5-Aminolevulinic Acid guided resection improves the overall survival of patients with Glioblastoma - A comparative cohort study of 343 patients

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Abstract

Background: 5-Aminolevulic Acid guided surgery (5-ALA-GS) improves the extent of resection and progression free survival in patients with glioblastoma multiforme (GBM).

Methods: Single-center retrospective cohort study of adult patients with GBM who had surgical resection between 2013 and 2019, 5-ALA guided versus a non 5-ALA cohort. Primary outcome was the overall survival (OS). Secondary outcomes were extent of resection (EoR), performance status (PS), and new focal neurological deficit.

Results: 343 patients were included: 253 patients in 5-ALA-GS Group and 90 patients in the non-5-ALA-GS Group. The OS (17.47 versus 10.63 months, p<0.0001), post-operative PS (p<0.0001), PS at 6 months (p=0.002), new focal neurological deficit (23.3% versus 44.9%, p<0.0001) and radiological EoR (gross total resection (GTR) - 47.4% versus 22.9%, p< 0.0001) were significantly better in the 5-ALA-GS Group compared to non-5-ALA-GS Group. In multivariate analysis, use of 5-ALA (p=0.003) and MGMT promoter methylation (p=0.001) were significantly related with a better OS. In patients with radiological GTR, OS was also significantly better (p<0.0001) in the 5-ALA-GS Group compared to the non-5-ALA-GS Group.

Conclusions: 5-ALA guided surgery is associated with a significant improvement in the OS, PS after surgery and at six months, larger EoR, and fewer new motor deficits in patients with GBM.

Keywords: 5-Aminolevulinic Acid, Glioblastoma, Resection, Overall Survival, Performance Status
Key points:

5-ALA guided resection improves overall survival of WHO grade 4 glioblastoma patients when compared with non-5-ALA guided surgery.

5-ALA guided surgery improves EoR and PFS, when compared with non-5-ALA guided surgery in WHO grade 4 glioblastoma patients.

Importance of the Study

Whilst 5-Aminolevulic Acid (5-ALA) use is now a common method of fluorescence guided surgery as it has been shown to improve rate of resection and progression free survival compared to white light, mixed evidence has been published on longer-term overall survival mainly derived from highly selected cohort of patients. Of the literature that currently exists, most are generally small-scale studies, creating potential for larger variation and bias of results. We believe our study presents data from the largest single-center cohort of patients operated on under 5-ALA to date. With this number of patients, we hope we can reduce the risk of bias as a result of small sample sizes and thus provide more reputable data.
Abbreviations:

5-ALA – 5-Aminolevulnic Acid

5-ALA-GS – 5-Aminolevulinic Acid Guided Surgery

EoR – Extent of Resection

FLAIR – Fluid-attenuated inversion recovery

GBM – Glioblastoma

GTR – Gross Total Resection

IONM – Intraoperative Neurophysiological Monitoring

IDH – isocitrate dehydrogenase

MGMT – O-6-methylguanine-DNA methyltransferase

OS – Overall Survival

PCV – Procarbazine, Lomustine and Vincristine

PFS – Progression-Free Survival

PS – Performance Status

STR – Subtotal Resection
Introduction

The main goals of surgical treatment in glioblastoma patients are maximal safe resection whilst preserving the quality of life and providing an accurate histopathological and molecular diagnosis that will help guiding the adjuvant treatment. This would ideally translate in the resection of the radiologically defined lesion, a situation that is difficult to reproduce in the intraoperative setting, due to the surgeon’s limitation of confidently distinguishing the brain-tumor interface under conventional white-light microscopy. Several intraoperative techniques, including neuro-navigation, intra-operative MRI and ultrasound, have been developed and applied, in an attempt to encourage and ensure greater tumor resection, although each technique has its own limitations.

5-aminolevulinic acid (5-ALA) is the cornerstone of fluorescence-guided brain surgery. It is a precursor of the protoporphyrin IX molecule, which in high concentrations allows fluorescence. This oral chemical agent that is administered pre-operatively, demonstrates high selective accumulation within the pathological tissue, and in particular glioblastomas. As a result, tumor tissue becomes fluorescent under blue-light microscopy. This fluorescence is independent of brain volume changes and brain shift, providing truly real-time guidance to the surgeon. Integration of this adjunct with pre-operative and intraoperative mapping has revolutionized the surgical approach to glioblastomas (Fig.1).

Intra-operative fluorescence allows identification of pathological tissue and a clearer visualization of the brain-tumor interface, allowing the neurosurgeon to extend the resection towards or beyond the contrast-enhancement areas on MRI (Fig.1). Indeed, when compared to conventional white-light resection, 5-ALA use has demonstrated improved extent of resection (36% in white-light compared to 65% with 5-ALA), which translated into a near twice improvement in the 6-month progression free survival (41% vs 21%) and a more than 3 month increase in the overall survival rates with no worsening of neurological deficits.

Nevertheless, the majority of the data on 5-ALA guided surgery (5-ALA-GS), stem from small case series with short follow up periods, making any recommendations less robust. In this report, we
present our institution’s experience with 5-ALA-assisted resection of glioblastomas, which to our knowledge is the largest to date.

**Methods:**

This is a single center retrospective cohort study between January 2013 and January 2019 of patients operated with 5-ALA for glioblastomas (GBM). The inclusion criteria were: ≥18 years old, 5-ALA-GS, pathology consistent with WHO grade 4 GBM and consent form signed for the surgical procedure. The exclusion criteria were non-glial tumor, non-WHO grade 4, surgical biopsies, and incomplete medical records.

