Australian Government
Department of Health
Therapeutic Goods Administration

AusPAR Attachment 2

Extract from the Clinical Evaluation Report for Aminolevulinic acid HCl

Proprietary Product Name: Gliolan

Sponsor: Specialised Therapeutics Australia Pty Ltd

Date of CER: December 2012–February 2013

TGA Health Safety Regulation

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About the Extract from the Clinical Evaluation Report

- This document provides a more detailed evaluation of the clinical findings, extracted from the Clinical Evaluation Report (CER) prepared by the TGA. This extract does not include sections from the CER regarding product documentation or post market activities.
- The words [Information redacted], where they appear in this document, indicate that confidential information has been deleted.
- For the most recent Product Information (PI), please refer to the TGA website <http://www.tga.gov.au/hp/information-medicines-pi.htm>.

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List of abbreviations

Abbreviation	Meaning
AA	Anaplastic astrocytoma
5-ALA	5-Aminolevulinic acid hydrochloride
ALAD	Aminolevulinic acid dehydratase
ALT (GPT)	Alanine transaminase
AP	Alkaline phosphatase
ARE	Amount of drug to be renally excreted
ASCO	American Society of Clinical Oncology
AST (GOT)	Aspartate transaminase
AO	Anaplastic oligodendroglioma
a.u.	Arbitrary units
AUC	Area under the curve
CI	Confidence interval
ClCr	Creatinine clearance
CNS	Central nervous system
CRF	Case report form
CT	Computed tomography
ECOG	Eastern Cooperative Oncology Group
FL (-group)	Fluorescence light group (arm A)
GABA	Gamma-aminobutyric acid
GBM	Glioblastoma multiforme
GCP	Good clinical practice
GI	Gastrointestinal
h	Hour
HPLC	High performance liquid chromatography

Abbreviation	Meaning
IEC	Independent Ethics Committee
IM	Intramuscularly
IRB	Institutional review board
ITT	Intention-to-treat
IU	International unit
IV	Intravenously
KPS	Karnofsky Performance Scale
MED	Minimal Erythema Dose
Min	Minute
MR	Magnetic resonance
MRI	Magnetic resonance imaging
MRT	Magnetic resonance tomography
ND	Not done
NIH-SS	National Institute of Health stroke score
NOS	Not otherwise specified
NR	Not reported
NS	Not statistically significant
PBG	Porphobilinogen
PDD	Photodynamic diagnosis
PDT	Photodynamic therapy
PO	Per os
PPIX	Protoporphyrine IX
Pts.	Patients
RPM	Risk Management Plan
SAE	Serious adverse event

Abbreviation	Meaning
sAP	Serum alkaline phosphatase
SEER	Surveillance, Epidemiology, and End Results database from the NCI
SD	Standard deviation
vs	versus
WHO	World Health Organisation
WL (-group)	White light group (control group, arm B)

1. Introduction

Aminolevulinic acid (5-aminolevulinic acid HCl; 5-ALA or ALA) is a naturally occurring, endogenous substance, which belongs to the group of sensitizers used in photodynamic/radiation therapy. It has been previously developed and approved for local (topical) treatment of some kinds of skin cancer and pre-cancerous conditions. It is a pro-drug that is metabolised intracellularly to form the fluorescent molecule, protoporphyrin (PPIX). The exogenous application of 5-ALA leads to a highly selective accumulation of PPIX in tumour cells and epithelial tissues. Following excitation with blue light (λ= 400 to 410 nm), the PPIX, which has accumulated selectively in the malignant tissue emits a red-violet light.

The proposed indication reads

> *'Gliolan is indicated in adult patients for visualisation of malignant tissue during surgery for malignant gliomas that are glioblastoma multiforme (GBM), and intended for gross macroscopic resection of all visible tumour.'*[1]

The proposed Product Information states "Gliolan should only be used by experienced neurosurgeons conversant with surgery of malignant gliomas and in-depth knowledge of functional brain anatomy who have completed a training course in fluorescence-guided surgery.

The recommended dose is 20 mg aminolevulinic acid hydrochloride per kilogram body weight. The solution should be administered orally three hours (range 2 to 4 hours) before anaesthesia. Use of ALA under conditions other than the ones used in the clinical trials entail an undetermined risk.

In the absence of compatibility studies, Gliolan must not be mixed with other medicinal products.

The oral solution is prepared by dissolving the amount of powder of one vial in 50 ml of drinking water. The reconstituted solution is a clear and colourless to slightly yellowish fluid.

Gliolan is for single use only and any content remaining after first use must be discarded.

2. Clinical rationale

Outline of condition to be treated: Gliomas comprise a heterogeneous group of neoplasms that differ in location within the central nervous system (CNS). In adults, the most frequently encountered of these are high-grade or malignant neoplasms of astrocytic and oligodendrocytic lineage, such as anaplastic astrocytoma (AA), anaplastic oligodendroglioma (AO), and glioblastoma multiforme (GBM), respectively.

GBM is by far the most common and most malignant of the glial tumours, accounting for approximately 12-15% of all intracranial neoplasms and 50-60% of all astrocytic tumours. In most European and North American countries, incidence is approximately 2-3 new cases per 100,000 people per year. GBMs primarily affect adults, and they are located preferentially in the cerebral hemispheres. Much less commonly, GBMs can affect the brain stem in children and the spinal cord. Like other brain tumours, they produce symptoms by a combination of focal neurological deficits from compression and infiltration of the surrounding brain, vascular compromise, and raised intracranial pressure.

