# Fluorescence Guided Resection and Photodynamic Therapy in Meningiomas

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**Abstract:** Meningiomas represent 30% of all intracranial neoplasms. They are predominantly slow growing, extra axial brain tumours arising from arachnoidal cells and usually have an excellent prognosis if completely removed. However, radical tumour removal near vital functional structures and areas of infiltration is not always possible and histologically "benign" meningiomas may exhibit aggressive behaviour like infiltration and early recurrence. Biological markers to make areas of infiltration visible by fluorescence might therefore have a significant impact on patient survival and quality of life.

5-Aminolevulinic acid (5-ALA) is a precursor in the cellular heme biosynthesis. The application of exogenous 5-ALA leads to an intracellular accumulation of protoporphyrin IX (PpIX), causing PpIX saturated cells to become fluorescent and photosensitive under light of an appropriate wavelength. 5-ALA induced PpIX fluorescence has the ability to define infiltration zones into dural structures and bone and mark residual meningioma tissue. It has the potential to facilitate meningioma resection and to individualize the extent of dural resection for each patient.

Specific intracellular accumulation of PpIX can be used for photodynamic therapy (PDT). Induction of selective apoptosis, reduction of tumour vessel density and no risk of secondary carcinogenesis make PDT an ideal treatment option for meningiomas. Due to the variable potential for PpIX accumulation within different meningioma subtypes, further research is required to ensure sufficiently intense fluorescence to enable PDT in these cases.

**Keywords:** Meningioma, photodynamic therapy, 5-aminolevulinic acid, fluorescence, resection, intraoperative imaging, ferrochelatase, tumour heterogeneity, dural tail, bone invasion.

## INTRODUCTION

Meningiomas are predominantly slow growing, extra axial brain tumours arising from arachnoidal cells. They have a female predominance of middle to late adult life and make up nearly 30% of all intracranial neoplasms [1]. Ninety percent of these lesions are of WHO grade I and have an excellent prognosis once the tumour is completely removed [2].

The importance of radical resection on the outcome of patients with meningiomas, regardless of histological grade, is well established. Non-radical resections show a 4.2 fold higher mortality during the second to the 15$^{th}$ postoperative year as compared to patients in whom the tumour was completely removed [3]. However, complete tumour removal is often not achieved since infiltration into bone and dura is hard to judge intraoperatively and complex topographical arrangements of vital functional structures frequently make surgery challenging [4]. Accordingly, recurrent tumour is sometimes found at multiple sites, indicating tumour remnants left behind during a previous resection [5].

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Not only atypical and anaplastic meningiomas, but also "benign" grade I meningiomas may present with an aggressive behaviour such as penetration of the arachnoid membrane, destruction of bone and regrowth of residual tumour. This specific aggressive "subtype" within grade I meningiomas is indistinguishable *in vivo* from the more benign grade I meningioma [6], and the infiltrating portion is easily missed in all types as demonstrated by the up to 20% recurrence rate of these tumours.

The extent of resection according to the Simpson grading system [2] is currently the standard by which surgical quality is established and continues to be used as a basis for clinical outcome. Small tumour remnants may be impossible to identify on MRI scans, and dural infiltration, bone invasion and arachnoid border destruction can be missed even with intraoperative imaging and navigation techniques. Biological markers that would make these areas of infiltration visible by fluorescence might therefore have a significant impact on patient survival and quality of life.

## PHOTODYNAMIC DIAGNOSIS (PDD) IN MENINGIOMAS

Several case reports, as well as small illustrative series, where fluorescence was used for resection guidance in meningioma patients have been published.

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The reports were encouraging: a strong, sometimes homogenous fluorescence marked the tumour in most cases, regardless of histopathological subtype, tumour grading or proliferation indices [7].

Visualizing the main tumour bulk in meningiomas can usually be achieved macroscopically, or with the use of an operating microscope, without adjuvant visualisation tools such as fluorescence. Without a pressing need to more specifically mark neoplastic tissue, fluorescence guided resection in meningiomas was hardly ever published. Using 20mg/kg body weight of 5-aminolevulinic acid (5-ALA) 4 hours prior to surgery to induce PpIX accumulation in their meningioma patients, Kajimoto, Morofuji and others demonstrated positive fluorescence not only within the main tumour bulk, but also within areas of meningeal tumour infiltration and areas of bone invasion, regardless of tumour grading [7, 8]. In accordance with the well known finding that dura infiltration shown in enhanced MRI and CT scans mostly represents venous congestion [9], they could elegantly demonstrate that within the areas of "hypertrophic dura" only limited fluorescence is encountered and that these regions of fluorescence could be clearly distinguished from non-tumoral hypertrophic dura [7].