5-ALA was administered via oral route with a dosage of 20mg/Kg to a maximal dose of 1500mg per patient. The ideal time of administration was 2-4 hours prior to surgery (even though administration outside this time frame was not considered an exclusion criterion for this study). Intraoperatively, two different microscopes were used during the 5-ALA-assisted procedures – Pentero 900 and KINEVO – with the BLUE 400 Filter from ZEISS Medical Technology®.

Demographic and clinical data were collected from patients’ medical records. The primary outcome was to assess the impact of 5-Aminolevulinic Acid Guided Surgery (5-ALA-GS) on the overall survival in patients diagnosed with GBM. The secondary outcomes were its impact on the post-operative performance status (PS), PS at 6 months after surgery, the extent of resection (EoR), new focal neurological deficit after surgery and length of hospital stay. Gross Total Resection (GTR) was defined as no residual contrast enhancement detected on the post-operative MRI scan performed within 72 hours of surgery whilst subtotal resection was defined as residual contrast enhancement.17 The results were compared with a cohort of patients treated by the same team in our institution, prior to implementation of a regular program of 5-ALA-GS for glioblastomas (January 2009- January 2013). Similar inclusion and exclusion criteria apart from 5-ALA-GS were applicable for the control cohort.
A literature review of the case series published in the last 10 years was also performed. We have excluded those where the survival outcomes were not reported as per EoR (GTR versus subtotal resection (STR)); studies were divided according to the intraoperative use of 5-ALA.

Regarding ethical approval, all procedures performed in studies involving human participants were in accordance with the ethical standards of our institution and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. For this type of study where data were collected during routine clinical care of patients, formal consent is not required. The use of 5-ALA was approved by our institution’s New and Novel Procedures Committee.

STATA 13.0® statistical software was used for the statistical analysis. Chi-square, T-test, and regression analysis (multinomial, ordered and logistic) were performed to investigate the relationship between the variables considered. Multinomial Cox-Hazard statistics was used for survival analysis. A p value < 0.05 was considered statistically significant. An adjusted regression model for confounding factors – age, gender, pre-operative PS, use of intraoperative neurophysiological monitoring (IONM), isocitrate dehydrogenase (IDH) mutation status, O-6-methylguanine-DNA methyltransferase (MGMT) methylation status, radiological extent of resection, post-operative PS, PS at 6 months, adjuvant treatment (radiotherapy and chemotherapy) and the use of 5-ALA was performed.

**Results:**

**Patient Demographics**

343 patients fulfilled the inclusion criteria: 253 patients had 5-ALA-GS and 90 patients had non-5-ALA-GS. Both groups had a predominantly male gender, similar tumor locations, a comparable mix of first and redo surgery, and a similar age distribution (5-ALA-GS: 56.69±0.75 versus non-5-ALA-GS: 54.43±1.73, p=0.234). the 5-ALA-GS group had lower pre-operative PS (p<0.0001), post-operative PS (p<0.0001), PS at 6 months (p=0.002) and higher utilization of intraoperative
neuromonitoring (31.8% versus 12.3%, p <0.0001), reflecting the gradual change in our practice over time. Both groups had a similar distribution of ATRX and IDH mutations. The 5-ALA-GS Group had a lower rate of MGMT promoter methylation (50.6% versus 68.3%, p= 0.015) (Table 1).

The time interval between administration of 5-ALA and the start of the surgery did not significantly affect the EOR (p=0.102). The use of IONM was related with larger EoR in the 5-ALA-GS Group (p=0.042) but not in the non-5-ALA-GS Group (p=0.710).

Chemotherapy regimen and radiotherapy doses were the same for the historical control group and the 5-ALA group. These were based on the STUPP protocol for first presentation cases and consisted of concomitant six weeks of radiotherapy with 60 Gy combined with temozolomide chemotherapy followed by 6 cycles of adjuvant temozolomide chemotherapy. For patients with recurrent GBM, procarbazine, lomustine and vincristine (PCV) combination chemotherapy was the regimen of choice.

Primary and Secondary Outcomes

The overall survival was significantly better with 5-ALA-GS (17.47 versus 10.63 months, p<0.0001). Additionally, post-operative PS (p<0.0001), PS at 6 months after surgery (p=0.002), new temporary focal neurological deficit (23.3% versus 44.9%, p<0.0001) and the radiological extent of resection (GTR - 47.4% versus 22.9%, p < 0.0001) were significantly better in the 5-ALA-GS Group (Table 2). Furthermore, significantly more patients in the 5-ALA-GS Group were able to complete post-operative chemotherapy and radiotherapy (92.8% versus 79.8%, p=0.001; 93.9% versus 83.5%, p=0.004, respectively) (Fig.2 & Table 2).

Risk Factor Analysis – Adjusted and Unadjusted Analysis

Older age, higher preoperative PS, non-use of IONM, unmethylated MGMT promotor, STR, higher postoperative PS, higher PS at 6 months, a new temporary motor deficit, lack of adjuvant treatment (radiotherapy and/or chemotherapy) and non-5-ALA-GS were related with a worse overall survival (Table 2). When the model was adjusted, MGMT promoter methylation and 5-ALA-GS emerged as the only factors related with an improvement in the overall survival (Table 2).
Subgroup Analysis – GTR versus STR

The 5-ALA-GS Group had a better overall survival both for those in whom GTR was achieved (17.77 months versus 14.03 months, p<0.0001) and in those undergoing STR (15.67 months versus 10.4 months, p=0.016). Post-operative PS was also significantly better in the 5-ALA-GS Group both for those undergoing GTR (p=0.014) and those with STR (0.029) although new transitory focal deficit was less common in STR compared to GTR group (18% versus 45%, p<0.0001), (Supplementary Material 1). Post-operative FLAIR images were only available in a limited number of patients who underwent GTR, 57 in 5-ALA-GS group and 3 in the historical controls. Within clear constraints of such small numbers, the cavity of resection was noted to include at least some of the FLAIR volume, beyond the contrast enhancement, in 80% when surgery was guided by 5-ALA versus 30% in the control group.