[1] This indication was the initial proposed indication. Prior to registration the proposed indication was changed to *'Gliolan is indicated in adult patients for visualisation of malignant tissue during surgery for malignant gliomas that are glioblastoma multiforme (GBM) on preoperative imaging, and who are intended for resection of the tumour'*

Though the prognosis of GBM is uniformly poor, treating patients in an attempt to improve the quality of life is worthwhile. Available treatment options are surgery, radiotherapy, and chemotherapy.

Surgery: The goal of surgery is to remove as much of the tumour as possible without damaging the neighbouring healthy brain tissue. Removal is often complicated by the nature of the tumour (especially if the tumour is invasive or highly vascularised) and by its location. Sometimes only partial removal (debulking) of the tumour is possible; nevertheless, debulking can improve a patient's quality of life by alleviating symptoms and possibly improving the chances for other treatments, such as radiation therapy or chemotherapy, to be effective.

Whether gross total versus subtotal tumour resection prolongs survival is debatable. Several studies have found a beneficial effect of the removal of all enhancing tumour on patient survival and/or progression-free intervals in patients with high-grade gliomas, whereas some other studies and a meta-analysis were not able to confirm these findings.

Although gross total tumour resection might be associated with prolongation of the survival time of patients with GBM, it has to be kept in mind that the risk of postoperative neurological deficits may increase with radical tumour resection, especially if the tumour is located adjacent to so-called “eloquent” areas. "Eloquent" refers to those areas, which control speech, motor functions, and senses. Therefore, the ability to achieve a complete resection must always be weighed against the potential for causing an important neurologic deficit.

Radiation: Radiation is used when the entire primary tumour cannot be surgically removed. Moreover, most malignant brain tumours are treated with external-beam radiation even if the entire primary tumour has been surgically removed, because hidden tumour cells often remain in the brain tissue. The survival rate for patients with anaplastic astrocytoma and glioblastoma multiforme, more than doubles with adjuvant radiation therapy, and it can prolong life for patients with low-grade gliomas as well.

Chemotherapy: A meta-analyses of data from 12 randomised trials and 3004 patients showed a significant prolongation of survival associated with chemotherapy, with a hazard ratio of 0.85 (95% CI 0.78-0.91, P = 0.00004) or 15% relative decrease in the risk of death. A randomised study of radiation therapy versus radiation therapy plus temozolomide followed by 6 months of adjuvant temozolomide in 573 patients with newly diagnosed GBM demonstrated a statistically significant increase in median survival of 2.5 months in the combination-treated group (12.1 vs 14.6 months). Today, adjuvant radiochemotherapy with temozolomide is recommended as standard treatment for all patients ages 18-70 with newly diagnosed glioblastoma multiforme.

Recurrence: Most glioblastomas recur in and around the original tumour bed, probably as a result of tumour branches infiltrating the adjacent tissue that were not removed by surgery. Reoperation generally is considered in the face of a significant recurrent mass. In all other cases, palliative chemotherapy can be used. The combination of procarbazine, carmustine, and vincristine (PCV) has shown activity at first relapse in patients who have not received adjuvant chemotherapy. Temozolomide has shown activity at both first and second relapse in patients who have received prior nitrosourea-based regimen.

Prognosis: GBMs are among the most malignant human neoplasms, with a median survival despite optimal treatment of less than 1 year. In a series of 279 patients receiving aggressive radiation and chemotherapy, only 5 of 279 patients (1.8%) survived longer than 3 years. Survival rates for GBM are also available from the population-based cancer registries of 18 European countries in the EUROCARE study as well as the SEER database. Prognosis for GBM is very poor. Relative survival for adults diagnosed with GBM was, in both European and US populations, less than 30% at one year, 5% at three years, and 3% at five years, with no difference between men and women. Five-year relative survival decreased markedly with age from 13% to less than 1% from the youngest (15-45 years) to the oldest age group of patients (75 years and over).

Data from a more recent randomised phase III trial and a meta-analysis give substantially better survival rates than population-based registries, showing a two years survival rate of 13-26.5% (1). Data from clinical trials may be due in part to improvement in therapeutic options, but may also reflect survival in selected patients with more favourable prognostic factors.

Clinical rationale: The margins of a GBM tumour are difficult to define during surgery because in many cases, there is no sharp demarcation between tumour and normal tissue. This can result in unintentional removal of healthy tissue or failure to remove malignant tissue. Therefore a method that improves intraoperative visualisation of malignant tissue would be helpful. In the past, numerous attempts have been made to develop optical markers for the detection of tumours in order to improve the clinical results of cancer surgery. The substances studied (tetracycline, methylene blue, semi-synthetic porphyrins like Photofrin®) showed low sensitivity as well as an unfavourable benefit/risk ratio due to side effects.

Malignant glioma tissue (WHO-grade III and IV, e.g. glioblastoma multiforme, gliosarcoma or anaplastic astrocytoma) has also been demonstrated to synthesize and accumulate porphyrins in response to 5-ALA administration. The concentration of PPIX is significantly lower in white matter than in cortex and tumour. Tissue surrounding the tumour and normal brain may also be affected. However, 5-ALA induced PPIX formation is significantly higher in malignant tissue than in normal brain.