More interesting still was the fact that corresponding areas of tumour invasion into bony structures of the skull showed positive fluorescence, visible through a specially fitted operating microscope. Although in most cases of meningiomas hyperostosis is due to tumour infiltration [10], invasion of meningioma cells into bony structures has also been demonstrated in patients without radiographically identified hyperostosis [10]. The extent of bone involvement in meningioma patients is therefore impossible to delineate reliably with neuronavigation or intraoperative imaging. Moreover, intraoperative pathological diagnosis cannot be accurately performed since bone specimens need demineralization prior to histological examination. Bone invasion, however, directly affects outcome in meningioma patients [11]: an accurate visualization of tumour infiltration within these hyperostotic areas would therefore facilitate complete resection and avoid unnecessary openings of nasal sinuses and pneumatised bone at the skull base and thus directly improve outcome. Tumour specific biomarkers, such as 5-ALA induced PpIX fluorescence, could therefore provide a new visualisation modality, as shown by preliminary data and quantitative fluorescence analysis of bone invasion within selected patients [8, 7].

## 5-AMINOLEVULINIC ACID

5-Aminolevulinic acid (5-ALA) is a precursor in the cellular heme biosynthesis, regulated by a negative feedback mechanism through the control of ALA synthetase by free heme. The negative feedback mechanism can be overcome by providing the cells with an excess amount of exogenous 5-ALA, resulting in intracellular accumulation of fluorescent porphyrins, mainly PpIX, which causes the cells to become fluorescent and photosensitive.

Cellular uptake of 5-ALA, increased PpIX synthesis, reduced PpIX conversion into heme and low availability of iron are probably the most relevant factors affecting the potential amount of PpIX accumulation within a cell. Available data show that even though all these factors play a role in the heme synthesis in meningioma cells, the highest impact on PpIX accumulation stems from the lowered conversion of PpIX into heme by the reduced activity of the enzyme ferrochelatase (FCH) [12].

## INTRATUMORAL HETEROGENEITY AND FLUORESCENCE INTENSITY IN MENINGIOMAS AFTER 5-ALA PRE-TREATMENT

Differences in fluorescence intensity after 5-ALA application cannot only be seen between different meningiomas [13], but also on an intra-tumoral level [14]. Even histologically confirmed tumour tissue can show no visible level of fluorescence, but still accumulate significant amounts of PpIX to allow for a distinction from the surrounding normal tissue when a more sensitive method than the human eye is used for detection [13]. This phenomenon has already been extensively described in high grade gliomas, where it is mostly due to infiltration, necrosis and areas of lower malignancy [15]. The exact mechanisms for the variable PpIX accumulation between meningioma cells within the same tumour are, however, not yet understood.

Differences in FCH activity and synthesis were shown to be the main reason for varying concentrations of 5-ALA induced PpIX accumulation between two different benign meningioma cells lines [12]. Yet, there may be multiple other reasons for these differences outside of the actual tumour biology, including tumour vascularity and hemodynamics.

In terms of tumour biology, meningiomas are genetically heterogeneous entities that display different patterns of chromosomal changes, with the presence of

more than one tumour cell clone in almost half of the cases [16]. These molecular changes may precede histopathological changes and therefore have clinical implications in as far as removal of the more malignant areas of a heterogeneous tumour has significant impact on patient survival [17].

The question whether heterogeneity in fluorescence intensity and molecular heterogeneity correlate to some degree is crucial in defining the usefulness of 5-ALA induced fluorescence for resection control in meningiomas [18]. For, if 5-ALA induced PpIX fluorescence would not only show infiltration reliably (Figure **1**), but also with an intensity that coincides with more malignant tumour areas (with extremely low or no fluorescence in benign areas), 5-ALA induced fluorescence would be a promising tool for resection guidance, indeed.

## PHOTODYNAMIC THERAPY (PDT) IN MENINGIOMAS

Although Simpson Grade I and II resections can be achieved with microsurgical techniques in deep seated skull base lesions, sometimes complemented by endoscopy and wide bone removal, extensive surgery in these areas often comes at a price of new neurological deficits. Eloquent anatomical structures, the brainstem, cranial nerves (to which the tumour is often adherent), blood vessels and dural sinuses are regularly compromised [4]. Alternative strategies are therefore used to achieve local tumour control after intentional subtotal resection, mostly in the form of radiation therapy either as stereotactic radiosurgery or using radionucleotides [19]. While generally yielding satisfactory to good results, high-energy irradiation has the potential of causing extensive DNA damage within the targeted cells, triggering mutations and carcinogenesis. As meningioma patients have a reasonable 10-15 year survival rate, this potential may eventually lead to tumour induction in itself.