Discussion:

The use of 5-ALA received FDA approval in 2017 and became routinely reimbursed in the United Kingdom in 2019. In our center, however, 5-ALA assisted surgery was introduced on an ad-hoc basis in 2010 and then became part of the routine practice of managing glioblastomas from 2014. Our data here shows that our practice of 5-ALA-GS has been associated with significant improvement in overall survival, EOR, post-operative PS, PS at 6 months after surgery and completion of adjuvant therapy in patients with WHO Grade 4 glioblastomas. Moreover, the adjusted regression model for confounding factors excluded other technical adjuncts (such as the use of IONM) as the reason for the improvement in survival. The biological signature of the tumors (MGMT) and the use of 5-ALA were shown to be the only factors related to survival in the multifactorial analysis.

Although the impact of 5-ALA on enhancing the intraoperative visualization of tumors, improving the EOR and progression free survival have been well established in the literature, data on its influence on overall survival is limited. Our study, the largest series thus far on 5-ALA-GS, indicated
improved overall survival in patients with glioblastomas undergoing 5-ALA-GS even after adjustment for other factors known to influence survival in these patients. Table 3 summarizes the surgical case series published in the last 10 years on the impact of surgery on the overall survival of patients with grade 4 gliomas.\textsuperscript{12,22-40} These indicate a general trend towards an increase in utilization of 5-ALA-GS in glioblastomas with an associated increase in EoR and a higher percentage of patients undergoing GTR. The patient numbers in these series, however, remain small with a median of 33 (range: 13-103), often with no adequate control group, limiting the conclusions on progression-free survival (PFS) and especially overall survival.

5-ALA-GS was found to improve the overall survival in both GTR and STR cohorts. Whilst the improvement in overall survival of patients with WHO Grade 4 gliomas in the 5-ALA-GS group compared to the controls might be attributable to improved EoR in patients with STR, the better overall survival in those with GTR in the 5-ALA-GS group is less readily explained. In part this may reflect changes in the overall care of patients during our relatively long study period given the sequential nature of the recruitment. In part though, the observed difference might highlight the challenges in how GTR is defined. Radiologically for GBMs this has been based on complete resection of enhancing tumor. Literature, however, is emerging to show that the 5-ALA dependent fluorescent tissue tends to extend beyond the limits of contrast enhancing tumor on the MRI.\textsuperscript{41,42} Indeed Yamada et al reported that the GTR achieved with the use of 5-ALA, in terms of the relationship between the size of the surgical cavity versus the size of the preoperative contrast enhancing tumor, was substantially different in patients undergoing combined 5-ALA/intra-operative MRI surgery versus those with intra-operative MRI alone.\textsuperscript{43} In fact, more recently, better outcomes in glioblastomas have been reported when the resection was extended beyond the contrast-enhancement limits to incorporate the FLAIR volume.\textsuperscript{44,45} In our cohort, analysis of the limited number of post-operative FLAIR images available, also signaled in favor of a larger resection cavity beyond the contrast enhancement when achieving GTR with 5-ALA-GS. Thus, 5-ALA-GS might result in a “more” maximal resection than that assessed based purely on contrast enhancement on MRI (Fig.3).
Large prospective studies with detailed volumetric MRI analysis are, however, required to fully explore this concept.

The impact of time between 5-ALA administration and the surgery on the outcomes were assessed. This is particularly significant in public health systems were urgent and emergent admissions can be responsible for delays in surgery. Although others have found significant correlation between the EoR and the time interval between 5-ALA administration and onset of surgery, no such correlation was identified in our patients undergoing craniotomy with a mean time of 4 hours and 25 minutes between administration of 5-ALA and surgery.

The ultimate goal of glioblastoma treatment is improvement or preservation of quality of life. A good performance status is crucial for tolerance, ability to complete and thus maximal benefit from adjuvant treatment. 5-ALA-GS was associated with a better PS at 6 months after surgery in both unadjusted and adjusted analysis and a higher probability of patients completing their adjuvant treatment. This further supports the use of this adjunct in glioblastoma surgery, not only for its direct effect on EoR and survival but also for its impact on performance status and facilitating adjuvant therapies.

Limitations:

Our study is prone to the limitations of a retrospective series spanning a long recruitment period. Data on the molecular tumor markers, particularly IDH, 1p19q and MGMT promoter methylation, were not available for all patients. These became routinely available since publication of the new classification of Tumors of the Central Nervous system in 2016 but were not systematically available before that date. Where historical tumor specimens were available, we carried out the assessments retrospectively but this was not the case for all.

Our control group was historical and therefore prone to the biases related to the evolution of our practice. For example, more patients in the 5-ALA group received adjuvant therapy than those in the control cohort which may have contributed to the differences seen in the survival outcomes although
not in EOR, post-operative neurological deficit or PS. The surgical learning curve may have also played a role here given that the control group were operated in the years prior to the 5-ALA group. Nonetheless the surgical team had over two decades of experience beyond the steep section of such learning curve and proficient in surgical techniques for tumor resection. Furthermore 5-ALA-GS technique itself is open to a learning curve which needs to be borne in mind. Nonetheless, by adjusting our analytic model, we addressed these limitations where possible, showing the MGMT status of the tumor and 5-ALA-GS as the only factors related with an improvement in the overall survival, rather than, for example the use of IONM.