In contrast, in low-grade tumours (WHO-grade I and II, e.g. medulloblastoma, ligodendroglioma) no fluorescence could be observed after application of the active substance. Brain metastases revealed inconsistent or no fluorescence.

The phenomenon of PPIX accumulation in WHO grade III and IV malignant gliomas may be explained by higher 5-ALA uptake into the tumour tissue or an altered pattern of expression or activity of enzymes (e.g. ferrochelatase) involved in haemoglobin biosynthesis in tumour cells. Explanations for higher 5-ALA uptake include a disrupted blood-brain barrier, increased neo-vascularisation, and the over-expression of membrane transporters in glioma tissue.

Upon exposure to violet-blue light, PPIX becomes activated resulting in red-light fluorescence.

The purpose of the administration of 5-ALA is neither to diagnose malignant gliomas nor to test the tumour stage or WHO grading. The aim of the use of 5-ALA is to visualise malignant lesions to facilitate surgery and improve completeness of resection. More complete resection of malignant gliomas may result in statistically significant prolongation of progression-free survival.

Alternative methods to increase the extent of tumour resection: Ultrasonography (US), computed tomography (CT), and magnetic resonance imaging (MRI) have been used to allow intermittent monitoring of the progress of surgery. Image-guided neuronavigation, utilising the principle of stereotaxis, has also been used. However, none of these methods has been validated in adequate, controlled phase III trials, and each has disadvantages, including intra-operative MRI.

Comment: The rationale provided is acceptable. Australian neurosurgeons have used ALA to demarcate GBM during surgery under the Special Access Scheme, and the sponsor has provided in an Annex to the RMP, a strategy for training Australian surgeons for this proposed procedure using ALA.

2.1. Orphan drug designation

Gliolan was granted Orphan Designation for the requested indication on 30 March 2012.

2.2. Guidance

Compliance with pre-submission issues and requirements: A document was enclosed listing these issues and in each case indicated the sponsor had or would comply with the requirements before the application was finalised.

Compliance with TGA Guidelines: The relevant guideline is that of the EMEA: "GUIDELINE ON CLINICAL EVALUATION OF DIAGNOSTIC AGENTS EU"

Comment: The present application complies with these guidelines when the additional analyses of clinical outcome as requested by the EMEA are included. The application did not include a determination of sensitivity and specificity as required in the guidelines, but I have calculated these from biopsy data that I regard as Standard of Truth as defined in the Guidelines (Section 5.4, p9 of guidelines).

3. Contents of the clinical dossier

3.1. Scope of the clinical dossier

The clinical dossier documented a full clinical development program of pharmacology, efficacy and safety studies.

The submission contained the following clinical information:

Module 5:

- One clinical pharmacology study (MC-ALS. 20/BV) in normal subjects provided absolute bioavailability data, pharmacokinetic data, and the extent and duration of skin photosensitisation after oral administration of ALA. Different doses were used in the latter study.
- No population pharmacokinetic analyses were provided.
- The pivotal efficacy/safety study, MC-ALS.3/GLI, randomised 415 patients with glioma multiforme (GM), 207 of whom received a single dose of ALA prior to surgery.
- One dose-finding study, MC-ALS. 8-I/GLI, enrolled 21 patients with GM, who received single doses of 0.2, 2 or 20 mg/kg to determine dose efficacy relationships.
- Two other studies (MC-ALS. 28/GLI and MC-ALS. 30/GLI) of safety and efficacy were performed in patients with GM. One study (MC-ALS. 32/GLI) assessed safety only.
- A PSUR from 8.3.2008 to 7.9.2008 was submitted, and a Australian Specific Annex to the EU Risk Management Plan (Module 1, 13.1).

Module 1

- Application letter, table of contents, , application form draft Australian PI and CMI, , expert's CV, drug master file certificate, GMP certificate, Declaration of Compliance with Pre-Submission Planning Form, Statement on Providing Individual Patient Data, Overseas Regulatory Status, Summary of Bioequivalence of Bioavailability Study (TGA form), Statement on use in paediatric patients (see next section), Pharmacovigilance, and CHMP Assessment Report and related issues, both non-clinical and clinical.

Module 2

- Introduction (2.2), Quality of drug (2.3), Non-Clinical Overview (2.4), Clinical Overview (2.5), Non-Clinical Summary (2.6), Clinical Summary (2.7), and Table of Contents.

3.2. Paediatric data

The submission did not include paediatric data. Justification was provided in Module 1.12, based on the Orphan Drug status of the product, and therefore on the difficulty in studying the drug in an even smaller population of children compared to an adult patient population.

Comment: The justification provided used Australian Institute of Health and Welfare data including the incidence, mortality and prevalence of brain cancers in Australia. The justification is acceptable.

3.3. Good clinical practice

Independent Ethics Committee (IEC) or Institutional Review Board (IRB): The protocol and the three amendments for this study were reviewed and approved by an appropriately constituted Independent Ethics Committee (IECs) of the Ludwig-Maximilian University Munich before start of the study.

