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What is the Surgical Benefit of Utilizing 5-ALA for Fluorescence-Guided Surgery of Malignant Gliomas?

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Abstract

The current neurosurgical goal for patients with malignant gliomas is maximal safe resection of the contrast-enhancing tumor. However, a complete resection of the contrast-enhancing tumor is achieved only in a minority of patients. One reason for this limitation is the difficulty in distinguishing viable tumor from normal adjacent brain during surgery at the tumor margin using conventional white-light microscopy. To overcome this limitation, fluorescence-guided surgery (FGS) using 5-aminolevulinic acid (5-ALA) has been introduced in the treatment of malignant gliomas. FGS permits the intraoperative visualization of malignant glioma tissue and supports the neurosurgeon with real-time guidance for differentiating tumor from normal brain that is independent of neuronavigation and brain shift. Tissue fluorescence after oral administration of 5-ALA is associated with unprecedented high sensitivity, specificity, and positive predictive values for identifying malignant glioma tumor tissue. 5-ALA-induced tumor fluorescence in diffusely infiltrating gliomas with non-significant MRI contrast-enhancement permits intraoperative identification of anaplastic foci and establishment of an accurate histopathological diagnosis for proper adjuvant treatment. 5-ALA FGS has enabled surgeons to achieve a significantly higher rate of complete resections of malignant gliomas as compared to conventional white-light resections. Consequently, 5-ALA FGS has become an indispensable surgical technique and standard of care at many neurosurgical departments around the world. We conducted an extensive literature review concerning the surgical benefit of utilizing 5-ALA for FGS of malignant gliomas. According to the literature, there are a number of reasons for the neurosurgeon to perform 5-ALA FGS, which will be discussed in detail in the current review.

Keywords

5-aminolevulinic acid; glioma; GBM; fluorescence-guided surgery

Introduction

Over the past decade, a large interest in fluorescence-guided surgery (FGS) in patients with malignant brain tumors has surfaced globally¹. Just in the past 5 years, a surge in publications on FGS of brain tumors can be found in the worldwide literature¹. The majority of these publications focus on the use of 5-aminolevulinic acid (5-ALA) as a fluorescent pro-agent for FGS of malignant gliomas. Much of this interest has been based on the initial efforts by Dr. Walter Stummer when he first described the use of 5-ALA and FGS in human malignant glioma patients in 1998^{2,3}. A randomized, controlled phase III study confirmed a more complete resection of malignant gliomas and better progression-free survival after 5-ALA administration and FGS⁴⁻⁶. Thousands of patients have now undergone resection of their malignant brain tumor after 5-ALA administration worldwide (information by medac, Wedel, Germany). 5-ALA (Gliolan®) has been approved for human use in Europe, Asia, and Australia. After oral administration, 5-ALA has been found to be a safe compound with only minimal side effects.

5-ALA, a natural hemoglobin metabolite

5-ALA is a natural metabolite in the human body that is produced with the hemoglobin metabolic pathway⁷. Exogenous 5-ALA acts like a pro-agent that is orally administered and has unprecedented penetration of the blood brain barrier (BBB) and tumor interface in brain tumors⁸⁻¹⁰. So far, no other known oral agent is available for FGS that can accumulate within malignant brain tumors and surrounding infiltrating cancer cells outside of the tumor bulk. Once 5-ALA is taken up by malignant glioma cells, it is metabolized into the fluorescent metabolite, protoporphyrin IX (PpIX)^{11,12}. Elevated PpIX production within malignant brain tumor cells permits violet-red fluorescence visualization of malignant tumor tissue after excitation with 405 nm wavelength blue light. The preferential accumulation of 5-ALA within malignant glioma cells is felt to occur due to decreased levels of ferrochelatase (a heme production enzyme that produces heme with the addition of iron (Fe)) and selective uptake by an ATP-binding cassette transporter (ABCB6)¹³. Other factors that correlate with fluorescence induced by 5-ALA are cellular density, tumor cell proliferative activity, neovascularity of the tumor, and BBB permeability^{10,14}.