Our observation of improved survival in the 5-ALA-GS group, even in those undergoing GTR compared to controls, and the possible contribution of 5-ALA aided resection beyond the contrast enhanced tumor on MRI, requires further investigation in prospective volumetric MRI studies.

**Conclusion:**

Within limitations of a retrospective study, the data from this largest series on 5-ALA-GS in patients with glioblastoma show significantly better overall survival, PFS, EOR and post-operative PS with the use of 5-ALA compared to controls.
References:

1. Hadjipanayis CG, Widhalm G, Stummer W. What is the Surgical Benefit of Utilizing 5-Aminolevulinic Acid for Fluorescence-Guided Surgery of Malignant Gliomas? Neurosurgery. 2015; 77(5):663-673.

2. Divaris DX, Kennedy JC, Pottier RH. Phototoxic damage to sebaceous glands and hair follicles of mice after systemic administration of 5-aminolevulinic acid correlates with localized protoporphyrin IX fluorescence. Am J Pathol. 1990; 136(4):891-897.

3. Malik Z, Lugaci H. Destruction of erythroleukaemic cells by photoactivation of endogenous porphyrins. Br J Cancer. 1987; 56(5):589-595.

4. 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; discussion 525-516.

5. 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.

6. Pogue BW, Gibbs-Strauss S, Valdes PA, Samkoe K, Roberts DW, Paulsen KD. Review of Neurosurgical Fluorescence Imaging Methodologies. IEEE J Sel Top Quantum Electron. 2010; 16(3):493-505.

7. Valdes PA, Leblond F, Kim A, et al. Quantitative fluorescence in intracranial tumor: implications for ALA-induced PpIX as an intraoperative biomarker. J Neurosurg. 2011; 115(1):11-17.

8. Stupp R, Hegi ME, Mason WP, et al. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. Lancet Oncol. 2009; 10(5):459-466.

9. 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.

10. Diez Valle R, Slof J, Galvan J, et al. Observational, retrospective study of the effectiveness of 5-aminolevulinic acid in malignant glioma surgery in Spain (The VISIONA study). Neurologia. 2014; 29(3):131-138.

11. Li Y, Rey-Dios R, Roberts DW, Valdes PA, Cohen-Gadol AA. Intraoperative fluorescence-guided resection of high-grade gliomas: a comparison of the present techniques and evolution of future strategies. World Neurosurg. 2014; 82(1-2):175-185.

12. 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; discussion 920-911.

13. Babu R, Adamson DC. Fluorescence-guided malignant glioma resections. Curr Drug Discov Technol. 2012; 9(4):256-267.

14. Colditz MJ, Leyen K, Jeffree RL. Aminolevulinic acid (ALA)-protoporphyrin IX fluorescence guided tumour resection. Part 2: theoretical, biochemical and practical aspects. J Clin Neurosci. 2012; 19(12):1611-1616.

15. Stummer W, Novotny A, Stepp H, Goetz C, Bise K, Reulen HJ. Fluorescence-guided resection of glioblastoma multiforme by using 5-aminolevulinic acid-induced porphyrins: a prospective study in 52 consecutive patients. J Neurosurg. 2000; 93(6):1003-1013.
16. 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.
17. Almenawer SA, Badhiwala JH, Alhazzani W, et al. Biopsy versus partial versus gross
total resection in older patients with high-grade glioma: a systematic review and meta-
analysis. Neuro Oncol. 2015; 17(6):868-881.
18. Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and
adjuvant temozolomide for glioblastoma. N Engl J Med. 2005; 352(10):987-996.
19. Hadjipanayis CG, Stummer W. 5-ALA and FDA approval for glioma surgery. J
Neurooncol. 2019; 141(3):479-486.
20. Gandhi S, Tayebi Meybodi A, Belykh E, et al. Survival Outcomes Among Patients
With High-Grade Glioma Treated With 5-Aminolevulinic Acid-Guided Surgery: A
Systematic Review and Meta-Analysis. Front Oncol. 2019; 9:620.
21. Ontario Health. 5-Aminolevulinic Acid Hydrochloride (5-ALA)
Guided Surgical
Resection of High-Grade Gliomas: A Health Technology Assessment. Ont Health
Technol Assess Ser. 2020; 20(9):1-92.
22. Dobran M, Nasi D, Della Costanza M, et al. Characteristics of treatment and outcome
in elderly patients with brain glioblastoma: a retrospective analysis of case series.
Acta Clin Croat. 2019; 58(2):221-228.
23. Guler OC, Yildirim BA, Onal C, Topkan E. Retrospective comparison of standard and
escalated doses of radiotherapy in newly diagnosed glioblastoma patients treated with
concurrent and adjuvant temozolomide. Indian J Cancer. 2019; 56(1):59-64.
24. Dayani F, Young JS, Bonte A, et al. Safety and outcomes of resection of butterfly
glioblastoma. Neurosurg Focus. 2018; 44(6):E4.
25. Chan DT, Hsieh SY, Lau CK, et al. Ten-year review of survival and management of
malignant glioma in Hong Kong. Hong Kong Med J. 2017; 23(2):134-139.
26. Harris G, Jayamanne D, Wheeler H, et al. Survival Outcomes of Elderly Patients With
Glioblastoma Multiforme in Their 75th Year or Older Treated With Adjuvant
Therapy. Int J Radiat Oncol Biol Phys. 2017; 98(4):802-810.
27. Guden M, Ayata HB, Ceylan C, Kilic A, Engin K. Prognostic factors effective on
survival of patients with glioblastoma: Anadolu Medical Center experience. Indian J
Cancer. 2016; 53(3):382-386.
28. 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.
29. Hrabalek L, Kalita O, Vaverka M, et al. Resection versus biopsy of glioblastomas in
elloquent brain areas. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub.
2015; 159(1):150-155.
30. Qin JJ, Liu ZX, Wang JM, et al. Prognostic factors influencing clinical outcomes of
malignant glioblastoma multiforme: clinical, immunophenotypic, and fluorescence in
situ hybridization findings for 1p19q in 816 chinese cases. Asian Pac J Cancer Prev.
2015; 16(3):971-977.
31. Lombardi G, Pace A, Pasqualetti F, et al. Predictors of survival and effect of short (40
Gy) or standard-course (60 Gy) irradiation plus concomitant temozolomide in elderly
patients with glioblastoma: a multicenter retrospective study of AINO (Italian
Association of Neuro-Oncology). J Neurooncol. 2015; 125(2):359-367.
32. Chaichana KL, Jusue-Torres I, Navarro-Ramirez R, et al. Establishing percent
resection and residual volume thresholds affecting survival and recurrence for patients
with newly diagnosed intracranial glioblastoma. Neuro Oncol. 2014; 16(1):113-122.
33. Hong B, Wiese B, Bremer M, et al. Multiple microsurgical resections for repeated recurrence of glioblastoma multiforme. *Am J Clin Oncol.* 2013; 36(3):261-268.