Ethical conduct of the study: The study was conducted in accordance with ethical principles of the Declaration of Helsinki (Somerset West, South Africa 1996) and in compliance with all applicable local regulations and the International Conference on Harmonization (ICH), Good Clinical Practice (GCP) guidelines.

Patient information and consent: Before randomization, the participating investigators informed each patient about the nature of the study, its purpose, procedures, expected duration, and possible benefits and risks of participation. Before entering the study, patients were asked to sign a data clarification and an informed consent statement approved by the Independent Ethics Committee. The signed informed consent and data clarification forms were to remain in the files of the investigator.

4. Pharmacokinetics

4.1. Studies providing pharmacokinetic data

Table 1 shows the studies relating to each pharmacokinetic topic.

Table 1. Submitted pharmacokinetic studies

PK topic	Subtopic	Study ID
PK in healthy adults	General PK Single dose	MC-ALS. 20/BV
	Multi-dose	
	Bioequivalence† Single dose	MC-ALS. 20/BV
	Multi-dose	
	Food effect	
PK in special populations	Target population§ Single dose	
	Multi-dose	MC-ALS. 8-I/GLI

PK topic	Subtopic	Study ID
	Hepatic impairment	
	Renal impairment	
	Neonates/infants/children/ adolescents	
	Elderly	
Genetic/gender-related PK	Males vs. females	
Population PK analyses	Healthy subjects	

* Indicates the primary aim of the study. † Bioequivalence of different formulations. § Subjects who would be eligible to receive the drug if approved for the proposed indication.

None of the pharmacokinetic studies had deficiencies that excluded their results from consideration.

4.2. Summary of pharmacokinetics

The information in the following summary is derived from conventional pharmacokinetic studies unless otherwise stated.

4.2.1. Pharmacokinetics in healthy subjects

4.2.1.1. Bioavailability

Study MC-ALS.20/BV evaluated the absolute bioavailability (BA) of oral administration of ALA and also determined some PK parameters of the drug. The study included one oral treatment and one IV treatment during two treatment periods separated by 3days, and was not blinded.

The first subgroup of 12 subjects was investigated for absolute bioavailability and the second subgroup (the original 12 plus 9 additional subjects) for PK parameters. Both groups received an oral dose of ALA (20 mg/kg b.w.), administered on an empty stomach. For the IV administration, a 10-fold lower dose was used (2 mg/kg b.w.) because of limited experience with the IV use of ALA at that time.

For BA calculation, the primary target parameter $AUC_{0-\infty}$ as well as the amount excreted in the urine (Ae,ur) during 12 h following oral administration of ALA were compared with the corresponding parameters following intravenous (IV) administration of ALA to each patient to give the ratio, oral vs IV, after dose adjustment.

Additional PK/PD investigations during the same study on healthy subjects: The PK parameters determined were: Cmax, tmax, t½λz. A secondary objective of the study was to evaluate the duration of photosensitization of the skin by observing the Minimal Erythema Dose (MED) and corresponding PP-IX plasma concentrations after oral treatment.

4.2.1.1.1. Absorption and absolute bioavailability

Absorption from the gastro-intestinal tract was rapid with a tmax value of 0.76h after oral administration of the 20mg dose. Absolute BA was 100.02% as calculated from the AUC-comparison and 104.79% as calculated from renal excretion data. Plasma levels returned to baseline values 24 hours after administration of an oral dose of 20 mg/kg body weight.

Comment: The bioavailability of 100% in normal subjects is greater than that found in animal studies (86%) in this application and also greater than that in both normal subjects and patients with superficial bladder cancer. The reason for the difference is unknown. The Clinical Overview suggests that the reduced bioavailability calculated in the reported trial might be due to differences in the quality or formulation of the 5-ALA preparation used, the different administration schedule, and different analytical technique.

4.2.1.1.2. Influence of food

The influence of food was not studied because ALA is always given before general anaesthesia to fasting patients.

4.2.1.1.3. Dose proportionality

Dose proportionality was not studied in this trial.

4.2.1.2. Distribution

4.2.1.2.1. Volume of distribution

The volume of distribution was not studied. The following information was obtained from animal studies.

4.2.1.2.2. Plasma protein binding

From non-clinical data, the mean protein binding of 5-ALA was 12% in the concentration range of 500 – 5,000 µg/L. There was no influence of the plasma concentration on protein binding.

4.2.1.2.3. Tissue distribution: blood-brain barrier

5-ALA is preferentially taken up by the liver, kidney, endothelial cells and skin as well as by malignant gliomas (WHO grade III and IV) and metabolised to fluorescent PPIX.

Non-clinical studies have shown that that orally administered ALA is able to penetrate into brain tumour tissue in sufficient amounts to form intracellularly enough PPIX that is necessary to visualise the tumour. Penetration into normal brain tissue is much less. The better penetration of ALA into brain tumour tissue compared to normal brain cells might be due to a disturbance of the blood-brain barrier of the tumour and/or increased brain tumour vascularisation. Gliomas are characterised by very high levels of neo-vascularisation.

4.2.1.3. Metabolism

4.2.1.3.1. Sites of metabolism and mechanisms / enzyme systems involved

As described in the clinical overview, aminolevulinic acid dehydratase (ALAD) condenses 2 molecules of 5-ALA to form monopyrrole porphobilinogen (PBG). A series of biochemical reactions synthesize protoporphyrin IX (PPIX), into which ferrous iron is inserted to form heme. PPIX is the last step before incorporation of ferrous iron.