Tissue fluorescence signifies malignant tumor tissue

The diagnostic accuracy of 5-ALA-induced tissue fluorescence in malignant gliomas is a key benefit for 5-ALA FGS. Multiple studies have found a very high sensitivity, specificity, and positive predictive value (PPV) for tissue fluorescence and malignant glioma tumor tissue (see Table 1). Values close to and over 90% have been consistently described by most groups for sensitivity, specificity, and/or PPV of fluorescence and malignant tumor in both newly diagnosed as well as recurrent malignant gliomas^{3,15-24}. Different fluorescent qualities have been observed in malignant glioma patients. Solid red fluorescence is found in the bulk of malignant glioma tumors, while a pink fluorescence is found at the tumor margin where cancer cells infiltrate the normal brain^{3,15,18-20}.

Strong vs. weak fluorescence

Recently, a prospective study by Stummer et al. assessed the reliability of visible 5-ALA-induced fluorescence by spectrometry, pathology, and imaging ²⁵. During malignant glioma resections, tumor biopsies were taken from tissues with strong and weak fluorescence. “Strong” fluorescence corresponded to greater spectrometric fluorescence, solidly proliferating tumor, and high tumor cell densities, whereas “weak” fluorescence corresponded to lower spectrometric fluorescence, infiltrating tumor, and medium tumor cell densities (see Figure 1). The study showed a PPV of 100% for malignant glioma tumor in strongly fluorescing tissue and 95% for weakly fluorescing tissue.

False positive fluorescence

5-ALA-induced fluorescence not associated with malignant tumor tissue has also been reported in rare cases. Several groups have described the presence of fluorescence in tissue areas surrounding the resection cavity within the *immediate vicinity* of viable tumor cells ^{3,19,26-28}, but not in normal brain distant from the gross tumor. Furthermore, it has been described in patients with recurrent malignant gliomas who had completed prior adjuvant therapies ^{15,29}. Reactive astrocytes may play a role in those patients with recurrent malignant gliomas and false positive fluorescence ^{15,16,29}. Autofluorescence of normal brain tissue might be another rare cause for false positive fluorescence ¹⁵, at least on a microscopic level. In 313 patients operated on for suspected recurrent glioblastomas based on imaging, about 3% turned out to have pathology consistent with radiation necrosis in tissues demonstrating fluorescence ³⁰.

False negative fluorescence

Malignant glioma tumor tissue that is present and not visible by 5-ALA-induced fluorescence can be attributed to specific circumstances. First of all, since malignant gliomas are characterized by a diffusely infiltrative growth pattern within the brain, cancer cells can be found to a much lesser extent centimeters away from the contrast-enhancing tumor bulk as well. Therefore, it is not surprising that the negative predictive value (NPV) of 5-ALA-induced visible fluorescence in malignant gliomas is limited in regions with low-density tumor cell infiltration. The NPV is dependent on the distance of the tissue-sampling site from the tumor bulk and thus varies widely between 22% and 91% ^{3,15-19,21,23,25}. The NPV can be increased using spectrometry ^{19,25}, which helps identifying fluorescence in regions of low-density cell infiltration too weak to be visualized directly with the microscope. Confocal microscopy has also been used to detect PpIX fluorescence in low-grade gliomas which usually cannot be visualized with conventional violet-blue excitation light ³¹. On the other hand, visible fluorescence has been shown to be present beyond the contrast-enhancing areas of malignant gliomas ^{32,33}. Despite the relatively low NPV reported in most studies, 5-ALA induced fluorescence is a powerful technique for identification of the contrast-enhancing tumor (in addition to large portions of the infiltration zone) that represents the neurosurgical target in malignant gliomas.

Secondly, false negative 5-ALA induced fluorescence can be attributed to structural barriers that interfere with fluorescence visualization. Malignant fluorescent tissue is typically hidden by photobleaching, blood, or overhanging brain tissue obstructing the view of the

resection cavity. Photobleaching is the process by which PpIX fluorescence deteriorates under the influence of light (blue 400 nm or standard white light). A large drop in tissue fluorescence (bleaching to 36%) can occur with prolonged light exposure (>25 min with blue light and >87 min with standard white light)². During 5-ALA FGS, photobleaching plays a minor role since new tissue layers are exposed during tumor resection. Inadequate illumination by the blue light from the microscope due to a small corticotomy may be another reason that fluorescent tumor tissue is not visualized. Thus, fluorescence does not replace proper surgical technique with adequate visualization of the entire resection cavity. Use of an endoscope for closer inspection of deep resection cavities may help visualize fluorescent tumor tissue³⁴.