34. Yabroff KR, Harlan L, Zeruto C, Abrams J, Mann B. Patterns of care and survival for patients with glioblastoma multiforme diagnosed during 2006. *Neuro Oncol.* 2012; 14(3):351-359.

35. Della Puppa A, Lombardi G, Rossetto M, et al. Outcome of patients affected by newly diagnosed glioblastoma undergoing surgery assisted by 5-aminolevulinic acid guided resection followed by BCNU wafers implantation: a 3-year follow-up. *J Neurooncol.* 2017; 131(2):331-340.

36. Hauser SB, Kockro RA, Actor B, Samthein J, Bernays RL. Combining 5-Aminolevulinic Acid Fluorescence and Intraoperative Magnetic Resonance Imaging in Glioblastoma Surgery: A Histology-Based Evaluation. *Neurosurgery.* 2016; 78(4):475-483.

37. 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.

38. Kim SK, Choi SH, Kim YH, Park C-K. Impact of fluorescence-guided surgery on the improvement of clinical outcomes in glioblastoma patients. *Neuro-Oncology Practice.* 2014; 1(3):81-85.

39. 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; discussion 935-926.

40. Diez Valle R, Tejada Solis S, Idoate Gastearena MA, Garcia de Eulate R, Dominguez Echavarri P, Aristu Mendiroz J. 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.

41. Della Puppa A, Ciccarino P, Lombardi G, Rolma G, Cecchin D, Rossetto M. 5-Aminolevulinic acid fluorescence in high grade glioma surgery: surgical outcome, intraoperative findings, and fluorescence patterns. *Biomed Res Int.* 2014; 1(3):81-85.

42. Schucht P, Seidel K, Beck J, et al. Intraoperative monopolar mapping during 5-ALA-guided resections of glioblastomas adjacent to motor eloquent areas: evaluation of resection rates and neurological outcome. *Neurosurg Focus.* 2014; 37(6):E16.

43. Yamada S, Muragaki Y, Maruyama T, Komori T, Okada Y. Role of neurochemical navigation with 5-aminolevulinic acid during intraoperative MRI-guided resection of intracranial malignant gliomas. *Clin Neurol Neurosurg.* 2015; 130:134-139.

44. De Leeuw BI, Van Baarsen KM, Snijders TJ, Robe P. Interrelationships between molecular subtype, anatomical location, and extent of resection in diffuse glioma: a systematic review and meta-analysis. *Neuro Oncol Adv.* 2019; 1(1):vdz032.

45. Molinaro AM, Hervey-Jumper S, Morshed RA, et al. Association of Maximal Extent of Resection of Contrast-Enhanced and Non-Contrast-Enhanced Tumor With Survival Within Molecular Subgroups of Patients With Newly Diagnosed Glioblastoma. *JAMA Oncol.* 2020; 6(4):495-503.

46. Chan DTM, Yi-Pin Sonia H, Poon WS. 5-Aminolevulinic acid fluorescence guided resection of malignant glioma: Hong Kong experience. *Asian J Surg.* 2018; 41(5):467-472.

47. Dietterle J, Wendt T, Wilhelmy F, et al. The prognostic value of peri-operative neurological performance in glioblastoma patients. *Acta Neurochir (Wien).* 2020; 162(2):417-425.
48. Lee JH, Jung TY, Jung S, et al. Performance status during and after radiotherapy plus concomitant and adjuvant temozolomide in elderly patients with glioblastoma multiforme. *J Clin Neurosci.* 2013; 20(4):503-508.

49. Zanovello WG, Malheiros SM, Stavale JN, et al. Performance of adjuvant treatment correlates with survival in reoperated glioblastomas. *Arq Neuropsiquiatr.* 2016; 74(11):887-894.