4.2.1.3.2. Non-renal clearance

A comparison of the AUC values of 5-ALA and PPIX showed that less than 6% of systemically available 5-ALA was metabolised to PPIX.

4.2.1.3.3. Metabolites identified in humans

· Active metabolites

The metabolite, PPIX was measured in this trial, because it is responsible for the fluorescence used to help define the tumour margins. Other metabolites of ALA were not referred to in the clinical section of this application

· Pharmacokinetics of PPIX

PPIX is responsible for the fluorescence occurring in tumour tissue after treatment with 5-ALA*HCl. Maximum plasma levels of PPIX were reached at 4h after administration. In the next 20 hours, PPIX plasma levels rapidly declined and at 48 hours they were no longer detectable.

The PK parameters showed the tmax to be 4h, and the t1/2, 3.6h.

4.2.1.4. Excretion

Approximately 30% of an orally administered dose of 20 mg/kg ALA was excreted unchanged in the urine of normal subjects within 12 hours. The terminal half lives of ALA were 0.71 h and 0.88 h after the IV and oral doses respectively. The Cl_{ren} value for the 20mg oral dose and the 2mg IV dose of ALA were 8.06 and 16.01L/h, respectively.

Comment: The amount of ALA excreted unchanged in the urine was consistent with the published results of 25%. The difference in the renal clearance between the IV and the oral dose was explained by the saturation of renal excretion at lower plasma concentrations of ALA and slower urine flow.

The terminal half life of less than 1 hour in normal subjects is less than that of 3h found for patients with GM. The terminal half-life of PPIX in normal subjects, 3.6h, was similar to that in patients, 2.6h. The Clinical Overview comments "The difference in half-life between dose levels 1, 2 and 3 (approx. 1 hour versus 3 hours…) observed in study MC-ALS.8-I/GLI cannot be satisfactorily explained, because half-life in healthy subjects after 20 mg/kg (study MC-ALS.20/BV) was also 1 hour only."

4.2.2. Pharmacokinetics in the target population

Patients with GM in Study MC-ALS. 8-1 /GLI were administered ALA orally at three doses, 0.2mg/kg, 2.0mg/kg and 20mg/kg. The inclusion and exclusion criteria are shown below:

Inclusion criteria

- Radiological suspicion of a malignant glioma with distinct ring- or garland-shaped, contrast agent-enhancing tumour structures and a core area of reduced intensity in the MRI (tumour necrosis)
- Indication for surgical tumour resection
- First operation of the tumour, no other tumour-specific pretreatment
- Karnofsky ≥70 %
- Patient's written informed consent
- Age 18-75 years

Exclusion criteria

- Porphyria, hypersensitivity to porphyrins
- Renal insufficiency: Creatinine > 2.0 mg/dl
- Hepatic insufficiency: Bilirubin > 3 mg/dl, Quick test < 60 %, GT > 100 U/I
- Other malignant growths (except basaliomas)
- Women:

  Existing/planned pregnancy (to be checked by a pregnancy test if of child-bearing age)/lactation or inadequate contraception (hormone cycle regulation "pill" or condom)
- Men:

Inadequate contraception (condom)

- Dementia or mental condition making it impossible to understand the therapy and therefore prohibiting written consent
- Simultaneous participation or participation in another clinical trial in the preceding 30 days

4.2.2.1. Absorption

ALA was rapidly absorbed after oral administration with geometric mean values for tmax of 0.50 h after an oral dose of 0.2 mg/kg b.w., 0.61 h after an oral dose of 2 mg/kg b.w., and 0.94 h after an oral dose of 20 mg/kg b.w., respectively.

Comment: The tmax value for the 20mg/kg dose was similar to that of 0.76h for normal patients and indicated rapid absorption.

4.2.2.2. Plasma concentrations

The Cmax values after an IV dose of 2.0mg/kg and an oral dose of 20mg/kg were 6.77 and 20.76mg/L. The PK parameters for ALA are shown in Table 2.

Table 2. PK parameters of ALA at varying oral doses in patients with GBM

Parameter		Dose level of 5-ALA*HCl		
		0.2 mg/kg	2 mg/kg	20 mg/kg
Number of patients:		7	7	7
$t_{1/2}$ [h]	Geo Mean (DF)	0.85 (1.71)	1.12 (2.00)	3.05 (2.09)
	Median (range)	0.75 (0.51 – 2.38)	0.84 (0.45 – 2.44)	1.94 (1.60– 10.04)
t_{max} [h]	Geo Mean (DF)	0.50 (1.75)	0.61 (1.77)	0.94 (1.51)
	Median (range)	0.50 (0.23 – 1.00)	0.50 (0.25 – 1.47)	1.00 (0.52 – 2.00)
c_{max} [mg/L]	Geo Mean (DF)	256.9 (1.20)	2,103.6 (1.57)	8,272.2 (1.11)
	Median (range)	274.7 (196.2 – 310.8)	1,862.6 (987.3 – 3,757.9)	8,239.1 (7,416.8 – 9,700.16)
AUC_{inf} [mg x h/L]	Geo Mean (DF)	539.9 (1.98)	3,326.0 (1.60)	26,914.9 (1.19)
	Median (range)	424.2 (246.8 – 1,779.7)	2,901.3 (1,602.2 – 5,880.1)	27,143.5 (20,413.0 – 34,625.8)
Geo Mean = geometric mean; DF = dispersion factors				