Photodynamic therapy (PDT) consists of the administration and tumour uptake of a photosensitizer, followed by irradiation with light of an appropriate wavelength. PDT has the potential to produce selective necrosis and apoptosis of tumour cells without causing damage to the surrounding normal tissue [20]. It thus offers a very selective treatment modality, and consequently seems ideal for brain tumours that often infiltrate normal brain and /or are seated in close vicinity to eloquent areas. As photosensitizers tend not to accumulate in cell nuclei, but at the inner mitochondrial wall in the case of 5-ALA mediated PpIX

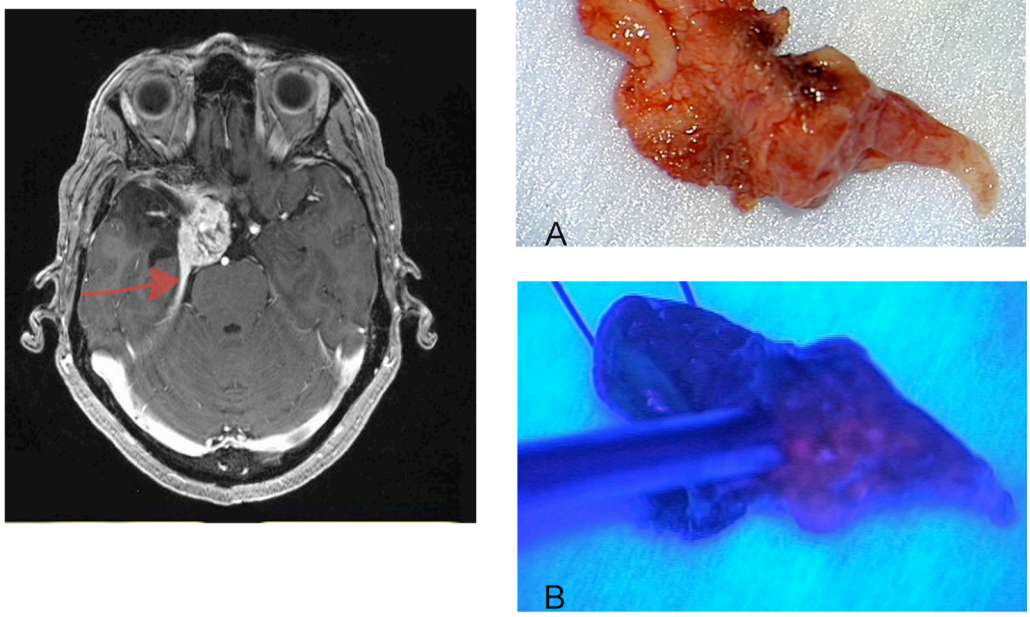


**Figure 1:** Axial T1 sequence with Gadolinium enhancement of a medial sphenoid meningioma infiltrating the cavernous sinus and the tentorial fold. The red arrow shows the area resected as "dural tail" according to intraoperative navigation. Dural tail under withe light (**A**) and illuminated with blue light (**B**) (the patient was pre-treated with 20mg/kg ALA (Gliolan©) 4h previous to surgery). The lesser part of the dural tail shows active fluorescence. Fluorescent tissue could be histopathologically correlated to tumor infiltration, whereas non-fluorescent tissue showed no tumour infiltration.

accumulation, PDT has a low potential for causing DNA damage, chromosomal mutations or carcinogenesis. Experimental and clinical studies demonstrated that PDT complements the existing traditional tumour therapies like surgical resection, radiation therapy and chemotherapy [21]. The effect of PDT is not only related to apoptosis and/or necrosis, but also to tumour vessel damage leading to secondary cell death and consequently a complete resistance to PDT has yet to be encountered [22]. PDT could reduce the necessity for aggressive and potential hazardous surgery in the vicinity of vital structures and carries no risk of secondary carcinogenesis in a disease with a relevant life expectancy. It does not interfere with established therapies and targets tumour vascular structures. These characteristics would make meningiomas an ideal candidate for PDT.

Early preclinical studies on PDT in brain tumours begun in 1972 with glioma cell cultures [23]; subsequent clinical studies on PDT in brain tumours were published in growing numbers over the last three decades. Clinical, as well as experimental studies, almost exclusively involve malignant gliomas and their recurrences with the odd exception of solitary anaplastic meningioma cases published as illustrative reports [24, 25].