50. Louis DN, Perry A, Reifenberger G, et al. The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. *Acta Neuropathol.* 2016; 131(6):803-820.
**Figure Captions:**

**Figure 1** – Integrated intraoperative US and MRI (1A and 1B) and 5-ALA in the surgical cavity (1C) global assessment: fusion of MRI with integrated pre-operative dissection of the fronto-aslant tract, intraoperative ultrasound and 5-ALA to assess the extent of resection. *White Arrow* – Contrast enhancing tumor identified in the US-fMRI fusion and confirmed with 5-ALA fluorescence.

**Figure 2** – Kaplan-Meier curves of our results. 5-ALA-GS = 5-Aminolevulinic Acid Guided Surgery.

**Figure 3** – Pre (A) and post (B) operative axial contrast enhanced MRI showing the size of the resection cavity beyond the limits of contrast enhancement.

**Table 1** – Overall characteristics of non-5-ALA-GS and the 5-ALA-GS groups. 5-ALA-GS = 5-Aminolevulinic Acid Guided Surgery; PS = Performance Status; ATRX = Alpha-thalassemia x-linked intellectual disability; IDH = isocitrate dehydrogenase; MGMT = O-6-methylguanine-DNA methyltransferase.

**Table 2** – Outcomes and Risk Factor Analysis in glioblastomas. 5-ALA-GS= 5-Aminolevulinic Acid Guided Surgery; PS = Performance Status; EoR = Extent of Resection; FU = Follow-up; IONM = Intraoperative Neurophysiological Monitoring; IDH = isocitrate dehydrogenase; MGMT = O-6-methylguanine-DNA methyltransferase.

**Table 3** – Literature Review of the surgical case series published with Non-5-ALA-GS and 5-ALA-GS for WHO grade 4 gliomas in the last 10 years in patients treated with Stupp Protocol (enrolment after 2006). EoR = Extent of resection; GBM = Glioblastoma Multiforme; bGBM = Butterfly Glioblastoma Multiforme; GTR = Gross Total Resection; STR = Subtotal Resection.
|                                | Non-5-ALA-GS Group (n=90) | 5-ALA-GS Group (n=253) | p value |
|--------------------------------|---------------------------|------------------------|---------|
| **Gender**                     |                           |                        |         |
| Male                           | 57                        | 174                    |         |
| Female                         | 33                        | 79                     | 0.406   |
| **Age (years old)**            | 54.43±1.73                | 56.69±0.75             | 0.234   |
| **Surgical Resection**         |                           |                        |         |
| First Craniotomy               | 68                        | 211                    |         |
| Redo Craniotomy                | 22                        | 42                     | 0.197   |
| **Pre-Operative PS**           |                           |                        |         |
| 0                              | 32                        | 128                    |         |
| 1                              | 27                        | 95                     |         |
| 2                              | 21                        | 23                     |         |
| 3                              | 4                         | 5                      | <0.0001 |
| 4                              | 6                         | 2                      |         |
| **Post-Operative PS**          |                           |                        |         |
| 0                              | 28                        | 94                     | <0.0001 |
| 1                              | 30                        | 120                    |         |
| 2                              | 11                        | 21                     |         |
| 3                              | 13                        | 13                     |         |
| 4                              | 8                         | 5                      |         |
| **PS at 6 months**             |                           |                        | 0.002   |
| 0                              | 14                        | 37                     |         |
| 1                              | 17                        | 67                     |         |
| 2                              | 4                         | 25                     |         |
| 3                              | 6                         | 19                     |         |
| 4                              | 9                         | 5                      |         |
| 5                              | 13†                       | 12††                   |         |
| Location                       |                           |                        | 0.830   |
| Frontal Lobe                   | 30                        | 83                     |         |
| Temporal Lobe                  | 32                        | 92                     |         |
| Parietal Lobe                  | 21                        | 48                     |         |
| Occipital Lobe                 | 5                         | 26                     |         |
| Others                         | 2                         | 4                      |         |
| **Intraoperative Neuromonitoring**| 7 (7.8%)               | 77 (30.4%)             | <0.0001 |
| **Molecular Markers**          |                           |                        |         |
| ATRX                           | 37 (88.1%) *              | 167 (90.6%) ^          | 0.624   |
| IDH                            | 5 (7.5%) **               | 19 (7.7%) ^ ^          | 0.981   |
| MGMT                           | 41 (68.3%) ***            | 120 (50.6%) ^ ^ ^      | 0.015   |
| **Chemotherapy**               | 43 (74.1%) ****           | 174 (95.1%) ^ ^ ^ ^ ^  | <0.0001 |
| Radiotherapy                   | 48 (76.2%) *****          | 181 (96.3%) ^ ^ ^ ^ ^ ^| <0.0001 |

† 27 patients lost for follow-up
†† 88 patients lost for follow-up
* 48 patients with no data
** 24 patients with no data
*** 30 patients with no data
**** 32 patients with no data
***** 27 patients with no data

^ 72 patients with no data
^ ^ 5 patients with no data
^ ^ ^ 16 patients with no data
^ ^ ^ ^ 70 patients with no data
^ ^ ^ ^ ^ 65 patients with no data
Table 1 – Overall characteristics of non-5-ALA-GS and the 5-ALA-GS groups. 5-ALA-GS = 5-Aminolevulinic Acid Guided Surgery; PS = Performance Status; ATRX = Alpha-thalassemia x-linked intellectual disability; IDH = isocitrate dehydrogenase; MGMT = O-6-methylguanine-DNA methyltransferase.
| Primary Outcome                  | HR     | 95% CI       | p value |
|---------------------------------|--------|--------------|---------|
| Overall Survival                | 2.07±0.36 | [1.47 – 2.93] | <0.0001 (Cox) |