Comment: Although the Cmax after IV administration was proportionately lower than the 10 fold difference in dose, the AUC values in each case did have a ratio of 10. In patients, the Cmax was also lower after an oral dose of 20mg/kg, with a Cmax value of 8.67mg/L. The difference was explained in the Clinical Overview as possibly due to glioma patients being sedated before surgery, and later anaesthetized, resulting in a difference in the distribution and elimination of 5-ALA. No evidence to support this was given.

4.2.2.3. Dose linearity and proportionality

By regression analysis, AUC values of ALA versus dose were linear over the dose range (0.2, 2, and 20 mg/kg b.w.) studied. However, for the three dose levels, AUC increased between dose level 1 and 2 only 6.8 fold, and between dose level 2 and 3, only 9.3fold (instead of 10 fold between each level). In contrast, a 10-fold ALA dose (20 mg/kg vs 2 mg/kg) resulted in only a 3-fold increase of AUC values of PPIX in plasma. The study report proposed that this indicated the metabolic capacity to produce PPIX from 5-ALA in humans is limited.

Comment: The results show a linear relationship of dose and AUC values, but not dose proportionality.

This evaluator could not find animal studies of dose proportionality for comparison. The suggestion of a saturable metabolism in the conversion of ALA to PPIX is reasonable and consistent with the data. If the results for the lowest dose of ALA of 0.2mg/kg are disregarded because of uncertainty about the low

values of the serum concentrations, the dose proportionality of the AUC values for the two other doses, 2mg/kg and 20mg/kg of 9.3-fold for a 10-fold increase respectively can be accepted as proportional. The low proportionality found for the AUC of PPIX with increasing dose of ALA was more marked with only a 3-fold increase in AUC value with a 10-fold increase in oral dose of ALA. Again this is consistent with a saturated mechanism in the formation of PPIX. The clinical significance is not great, as animal studies have found that plasma levels of PPIX do not reflect intracellular formation of this metabolite in different tissues and tumour.

4.2.2.4. Drug Elimination

ALA was eliminated quickly with terminal elimination half lives of 0.85, 1.12 and 3.05 h for doses 0.2mg/kg, 2.0mg/kg and 20.0mg/kg respectively.

Comment: The study report proposed that the lower values of the terminal elimination half life with the lower doses of ALA were because the concentration of ALA in the plasma was too low for reliable measurements in the terminal phases, so that the measured profiles did not represent the terminal elimination rate constants λz.

4.2.2.4.1. Metabolites identified in humans

· Active metabolites

The only metabolite measured was PPIX, because it is responsible for the fluorescence used to help define the tumour margins.

4.2.2.4.2. Pharmacokinetics of metabolites

The PK characteristics of PPIX were determined after 0.2mg/kg, 2.0mg/kg, and 20mg/kg oral doses of ALA in patients with GM and are shown Table 3. Values at the lowest dose could not be determined as they were below the LLQ.

Table 3. PK parameters of PPIX at varying oral doses of ALA in patients with GBM

Parameter		Dose level		
		0.2 mg/kg	2 mg/kg	20 mg/kg
Number of patients:		7	7	7
$t_{1/2}$ [h]	Geo Mean (DF)	Not calculated	2.90 (1.36)	2.61 (1.63)
	Median (range)		3.19 (1.62 – 3.83)	3.38 (1.52 – 4.08)
t_{max} [h]	Geo Mean (DF)	Not calculated	4.81 (1.37)	5.73 (1.58)
	Median (range)		4.92 (2.90 – 6.92)	5.48 (2.97 – 11.92)
c_{max} [mg/L]	Geo Mean (DF)	Not calculated	32.28 (2.28)	Not calculated
	Median (range)		27.44 (9.87 – 83.20)	101.71 (0.00-258.83)
AUC_{inf} [mg x h/L]	Geo Mean (DF)	Not calculated	255.80 (2.46)	779.90 (2.73)
	Median (range)		318.94 (54.97 – 572.60)	862.04 (247.96 – 2,655.06)

Geo Mean = geometric mean; DF = dispersion factors

Comment: As for the PK parameters of ALA, values for PPIX were different in patients from those found in normal subjects, as shown in the following table. The values of Cmax and AUC are markedly lower and unexplained.