Although experimental studies demonstrated PDT efficacy on benign as well as on malignant primary meningioma cell cultures [26, 27], enthusiasm for PDT in a less “dismal” brain tumour than high grade gliomas remained low.

This is partly due to the specific side effects of PDT, like increased intracranial pressure, oedema and prolonged skin photosensitization. These side effects depend on the type of photosensitizer, its concentration, the duration of light exposure and the light intensity used (e.g.: heamatoporphyrin derivates (HPD), meta-tetrahydroxyphenylchlorin (mTHPC, Foscan)) [28].

5-ALA has the advantage of an improved ‘‘tumour to normal cell’’ uptake ratio and a skin photosensitization duration of only a few hours, as compared to other exogenous porphyrins. This translates into less or no damage to surrounding structures and significantly less brain oedema [29], making it an ideally suited photosensitizer for intracranial lesions.

Insufficient 5-ALA induced PpIX accumulation in major tissues of mesoderm origin was demonstrated in 1992 [30]. Meningiomas are neoplasms derived from the arachnoidal cap cells and thus considered by most of mesoderm origin. Consistent with these findings, later *in vitro* studies showed inadequate concentrations of PpIX in meningioma cells after pre-treatment with 5-ALA in various concentrations [31]. To make matters even worse, PpIX is less phototoxic than the exogenous HPD or mTHPC, resulting in lower photodynamic effects at comparable concentrations and light doses [32]. Low PpIX concentrations and low phototoxic potential of PpIX made susceptibility to PDT in 5-ALA pre-treated meningioma cells marginal. Meningiomas were therefore not considered a suitable target for 5-ALA based PDT [31].

However, reports of consistent and frequent PpIX induced fluorescence in meningiomas after 5-ALA application encouraged further research on PDT in these tumours. It was found that the inadequate PpIX synthesis after 5-ALA pre-treatment of HBL-52 meningioma cells was related to a high ferrochelatase (FCH) activity. In comparison, PpIX synthesis was abundant in a benign meningioma (BEN-MEN-1) of low FCH activity. HBL-52 cells were irresponsive to PDT, whereas over 95% of the BEN-MEN-1 cells showed loss of vitality at light doses as low as $2J/cm^2$ [12]. Our findings show that FCH activity plays a crucial part in the accumulation of PpIX in meningioma cells, and that when the FCH activity is low, PDT for meningioma is feasible even under physiological conditions. This data further implies that FCH inhibiting substances could ensure sufficiently intense fluorescence after 5-ALA administration to allow local PDT of tumour remnants too risky to remove surgically, even in meningiomas normally not prone to PpIX accumulation.

Skull base meningiomas infiltrating the cavernous sinus, the orbit, the tentorial fold, Dorello's canal or other anatomical regions where neurological functional integrity is at risk, could be treated with PDT without the hazard of future dedifferentiation or carcinogenesis. 5-ALA based PDT could be a useful adjunct in the treatment of meningiomas, but as with PDD, the question whether heterogeneity in fluorescence intensity and molecular heterogeneity correlate to some degree will be the key to define the usefulness of PDT in these challenging tumours.

## CONCLUSION

Meningioma specific biomarkers that fluoresce have the ability to define infiltration zones into dural structures and bone and mark residual tumour tissue

during surgical procedures. Specific biomarkers have the potential to facilitate meningioma resection; they can individualize the extent of dural resection for each patient and may thus prevent morbidity. Tumour specific PpIX accumulation induced by 5-ALA seems the most promising candidate, to date, not only to mark meningioma cells reliably, but to be used for meningioma specific therapy during the same procedure. PpIX based PDT does not cause significant DNA damage, chromosomal mutations or carcinogenesis; it's a therapy that complements traditional tumour therapies and shows efficacy not only related to apoptosis and/or necrosis, but PpIX PDT also damages tumour vasculature. If sufficient PpIX is present, no complete resistance to PDT has been found in meningiomas, so far. In terms of tumour biology, meningiomas are genetically heterogeneous entities that display different patterns of chromosomal changes. These changes have clinical implications in as far as removal of the more malignant areas of a heterogeneous tumour has significant impact on patient survival. Intratumoural and intertumoural heterogeneity of fluorescence intensity after 5-ALA application has been found in meningiomas. The question whether heterogeneity in fluorescence intensity and molecular heterogeneity correlate to some degree will be crucial in defining the therapeutic impact of 5-ALA induced PpIX accumulation in meningiomas.

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