| Secondary Outcomes               | Coef.  | 95% CI       | p value |
|---------------------------------|--------|--------------|---------|
| Post-Operative PS               | 0.43±0.12 | [0.19 – 0.687] | <0.0001 (logit) |
| New Focal Neurological Deficit  | 1.26±0.29 | [0.69 – 1.82] | <0.0001 |
| EoR                             | 1.11±0.29 | [0.54 – 1.68] | <0.0001 |
| PS at 6 months of FU            | 0.28±0.09 | [0.10 – 0.46] | 0.002 |

| Risk Factors for Overall Survival (Unadjusted) | HR     | 95% CI       | p value |
|-----------------------------------------------|--------|--------------|---------|
| Gender                                        | 0.87±0.14 | [0.64 – 1.19] | 0.386 |
| Age                                           | 1.01±0.10 | [1.00 – 1.03] | 0.028 |
| Preoperative PS                               | 1.33±0.11 | [1.13 – 1.56] | <0.0001 |
| IONM                                          | 0.54±0.11 | [0.37 – 0.81] | 0.002 |
| IDH                                           | 0.66±0.22 | [0.35 – 1.26] | 0.213 |
| MGMT                                          | 0.42±0.07 | [0.31 – 0.59] | <0.0001 |
| Radiological Extent of Resection             | 1.42±0.22 | [1.04 – 1.93] | 0.028 |
| Post-Operative PS                            | 1.40±0.11 | [1.20 – 1.64] | <0.0001 |
| PS at 6 months of FU                         | 1.53±0.08 | [1.37 – 1.70] | <0.0001 |
| New Temporary Motor Deficit                  | 1.42±0.28 | [0.96 – 2.10] | 0.082 |
| Radiotherapy                                  | 0.20±0.06 | [0.11 – 0.37] | <0.0001 |
| Chemotherapy                                  | 0.04±0.1 | [0.02 – 0.08] | <0.0001 |
| Non-5-ALA-assisted Surgery                   | 2.07±0.36 | [1.47 – 2.93] | <0.0001 |

| Risk Factors for Overall Survival (Adjusted) | HR     | 95% CI       | p value |
|---------------------------------------------|--------|--------------|---------|
| Gender                                      | 0.77±0.21 | [0.45 – 1.32] | 0.347 |
| Age                                         | 1.01±0.13 | [0.98 – 1.03] | 0.605 |
| Preoperative PS                             | 0.81±0.16 | [0.55 – 1.21] | 0.319 |
| IONM                                        | 0.72±0.22 | [0.40 – 1.31] | 0.281 |
| IDH                                         | 1.54±0.89 | [0.50 – 4.75] | 0.451 |
| MGMT                                        | 0.39±0.11 | [0.22 – 0.68] | 0.001 |
| Radiological Extent of Resection            | 1.02±0.28 | [0.60 – 1.74] | 0.942 |
| Post-Operative PS                           | 1.34±0.27 | [0.91 – 1.98] | 0.144 |
| PS at 6 months of FU                        | 1.19±0.11 | [0.99 – 1.42] | 0.052 |
| Radiotherapy                                | 0.78±0.74 | [0.12 – 5.00] | 0.790 |
| Chemotherapy                                | 0.19±0.18 | [0.03 – 1.30] | 0.091 |
| Non-5-ALA-GS                                | 2.95±1.09 | [1.43 – 6.09] | 0.003 |

Table 2 – Outcomes and Risk Factor Analysis in glioblastomas. 5-ALA-GS= 5-Aminolevulinic Acid Guided Surgery; PS = Performance Status; EoR = Extent of Resection; FU = Follow-up; IONM = Intraoperative Neurophysiological Monitoring; IDH = isocitrate dehydrogenase; MGMT = O- 6-methylguanine- DNA methyltransferase.
## Non-Fluorescence Guided Surgery Literature

| Author (year)        | Years Included | No. of Patients | Median/Mea n Age (Years) | Pre-Op KPS | Tumour Type (WHO Grade) | Surgical Outcome | Post-Op KPS | Follow-up KPS | Progressio n-Free Survival (Months) | Overall Survival (Months) |
|----------------------|----------------|-----------------|--------------------------|------------|-------------------------|-----------------|-------------|--------------|------------------------------------|--------------------------|
| Dobran et al. (2019) | 2012-2015      | 40              | 60                       | N          | GBM (IV)                | GTR: 25 STR: 15 | 72          | 70           | N/A                                | GTR: 9 STR: 6             |
| Guler et al. (2019)  | 2007-2014       | 126             | 55                       | N/A        | GBM (IV)                | GTR: 31 STR: 85 Biopsy: 10 | 70-80: 51 90-100: 75 | N/A          | N/A                                | GTR: 23.6 STR/Biopsy y: 14.1 |
| Dayani et al. (2018) | 2004-2014       | 39              | 57.8                     | N          | bGBM (IV)               | Resection: 14 (median EOR: 83%) Biopsy: 25 | 80          | N/A          | N/A                                | GTR: 16 STR: 13 Biopsy: 4.5  |
| Chan et al. (2017)   | 2010-2012       | 42              | 55.1                     | N/A        | GBM (IV)                | GTR: 15 STR: 18 Biopsy: 9 | N/A          | N/A          | N/A                                | GTR: 12 STR/Biopsy y: 6.7 |
| Harris et al. (2017) | 2006-2016       | 108             | 78.7                     | N/A        | GBM (IV)                | GTR: 40 STR/Biopsy y: 30 | N/A          | N/A          | N/A                                | GTR: 29.4 STR: 29.8       |
| Guden et al. (2016)  | 2006-2015       | 92              | 59                       | <70: 17 >70: 74 | GBM (IV)                | GTR: 56 STR: 24 Biopsy: 12 | N/A          | N/A          | GTR-GTR: 30.8 STR-STR: 24.7       | GTR-GTR: 13.6 GTR-STR: 9.2 |
| Ringel et al. (2016) | 2006-2010       | 503             | 57                       | N          | Recurrent GBM (IV)      | GTR: 238 STR: 170 PR: 39 | 90          | N/A          | N/A                                | GTR-GTR: 13.6 GTR-STR: 10.2 |
## Fluorescence Guided Surgery Literature