Third, the timing of 5-ALA administration is important and may account for the lack of tumor fluorescence in limited cases. After 5-ALA oral ingestion, a patient is taken back to the operating room typically 3 h (2-4 h) later. Once ingested, 5-ALA is rapidly absorbed into the bloodstream within 1 h. However, PpIX plasma levels peak 4 h after oral administration of 5-ALA (20 mg/kg)^{2,11}. In animal studies, peak PpIX fluorescence in malignant glioma tumors was found 6 h after 5-ALA administration¹¹. Taking a patient too early to surgery (less than 2 h) after oral administration of 5-ALA may account for a lack of tumor fluorescence. Waiting too long to take a patient to surgery may also result in a lack of tumor fluorescence. However, according to the authors' experience, sufficient tumor fluorescence can be observed in malignant glioma patients even 12 h after oral 5-ALA administration.

Finally, necrotic portions of malignant gliomas have been described to have absent or less tumor fluorescence². Anaplastic oligodendrogliomas have also been reported to have less tumor fluorescence present after 5-ALA administration³⁵.

Diffusely infiltrating gliomas with malignant foci

Diffusely infiltrating gliomas (DIG) with non-significant MRI contrast-enhancement are presumed to be slowly growing low-grade gliomas (WHO Grade II). Nevertheless, close to 50% of radiologically suspected low-grade gliomas contain anaplastic or malignant foci with tumor tissue of World Health Organization (WHO) Grade III or IV histology³⁶. Since the postoperative management of malignant gliomas (WHO Grade III and IV) as compared to low-grade gliomas (WHO Grade II) differs significantly, intraoperative identification of anaplastic foci and thus establishment of an accurate histopathological diagnosis according to the WHO is essential. Properly diagnosing a malignant glioma would permit treatment of the patient with necessary adjuvant therapies and avoid any delay in treatment initiation. However, no clinically reliable intraoperative marker for detection of anaplastic foci in order to avoid histological undergrading has been available so far.

Recent studies demonstrated that 5-ALA-induced fluorescence is also capable of visualizing intraoperatively such anaplastic foci in the majority of cases³⁷⁻³⁹. Furthermore, histopathological analysis of intratumoral areas with focal 5-ALA-induced fluorescence showed that fluorescing tumor tissue significantly correlates with an increased proliferation rate and histopathological WHO parameters of anaplasia^{38,39}. An illustrative case of a DIG with non-significant contrast-enhancement on MRI with an anaplastic focus that was identified by 5-ALA-induced PpIX fluorescence is shown in Figure 2. Consequently,

application of 5-ALA to patients with DIG with non-significant MRI contrast-enhancement optimizes tissue sampling for precise histopathologic diagnosis and thus enables optimal postoperative patient management³⁸⁻⁴¹. A detailed literature overview of 5-ALA-induced fluorescence in low-grade and high-grade gliomas is provided in Table 2.

Real-time intraoperative guidance

Frameless stereotactic guidance, also known as neuronavigation, is routinely used to guide neurosurgeons in their resection of malignant gliomas⁴². Well-known limitations of neuronavigation include the use of a preoperative MRI scan and brain shift that occurs during surgical resection of malignant gliomas^{43,44}. Preoperative MRI scans are typically obtained days to over a week prior to actual surgery and used to register with the patient's skin surface anatomy or skin fiducials placed at the time of scanning. Registration of the preoperative MRI scan with the patient can be imprecise and thus results in inaccuracy of the neuronavigation prior to glioma removal. During the actual tumor resection, brain shift further compounds the inaccuracy of the neuronavigation system up to several more centimeters⁴³.

Currently, 5-ALA FGS of malignant gliomas permits real-time intraoperative guidance for tumor visualization. PpIX fluorescence provides the neurosurgeon with real-time information for differentiating tumor from normal tissue, independent of neuronavigation and brain shift¹⁹. Switching from the standard white light to the fluorescent mode on the microscope is performed with the push of a button, minimizing disruption to the flow of surgery. Intraoperative MRI (iMRI) and ultrasonography can be used to update the neuronavigation system to account for brain shift after tumor removal^{45,46}. In addition to being cost prohibitive, iMRI disrupts the flow of surgery, adding up to one additional hour of operative time.