Table 4. Comparison of PK parameters of PPIX in the two trials

Parameter		Study	
		MC-ALS.20/BV *Data from FSR table 5 / p 57*	MC-ALS.8/GLI *Data from FSR table 11.4.1.2C*
Number of patients:		12	7
$t_{1/2}$ [h]	Geo Mean (DF)	3.57 (1.82)	2.61 (1.63)
	Median (range)	4.04 (1.19 – 7.76)	3.38 (1.52 – 4.08)
t_{max} [h]	Geo Mean (DF)	n.a.	5.73 (1.58)
	Median (range)	4.00 (2.50 – 8.00)	5.48 (2.97 – 11.92)
c_{max} [µg/L]	Geo Mean (DF)	279.05 (1.36)	Not calculated
	Median (range)	259.18 (170.91 – 561.67)	101.71 (0.00 – 258.83)
AUC_{inf} [µg x h/L]	Geo Mean (DF)	1,875.66 (1.47)	779.90 (2.73)
	Median (range)	1,906.64 (970.73-3,431.63)	862.04 (247.96 – 2,655.06)

Geo Mean = geometric mean; DF = dispersion factors; Ari Mean = arithmetic means; SDEV = standard deviation

4.2.2.5. Excretion

4.2.2.5.1. Routes and mechanisms of excretion

The percentage of unchanged ALA excreted in the urine was not calculated in patients.

4.2.2.5.2. Renal clearance

The terminal half lives of ALA in patients were discussed above (See Drug Elimination section).

4.2.3. Pharmacokinetics in other special populations

These studies were not done.

4.2.4. Pharmacokinetic interactions

Pharmacokinetic interactions were not studied in this trial.

4.3. Evaluator's overall conclusions on pharmacokinetics

- The PK studies showed rapid and complete absorption of ALA after an oral dose in normal subjects and rapid absorption in patients with GM. About 6% of ALA was metabolised to PPIX, the active metabolite, which reached a maximum plasma concentration in 4h, and was not detectable at 48h. In normal subjects, renal excretion of ALA was slower at lower plasma concentration of ALA and slower urine flow rates.
- Values for the PK parameters for ALA and PPIX differed between normal subjects and patients, the latter having slower absorption, lower plasma concentrations (Cmax and AUC), and a longer half life for ALA. The half-life of PPIX was similar in both groups. The plasma concentration of PPIX did not correlate with PD effects of ALA (see next section).
- The PK studies had no important deficiencies but it is difficult to draw clinical conclusions from them. Such conclusions are better based on the PD (see next section).
- The PK information in the Proposed Product Information is correct and acceptable except that the section on dose proportionality should be expressed more cautiously as dose proportionality was only shown for two concentrations, 2.0 mg/kg and 20.0 mg/kg.

5. Pharmacodynamics

5.1. Studies providing pharmacodynamic data

The following table shows the studies relating to each pharmacodynamic topic and the location of each study summary.

Table 5. Submitted Pharmacodynamic Studies

PD Topic	Subtopic	Study ID
Primary Pharmacology	Effect on photosensitisation of the skin from the Minimal Erthyema Dose (MED) in normal subjects	M-ALS.20/BV
	Dose related effects on the efficacy of treatment with ALA as judged by the extent and quality of fluorescence in the tumour core	MC-ALS.8-I/GLI
Secondary Pharmacology	PK parameters as in PK section above	
Gender other genetic and Age-Related Differences in PD Response	Not applicable	
PD Interactions	Not done	
Population PD and PK-PD analyses	Not done	

None of the pharmacodynamic studies had deficiencies that excluded their results from consideration.

5.2. Summary of pharmacodynamics

The information in the following summary is derived from pharmacodynamic studies in humans unless otherwise stated.

5.2.1. Mechanism of action

As described previously, the PD effect of ALA depends on its conversion intracellularly to form the fluorescent molecule protoporphyrin (PPIX). The exogenous application of 5-ALA leads to a highly selective accumulation of PPIX in tumour cells and epithelial tissues. Following excitation with blue light (λ= 400-410 nm), the PPIX, which has accumulated selectively in the malignant tissue, emits a red-violet light. This phenomenon can be used to guide tumour resection.

5.2.2. Pharmacodynamic effects

5.2.2.1. Primary pharmacodynamic effects

The primary PD effect is to produce fluorescence in the relevant tissue when exposed to blue light. This primary effect was studied in patients with GM.

5.2.2.2. Secondary pharmacodynamic effects

The secondary PD effects are an immediate and a late reaction of the skin due to photosensitisation following ALA treatment. These are clearly different processes. The immediate reaction after irradiation is due to activation of the pigments in the skin shown by the development of erythema. This can be quantitated by measuring the minimal erythema dose (MED) and the concentration of PPIX at the site. The late reaction is interpreted as an bronzing effect over time following irritation, and is not related to any concentration of 5-ALA or PP-IX in the skin. These studies were carried out on normal subjects.

5.2.3. Time course of pharmacodynamic effects

Primary effects: The higher content of PPIX in tumour tissue compared to normal tissue was confirmed in study MC-ALS.8-I/GLI. After oral administration of 20 mg/kg 5-ALA*HCl to patients with malignant gliomas, fluorescence intensity (measured spectrophotometrically) in the tumour core as well as tumour margin was more than 10-fold higher than in the adjacent normal tissue. The subjective visual impression of a stronger fluorescence quality of the glioma tissue was paralleled (and therefore confirmed) by a higher spectrophotometrically measured fluorescence intensity. Fluorescence intensity was low in those areas with subjectively described missing (none) fluorescence, moderate in areas described as weak fluorescing and high in those areas described as strong fluorescing.