| Author (year) | Years Included | No. of Patients | Median/Mean Age (Years) | 5-ALA Pre-Op KS | Tumour Type (Grade) | Surgical Outcome | Post-Op KPS | Follow-up KPS | Progression-Free Survival (Months) | Overall Survival (Months) |
|---------------|---------------|----------------|-------------------------|----------------|-------------------|------------------|------------|--------------|-----------------------------------|--------------------------|
| Hrabalek et al. (2015) | 2007-2009 | 62.5 | N | 80 | GBM (IV) | GTR: 3 NTR: 6 STR: 7 PR: 6 | 80 | N/A | N/A | GTR: 11.3 NTR: 17.1 STR: 7.8 PR: 9.7 |
| Qin et al. (2015) | 2010-2014 | 816 | - | N | <80 | 748x Primary GBM (IV) 68x Secondary GBM (IV) | GTR: 209 STR: 607 | N/A | N/A | GTR: 12.5 STR: 8 | GTR: 19.9 STR: 14.8 |
| Lombardi et al. (2015) | 2007-2014 | 237 | 71 | N/A | GBM (IV) | GTR: 174 STR: 63 | N/A | N/A | N/A | GTR: 17.7 STR: 16.1 |
| Chaichana et al. (2014) | 2007-2011 | 259 | 59.6 | N/A | GBM (IV) | Unspecified, 81% average extent of resection | N/A | N/A | N/A | >70%: 14.4 ≤70%: 10.5 |
| Hong et al. (2013) | 2006-2010 | 42 | A:60 B:56.5 | N/A | GBM (IV) | A: GTR: 8 STR: 2 B: GTR: 26 STR: 6 | A: <80: 0 ≥80: 10 B: <80: 14 ≥80: 18 | N/A | N/A | GTR: 16 STR: 14 |
| Yabroff et al. (2012) | 2006 | 1202 | - | N/A | 1170x Glioblastoma (IV) 11x GCGBM (IV) 21x Gliosarcoma (IV) | GTR: 461 PR: 314 Biopsy: 29 Other: 120 No Surgery: 275 Unknown: 3 | N/A | N/A | N/A | GTR: 20 PR: 13 Biopsy: 14 No Surgery: 10 |
| Study                          | Year  | N  | 5-ALA Dose (%) | ALA or ALA + iMRI | GBM (IV) | GTR: %  | STR: %  | GTR: %  | STR: %  | GTR: %  | STR: %  | GTR: %  | STR: %  | Notes                         |
|-------------------------------|-------|----|----------------|-------------------|----------|---------|---------|---------|---------|---------|---------|---------|-----------------------------|
| Della Puppa *et al.* (2017)   | 2009-2013 | 20 | 55.3           | 20x 5-ALA + BCNU wafer | 100      | GTR: 100% | Not Stated | Not Stated | 11 | 22 |
| Hauser *et al.* (2016)        | 2009-2011 | 13 | 60.5           | Y                 | N/A      | GTR: 10 | STR: 3 | N/A      | N/A      | N/A      | GTR: 18.9 | STR: 8.3 | Not Stated                        |
| Coburger *et al.* (2015)      | 2008-2012 | 33 | 5%             | 5-ALA + iMRI      | N/A      | GTR 100% | Not Stated | Not Stated | 6  | 17 |
| Kim *et al.* (2014)           | 2009-2011 | 40 | 51             | <80               | GBM (IV) | GTR: 80% | STR: 20%| 82.7     | 84.7     | 18 | GTR: 24 | STR: 13 | Not Stated                        |
| Aldave *et al.* (2013)        | 2007-2011 | 52 | 58.7           | Y                 | GBM (IV) | GTR: 100% | N/A      | N/A      | N/A      | N/A      | nonresidual fluorescence = 27m, 17.5m with residual fluorescence |
| Schucht *et al.* (2012)       | 2008-2010 | 103 | 62             | Y                 | GBM (IV) | GTR: 53 | STR: 16 | Biopsy: 34 | 84 | N/A     | N/A     | GTR: 16.7 | STR: 11.8 | Not Stated                        |
| Diez Valle *et al.* (2011)    | 2007-2009 | 28 | 58             | Y                 | GBM (IV) | GTR: 23 | STR: 5  | 70 (mean) | Not Stated | GTR: 8.5 | STR: 10.7 | GTR: 10.6 | STR: 13 | Not Stated                        |

**Table 3** – Literature Review of the surgical case series published with Non-5-ALA-GS and 5-ALA-GS for glioblastomas in the last 10 years in patients treated with Stupp Protocol (enrolment after 2006). GBM = Glioblastoma Multiforme; bGBM = Butterfly Glioblastoma Multiforme; GTR = Gross Total Resection; STR = Subtotal Resection.
Figure 2

**Overall Survival**

- **WHO Grade 4 Glioma**
  - S-ALA-55 Group
  - Non-S-ALA-65 Group

**Gross Total Resection**

**Subtotal Total Resection**

Statistical significance: P < 0.0001