Malignant glioma tumor margin visualization

Multiple studies have shown that 5-ALA-induced tumor fluorescence extends past the area of gadolinium contrast-enhancement found on preoperative MRI in patients with malignant gliomas^{32,33}. Since gadolinium contrast-enhancement relies on disruption of the BBB, infiltrative glioma regions at the tumor margin are typically not shown with MRI⁴⁷. However, PpIX accumulation is found within infiltrating glioma cells at the tumor margin⁴⁸. A study by Roessler et al. was able to show that 5-ALA induced fluorescence is much more sensitive at detecting the tumor margin than neuronavigation³². Due to brain shift, however, a direct comparison between 5-ALA-induced tumor fluorescence and neuronavigation is difficult. Coburger et al. recently performed a comparison of gadolinium contrast-enhancement and 5-ALA-induced fluorescence at the tumor margin with the use of iMRI²¹. In their study of newly diagnosed and recurrent glioblastoma multiforme (GBM) patients, they found a higher detection rate for tumor infiltration at the tumor margin with 5-ALA-induced fluorescence as opposed to iMRI.

Is there tissue fluorescence after maximal white light microsurgical resection?

Five prospective studies have been reported examining the presence of tissue fluorescence after maximal white light microsurgical resection in patients with malignant gliomas (see Table 3). Nabavi et al. were the first to report the use of 5-ALA FGS in patients with recurrent malignant gliomas²⁶. In this multicenter study of 36 patients, surgeons performed standard microsurgical resection of recurrent malignant gliomas. After tumor resection, the resection cavity was examined with standard white light illumination. Abnormal, pathologically-appearing tissue and adjacent, normal-appearing tissue were identified. The microscope was switched to the fluorescent mode for fluorescence visualization. Fluorescent tissue was sampled for histopathologic analysis. In abnormal tissue, fluorescence had a PPV of 97% for malignant glioma tissue in 35 of 36 patients. More importantly, areas appearing normal under white light, but displaying strong fluorescence, had a high PPV of 92% for malignant glioma tissue in 22 of 24 patients.

Panciani et al. reported their multicenter study on 23 patients with newly diagnosed malignant gliomas¹⁵. For each patient, tissue sampling was collected from the following areas: a. fluorescent sample within the neuronavigation area, b. fluorescent sample outside the neuronavigation area, c. non-fluorescing sample within the navigation area, and d. non-fluorescing sample outside the navigation area. All 23 patients (100%) were noted to have fluorescence within the resection cavity included in the neuronavigation area that was pathologic for malignant glioma. It is of note that 18 of 23 patients (78%) were found to have tissue fluorescence even outside the area of neuronavigation that was consistent with tumor.

Stummer et al. conducted a prospective study on 33 patients with newly diagnosed malignant gliomas²⁵. During the tumor resection, the authors observed residual tumor fluorescence in 42 different regions. In 24% of cases, this residual tumor was also detectable with conventional white-light microscopy. However, in the remaining 76% of cases, the residual tumor was not detectable with conventional white-light and could only be identified by visible 5-ALA-induced fluorescence.

Another prospective study by Coburger et al. was completed in 34 patients with newly diagnosed and recurrent malignant gliomas²¹. After maximal white light tumor resection, spatial location of tissue fluorescence was noted in the resection cavity. Then, iMRI was performed in patients to determine if any residual gadolinium contrast enhancement was present suggestive of residual tumor. All areas were biopsied for histopathologic analysis. Tumor detection was significantly higher with 5-ALA than with iMRI especially at the tumor margin where both sensitivity (tumor positive) and specificity (tumor negative) were superior.

A more recent prospective study by Yamada et al. was completed in 99 patients with malignant gliomas (67 GBM and 32 anaplastic astrocytomas)²³. Eighty patients with newly diagnosed and 19 patients with recurrent malignant gliomas underwent maximal standard microsurgical resection of their tumor with updated iMRI neuronavigation. 5-ALA FGS was performed at the tumor margin where over 154 tissue specimens were obtained for histopathological analysis. 5-ALA-induced tissue fluorescence was revealed in all 99

patients. Of the 154 samples from the “peritumoral brain”, fluorescence intensity was strong in 107 and weak in 47 cases. In 133 of the biopsy specimens (86%), histopathologic analysis revealed tumor. Histopathology revealed tumor presence in 95 of 107 (89%) and in 38 of 47 (81%) biopsy specimens obtained from areas of strong and weak fluorescence, respectively. Overall, 5-ALA-induced tissue fluorescence had 92% PPV for presence of glioma on histology in the study.

