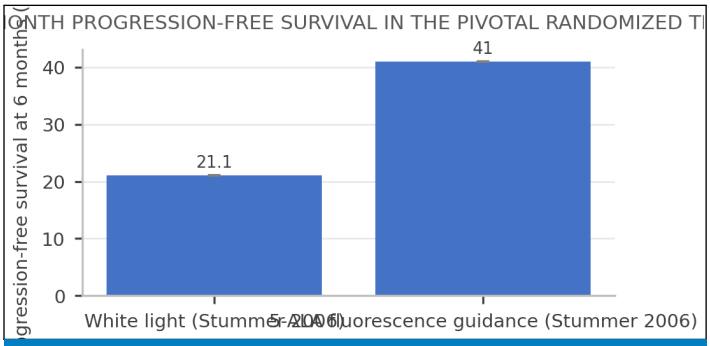

Figure 2: Six-month progression-free survival in the pivotal randomized trial: 21.1% under white light versus 41.0% with 5-ALA fluorescence guidance [7].

A supplemental analysis of the same trial cohort further examined the balance of risks and gains from more extensive resection, confirming that achieving complete resection of enhancing tumor was associated with a survival benefit that outweighed the modest increase in

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early postoperative deficits among patients undergoing more aggressive fluorescence-guided resection [8]. Complementary work from the same trial group addressing methodological bias in extent-of-resection analyses reinforced that the observed survival relationship was not simply an artifact of surgeon or tumor selection, since patients with more resectable, favorably located tumors might otherwise be expected to both undergo more complete resections and survive longer regardless of technique [9].

Together, these analyses transformed 5-ALA from a promising optical technique into a guideline-referenced standard adjunct for newly diagnosed high-grade glioma surgery. Long-term clinical experience since the pivotal trial has generally reinforced rather than overturned its central finding: fluorescence guidance reliably shifts the distribution of achieved resections toward completeness, and that shift is the mechanism through which the technique is presumed to influence survival, rather than any direct antitumor effect of the dye itself.

Extent of Resection and Survival Outcomes

Independent of fluorescence guidance specifically, a large single-institution analysis established that survival benefit from resection begins near a threshold of about 78% of tumor volume removed, with progressively greater benefit as resection approaches totality [10]. A systematic review and meta-analysis pooling multiple cohorts confirmed that greater extent of resection is consistently associated with improved overall survival in glioblastoma, supporting extent of resection as a primary surgical objective rather than an incidental outcome [11].

5-ALA fluorescence contributes to this objective specifically because visible fluorescence frequently extends beyond the margins defined by contrast enhancement on MRI, allowing resection of biologically active tumor that would otherwise be left behind [12]. Volumetric analyses correcting for intraoperative brain shift have shown that resections guided to the limits of visible fluorescence achieve greater completeness than resections guided to the limits of MR contrast enhancement alone [13]. This distinction matters most in tumors bordering eloquent cortex, where surgeons must weigh additional resection against the risk of new neurological deficit. A systematic review and meta-analysis of resections combining electrophysiological mapping with 5-ALA fluorescence near eloquent areas found that the two techniques together allowed more complete resection of contrast-enhancing tumor without a corresponding increase in permanent neurological morbidity, suggesting fluorescence guidance and functional mapping are complementary rather than competing strategies for maximizing safe resection [14].

Table 1: Key trials and pooled analyses of 5-aminolevulinic acid fluorescence-guided resection of high-grade glioma [7,8,9,10,11,19,16,17,18].

Study	Design	N	Key finding
Stummer 2006	Randomized controlled phase III trial	270	Complete resection 65% (5-ALA) vs 36% (white light)
Stummer 2011	Supplemental risk-benefit analysis of RCT cohort	270	Survival benefit of complete resection outweighs deficit risk
Stummer 2008	Bias-adjusted extent-of-resection analysis	270	Survival relationship persists after adjustment for selection bias
Sanai 2011	Single-institution retrospective cohort	500	Survival benefit begins near 78% resection threshold
Brown 2016	Systematic review and meta-analysis	Pooled cohorts	Greater extent of resection consistently improves overall survival

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Study	Design	N	Key finding
Zhao 2013	Systematic review and meta-analysis of prospective studies	Pooled cohorts	5-ALA guidance increases complete resection and PFS
Roder 2023	Prospective controlled multicenter trial	Multicenter	iMRI not superior to 5-ALA guidance
Coburger 2015	Retrospective cohort, combined modality	Single-center	5-ALA plus iMRI further increases resection completeness
Golub 2020	Network meta-analysis	Pooled trials/cohorts	No clear superiority of iMRI over 5-ALA