An illustrative case of a malignant glioma in which residual tumor tissue could be identified by 5-ALA-induced PpIX fluorescence despite assumed complete tumor resection with conventional white-light microscopy is shown in Figure 3.

Reported extents of resection using 5-ALA-induced fluorescence

Safe maximal resection is the commonly accepted goal of surgery for malignant gliomas, optimally defined as the complete resection of contrast-enhancing tumor⁴⁹. However, not all malignant gliomas can be maximally resected. Extent of resection depends on many factors, including tumor location in or adjacent to eloquent brain and motor tracts, size, use of intraoperative monitoring and mapping, and, finally, on the experience of the surgeon. One important confounder, however, is the difficulty in distinguishing viable tumor from normal adjacent brain even when using the normal surgical microscope⁵⁰. Even in newer series, the “complete” resection of contrast-enhancing tumor is only achieved in approximately 35% of cases^{4,51} if surgery is performed without fluorescence or iMRI.

In a first series of 52 patients who were operated on for malignant gliomas using 5-ALA-induced fluorescence, complete resection of the contrast-enhancing tumor was achieved in 63% of cases³. A subsequent randomized multicenter phase III trial on malignant gliomas confirmed that surgery using white light alone would result in 36% complete resections, which was virtually doubled to 65% if surgeons could exploit the information gained from tissue fluorescence⁴. Importantly, no differences in neurological functions were noted after surgery when comparing conventional to FGS. Nevertheless, the surgeons contributing to this study were inexperienced with FGS, operating on their first dozen or so patients, and resection rates differed widely. Today, higher rates of complete resections are generally reported, ranging from 73% for tumors located in eloquent brain regions to 89% in unselected patients⁵²⁻⁵⁴. These values reflect the increasing familiarity of more and more neurosurgeons with 5-ALA FGS, especially in combination with intraoperative monitoring and mapping. In addition, Diez Valle et al. performed an analysis of surgically removed tumor volumes in 36 unselected GBM patients using 5-ALA and reported removal of >98% of tumor volumes using 5-ALA FGS in all cases¹⁸. An overview of studies in the literature concerning the extent of resection in 5-ALA FGS of malignant gliomas is given in Table 4. A recent meta-analysis by Eljamel found a gross total resection rate (removal of 98% or more of the contrast-enhancing tumor) of 75.4% (418/565 patients) in GBM patients after 5-ALA FGS²⁴. Together, these data demonstrate that 5-ALA FGS helps neurosurgeons in achieving high rates of complete resections in malignant gliomas especially in combination with intraoperative monitoring and mapping.

Future detection of 5-ALA tumor fluorescence in low-grade gliomas

Current microscope fluorescence technologies are unable to detect visible PpIX fluorescence in the majority of low-grade gliomas³⁷⁻³⁹. Recently, it was shown that such non-fluorescing low-grade gliomas can be visualized intraoperatively by application of quantitative measurement of PpIX using a fiberoptic probe^{9,55}. Furthermore, intraoperative visualization of low-grade gliomas with confocal microscopy using a specific hand-held probe represents another promising technique³¹. Therefore, such hand-held devices that are capable of detecting fluorescence signals in a more sensitive fashion can permit in the future the visualization of PpIX within tumor tissue not visualized with the current fluorescence microscope.

Conclusion

The introduction of FGS represents one of the most important advances in the neurosurgical treatment of brain tumors over the past decade. The achievement of this innovative treatment is strongly linked to the only orally administered fluorescent dye 5-ALA that permits intraoperative visualization of the tumor bulk in addition to the surrounding zone of tumor infiltration present in malignant gliomas. During tumor resection, 5-ALA-induced fluorescence supports the neurosurgeon with real-time information for differentiating tumor from normal tissue that is independent of neuronavigation and brain shift. According to the data in the literature, most studies have found a very high sensitivity, specificity and PPV for PpIX fluorescence and the presence of tumor tissue during resection of both newly diagnosed and recurrent malignant gliomas. Recently, PpIX fluorescence was also identified as a novel marker for intraoperative detection of anaplastic foci in non-enhancing gliomas that ensures a precise histopathological diagnosis and optimal patient treatment. Furthermore, five prospective studies have confirmed that PpIX fluorescence is able to identify residual tumor tissue after assumed maximal resection of malignant gliomas with conventional white-light microscopy. Since intraoperative detection of malignant glioma tissue is significantly improved by 5-ALA FGS, high rates of complete tumor resections are achieved especially in combination with intraoperative monitoring and mapping.

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Abbreviations

5-LA	5-aminolevulinic acid
BBB	Blood brain barrier
DIG	Diffusely infiltrating gliomas
FGS	Fluorescence-guided surgery
GBM	Glioblastoma multiforme
iMRI	Intraoperative MRI
NPV	Negative predictive value
PpIX	Protoporphyrin IX
PPV	Positive predictive value
WHO	World Health Organization

Figure 1. Comparison of specific intratumoral areas in a malignant glioma (illustrative case): Contrast-enhanced MRI, conventional white-light microscopy, 5-ALA induced PpIX fluorescence and histopathology

The central necrotic part of the GBM (1a) on imaging and (1b) during white-light resection does (1c) not show any visible PpIX fluorescence and (1d) corresponding histopathology confirms the presence of tissue necrosis. (2a) In the region of the ring-like contrast-enhancement on MRI, (2b) greyish/soft tumor tissue is found under white-light microscopy that shows (2c) strong PpIX fluorescence and (2d) corresponding histopathology reveals solidly proliferating tumor tissue with high tumor cell density. (3a) Outside the contrast-enhancing part on MRI, (3b) the tissue showing only slight pathological appearance under conventional white-light can be (3c) clearly visualized by weak PpIX fluorescence and (3d) corresponding histopathology depicts infiltrating glioma tissue with medium tumor cell density. At the assumed end of the infiltration zone (4a) on imaging and (4b) under white-light, (4c) no visible PpIX fluorescence can be found and (4d) corresponding histopathology is not able to detect obvious infiltrating glioma cells in this case. Reprinted from Widhalm. Intra-operative visualization of brain tumors with 5-aminolevulinic acid-induced fluorescence. Clin Neuropathol. 2014 Jul-Aug; 33(4): 260-78 ⁵⁶, with permission Dustri-Verlag Dr. Karl Feistle GmbH & Co. KG

Figure 2. Detection of an anaplastic focus in a DIG with non-significant contrast-enhancement on MRI with visible 5-ALA induced fluorescence

A., During tumor resection, the glioma tissue shows a relatively homogenous appearance under conventional white-light microscopy. B., In contrast, a small circumscribed intratumoral area can be identified with 5-ALA induced PpIX fluorescence under violet-blue excitation light. Histopathological analysis of the fluorescing intratumoral area shows (C) malignant glioma tissue (D) with a high proliferation rate indicating the presence of an anaplastic focus. On the contrary, the surrounding non-fluorescing tissue reveals only (E) low-grade glioma tissue (F) with a low proliferation rate. Reprinted from Widhalm. Intra-operative visualization of brain tumors with 5-aminolevulinic acid-induced fluorescence. Clin Neuropathol. 2014 Jul-Aug; 33(4): 260-78 ⁵⁶, with permission Dustri-Verlag Dr. Karl Feistle GmbH & Co. KG

Figure 3. Detection of residual newly-diagnosed GBM tumor with PpIX fluorescence after maximal white-light conventional resection

A., Left, Microsurgical white-light visualization of GBM resection cavity after maximal conventional resection. Right, Visualization of PpIX fluorescence at tumor margin after conventional microsurgical resection. B., Left, Preoperative MRI scan with gadolinium enhancement in right parietal lobe consistent with malignant glioma. Center and Right, postoperative MRI scans with and without gadolinium enhancement confirming complete resection of the enhancing tumor. C, Histopathologic examination (hematoxylin and eosin staining) of fluorescent tissue at tumor margin confirming presence of infiltrating tumor extending away from tumor bulk at 10 × (left) and 20 × (right) magnification.

Studies with sensitivity, specificity, positive and negative predictive value of 5-ALA induced fluorescence and malignant glioma tissue

Table 1

Publication	n patients	Included tumor entities	Newly diagnosed / recurrent tumors	Sensitivity	Specificity	PPV	NPV
Yamada et al. (2015)	97	HGG	newly + recurrent	95%	53%	92%	69%
Coburger et al. (2014)	34	GBM	newly + recurrent	91%	80%	99%	22%
Stummer et al. (2014)	33	HGG	newly + recurrent	-	-	Overall 96% Strong PpIX 100% Vague PpIX 95%	40%
Panciani et al. I (2012)	23	GBM	newly	91%	89%	89%	91%
Panciani et al. II (2012)	18	GBM	newly	91%	89%	88%	91%
Diez-Valle et al. (2011)	36	GBM	newly + recurrent	-	-	Strong PpIX 100% Vague PpIX 97%	66%
Idoate et al. (2011)	30	GBM	newly + recurrent	-	-	Solid tumor 100% Invasive tumor 97%	67%
Roberts et al. (2011)	11	GBM	newly	75%	71%	95%	26%
Stummer et al. (2000)	52	GBM	newly + recurrent	89%	96%	99%	50%