Comparison with Intraoperative MRI and Other Guidance Modalities

Intraoperative MRI (iMRI) is the principal alternative technology for maximizing resection, using repeat scanning during surgery to detect residual enhancing tumor that the surgeon can then continue to resect within the same operative session. An early randomized trial found that iMRI guidance increased the rate of complete resection compared with conventional neuronavigation-guided surgery, establishing iMRI as a credible competitor to fluorescence-based approaches [15]. More recent prospective, multicentre trials have directly compared iMRI against 5-ALA fluorescence guidance in newly diagnosed glioblastoma and found that iMRI-guided resection was not superior to 5-ALA guidance, suggesting the two approaches achieve broadly comparable extents of resection despite very different infrastructure requirements, costs, and operative workflows [16]. Because iMRI requires a dedicated intraoperative scanner suite while 5-ALA requires only an oral agent and a filter-equipped microscope, this equivalence has practical implications for which technology a given center can realistically adopt.

Table 2: Guidance modalities for maximizing resection in high-grade glioma [4,15,16,21,20,25].

Modality	Mechanism	Evidence base	Key limitation
5-ALA fluorescence	Tumor-selective PpIX accumulation, visible under filtered light	Phase III RCT plus multiple meta-analyses	Weak signal at diffusely infiltrative margins
Intraoperative MRI	Repeat MRI scanning during surgery	RCT vs conventional surgery; RCT vs 5-ALA	High infrastructure cost, workflow disruption
Combined 5-ALA plus iMRI	Fluorescence with interval MRI confirmation	Retrospective cohort study	Added cost and operative complexity
Conventional white light plus neuronavigation	Preoperative imaging and surgeon judgment alone	Comparator arm across cited trials	Lowest completeness of resection

Combining both modalities has also been explored directly: a cohort study assessing 5-ALA together with iMRI found that the combination could further increase resection completeness relative to either technique used alone, though at added cost and operative complexity [17]. A network meta-analysis synthesizing this comparative literature across multiple trials and cohorts likewise found no clear superiority of one adjunct technology over the other in terms of achieved resection extent, framing the choice between iMRI and 5-ALA as one of institutional resources and surgical workflow rather than a proven efficacy difference favoring either modality [18].

Meta-Analyses, Pooled Evidence, and Remaining Limitations

Multiple systematic reviews and meta-analyses of prospective 5-ALA studies confirm the pattern established in the pivotal trial, reporting consistently higher rates of complete resection and, in several pooled analyses, improved progression-free survival with fluorescence guidance compared with conventional resection [19]. A Cochrane systematic review of image-guided technologies for brain tumor resection, and its subsequent update, similarly concluded that 5-ALA increases the likelihood of complete resection of contrast-enhancing tumor, while noting that evidence for a direct overall survival benefit

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remains less definitive than the resection and progression-free survival findings [20,21]. Narrative synthesis of the surgical-benefit literature reaches a comparable conclusion regarding consistent gains in resection completeness [22,23].

Important limitations temper this evidence. Fluorescence is least reliable exactly where it would be most useful: at diffusely infiltrating margins where tumor cell density falls below the threshold needed to generate visible PpIX accumulation, producing false-negative regions that leave infiltrative disease unresected despite an apparently complete fluorescence-guided operation [12]. Registry data from routine clinical practice outside the trial setting confirm that real-world effectiveness and resection rates track the randomized trial results reasonably well, though with the expected variability of observational cohorts [24]. Cost and regulatory access remain relevant barriers: economic evaluations have found 5-ALA-guided surgery to be a cost-effective addition to standard care despite the added expense of the agent itself, but availability differs by health system and regulatory approval pathway [25]. Adjunctive photodynamic approaches building on 5-ALA-induced fluorescence, including combined resection and intraoperative photodynamic therapy for recurrent disease, illustrate ongoing efforts to extend the technique's benefit beyond visualization alone, an extension building on earlier photodynamic therapy trials that established the biological plausibility of PpIX-mediated tumor cell injury [26,27].

Conclusion

5-ALA fluorescence-guided resection is among the most rigorously validated surgical adjuncts in neuro-oncology, supported by a positive phase III randomized trial, consistent long-term and supplemental analyses of that trial, multiple independent meta-analyses, and direct comparison against intraoperative MRI. Across this literature, fluorescence guidance reliably increases the rate of complete resection of enhancing high-grade glioma and appears cost-effective relative to conventional white-light surgery. Its principal limitation is biological rather than technical: fluorescence intensity declines at the diffusely infiltrating tumor margin, so a fluorescence-guided operation that appears complete may still leave infiltrative tumor behind. Combining 5-ALA with complementary technologies such as intraoperative MRI or functional mapping, and continuing to broaden regulatory and economic access worldwide, represent the most promising directions for extending the gains this technique has already demonstrated.

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