GBM... Glioblastoma, HGG... High-grade glioma (WHO grade III + IV), NPV... Negative predictive value, PPV... Positive predictive value, PpIX... Protoporphyrine IX fluorescence

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Table 2
Studies of 5-ALA induced fluorescence in low-grade and high-grade gliomas

Publication	n patients	Study design	Type of surgery	Visible PpIX fluorescence (n patients)	
				Gliomas with low-grade glioma tissue only	Gliomas with malignant tissue / areas
Widhalm et al. (2013)	59	In-vivo	Resection	4/33	23/26
Widhalm et al. (2012)	39	In-vivo	Stereotactic biopsy	0/6	32/33
Ewelt et al. (2011)	30	In-vivo	Resection	1/13	12/17
Valdes et al. (2011)	23*	Ex-vivo	-	1/6	15/15
Widhalm et al. (2010)	17	In-vivo	Resection	0/8	8/9
Ishara et al. (2007)	6	Ex-vivo	-	0/2	4/4

GBM...Glioblastoma, HGG...High-grade glioma (WHO grade III + IV), PpIX...Protoporphyrine IX

* in two cases of a recurrent glioma no WHO grade was available

Table 3
Studies investigating the presence of tissue fluorescence after maximal white light microsurgical resection of malignant gliomas

Publication	n patients	Included tumor entities	Newly diagnosed/recurrent tumors	Visible PpIX fluorescence after white-light resection	Histological analysis of tissue from remaining PpIX fluorescence
Yamada et al. (2015)	97	HGG	newly + recurrent	yes	yes
Stummer et al. (2014)	33	HGG	newly + recurrent	yes	no
Coburger et al. (2014)	34	GBM	newly + recurrent	yes	yes
Panciani et al. (2012)	23	GBM	newly	yes	yes
Nabavi et al. (2009)	36	HGG	recurrent	yes	yes
GBM...Glioblastoma, HGG...High-grade glioma (WHO grade III + IV), PpIX...Protoporphyrine IX					
Publication	n patients	Included tumor entities	Newly diagnosed/recurrent tumors	Visible PpIX fluorescence after white-light resection	Presence of tumor tissue within remaining PpIX fluorescence
Yamada et al. (2015)	97	HGG	newly + recurrent	yes	yes
Stummer et al. (2014)	33	HGG	newly + recurrent	yes	n.d.
Coburger et al. (2014)	34	GBM	newly + recurrent	yes	yes
Panciani et al. (2012)	23	GBM	newly	yes	yes
Nabavi et al. (2009)	36	HGG	recurrent	yes	yes
GBM...Glioblastoma, HGG...High-grade glioma (WHO grade III + IV), n.d....No data, PpIX...Protoporphyrine IX					

Table 4
Studies of Extent of resection after 5-ALA fluorescence-guided surgery in malignant gliomas

Publication	n patients	Study population	Included tumor entities	Newly diagnosed/recurrent tumors	Complete resection of contrast-enhancing tumor		Volumetric analysis
					5-ALA resection	Control group	
Schucht et al. (2014)	67	eloquent tumors	GBM	newly + recurrent	73%	-	-
Della Puppa et al. (2013)	31	eloquent tumors	HGG	newly + recurrent	74%	-	-
Schucht et al. (2012)	53	unselected tumors	GBM	newly + recurrent	89%	-	-
Diez Valle et al. (2011)	36	unselected tumors	GBM	newly + recurrent	83%	-	resection of >98% tumor volume in all cases
Stummer et al. (2006)	5-ALA group: 139 Control group: 131	unselected tumors	HGG	newly	65%	36%	-
Stummer et al. (2000)	52	unselected tumors	GBM	newly + recurrent	63%	-	-

5-ALA...5-aminolevulinic acid, GBM...Glioblastoma, HGG...High-grade glioma (WHO grade III + IV)