Since PPIX is a photolabile substance, depletion of fluorescence intensity over time of light exposure (commonly termed photobleaching) may occur during continued illumination. Stummer et al. (2) have shown that under operating light conditions, fluorescence decays to 36% in 25 minutes for violet-blue light and in 87 minutes for white light (measured in tumour tissue ex vivo). However, intraoperatively bleaching times are prolonged because not all tumour layers are exposed to light at the same time, and blood or cotton pads protect the tissue from light. Since glioma surgery usually lasts up to 5 hours, photobleaching is not significant.

Secondary effects: The MED was defined as the dose of irradiation following which a minimal skin reaction was visible in the exposed area. The skin reaction was observed by two investigators (one for the immediate reaction and another for the late reaction after 24 hours).

Comment: A higher dose of radiation (a higher value of MED) would indicate less photosensitivity of the exposed skin, and a lower value, greater photosensitivity. Greater sensitivity would be expected when the active metabolite (PPIX) of ALA reached maximum concentration after an oral dose of ALA, and less sensitivity when the PPIX concentration was low in target tissue. The maximum plasma concentration of PPIX was reached at 5.5 hours (median) after an oral dose of 20mg/kg of ALA, with a range from 3 hours to 12 hours, in patients with GM.

A caveat, however, is that the relationship of plasma concentration of PPIX to the concentration in tissue is unknown.

The MED for immediate reactions was evaluated 16 minutes after exposure and for the late reactions 24 hours after exposure. Exposures were made at baseline, and after 12, 24 and 48 hours.

The results showed that at baseline, values of MED were highest, showing lower photosensitivity. The value of the MEDs at 12 and 24 hours after administration of ALA, measured shortly after end of irradiation (immediate reaction) was significantly reduced compared to baseline ($P < 0.0001$). MED returned to baseline values at 48 hours, a time where PPIX plasma levels had already dropped below the limit of detection.

For late reactions, a decrease of MED, ie increased photosensitivity, was only observed 12 hours after administration of ALA. The study also showed that there was no correlation between PPIX plasma levels and immediate or late skin reaction to UVA-light.

5.2.4. Relationship between drug concentration and pharmacodynamic effects

See above

5.2.5. Pharmacodynamic interactions

The interactions were not studied. An interaction with light that caused photobleaching of the fluorescence was considered in Section 5.2.3 above.

5.3. Evaluator's overall conclusions on pharmacodynamics

Photosensitization of the skin in normal subjects after ALA administration: Photosensitisation of the skin as measured by the minimum dose of light (MED) needed to produce erythema occurred 12 and 24 hours after administration of ALA, and returned to baseline at 48 hrs. At this time the plasma concentrations of PPIX had dropped below the limit of detection. Late reactions were only observed 12 hours after administration of ALA.

Caveats regarding safety were that the PK parameters of both ALA and PPIX differed in normal subjects from those in patients, and photosensitisation of the skin was not measures in patients. Because the study also showed that photosensitisation of the skin did not relate to plasma levels of PPIX, this difference may not be clinically important.

Demonstration of greater fluorescence of tumor compared to normal brain tissue: After oral administration of 20 mg/kg ALA to patients with malignant gliomas, fluorescence intensity (measured spectrophotometrically) in the tumour core as well as tumour margin was more than 10 fold higher than in the adjacent normal tissue. The subjective visual impression of a stronger fluorescence quality of the glioma tissue was paralleled by a higher spectrophotometrically measured fluorescence intensity. Fluorescence intensity was low in those areas with subjectively described missing (none) fluorescence, moderate in areas described as weak fluorescing and high in those areas described as strong fluorescing. Additionally, biopsies taken from selected areas of tumour core and margin showed a significant correlation between tumour cellularity and fluorescence intensity only for the highest dose level of 20 mg/kg b.w.

6. Dosage selection for the pivotal studies

A single dose finding study was submitted (Study MC-ALS.8-I/GLI) that used 0.2mg/kg, 2.0mg/kg and 20 mg/kg of oral ALA and related these doses to the fluorescence produced in the tumour and normal brain. Statistically higher fluorescence was found at the highest dose (20mg/kg) with acceptable toxicity. This was therefore chosen as the dose to be used in further studies.

Comment: The difference between the 2.0mg/kg and the 20.0mg/kg is 10-fold so that intermediate doses may have been as effective. The Clinical Overview commented "It might be speculated that a dose of 10 mg/kg would have led to similar good results, however, since 20 mg/kg 5-ALA.HCL did not cause any substantial drug-related side effect in any of the clinical studies (which includes 548 subjects/patients treated at this dose level) this does not seem to be worth to be discussed further. Furthermore, there are some hints from the literature that 20 mg/kg better visualises malignant gliomas than 10 mg/kg 5-ALA.HCl. Doses of 5-ALA*HCl higher than 20 mg/kg are not necessary and only increase the frequency of side effects."

7. Clinical efficacy

The application contained one pivotal study (MC-ALS.3/GLI) of 415 patients (205 in the ALA arm and 208 controls), two supporting studies to determine the positive predictive value of tissue fluorescence and safety (MC-ALS.28/GLI and MC-ALS.30/GLI), and one supporting study of safety alone (MC-ALA.32/GLI).

7.1. Efficacy for the proposed indication

Proposed Indication: "*Gliolan is indicated in adult patients for visualisation of malignant tissue during surgery for malignant gliomas that are glioblastoma multiforme (GBM), and intended for gross macroscopic resection of all visible tumour*."

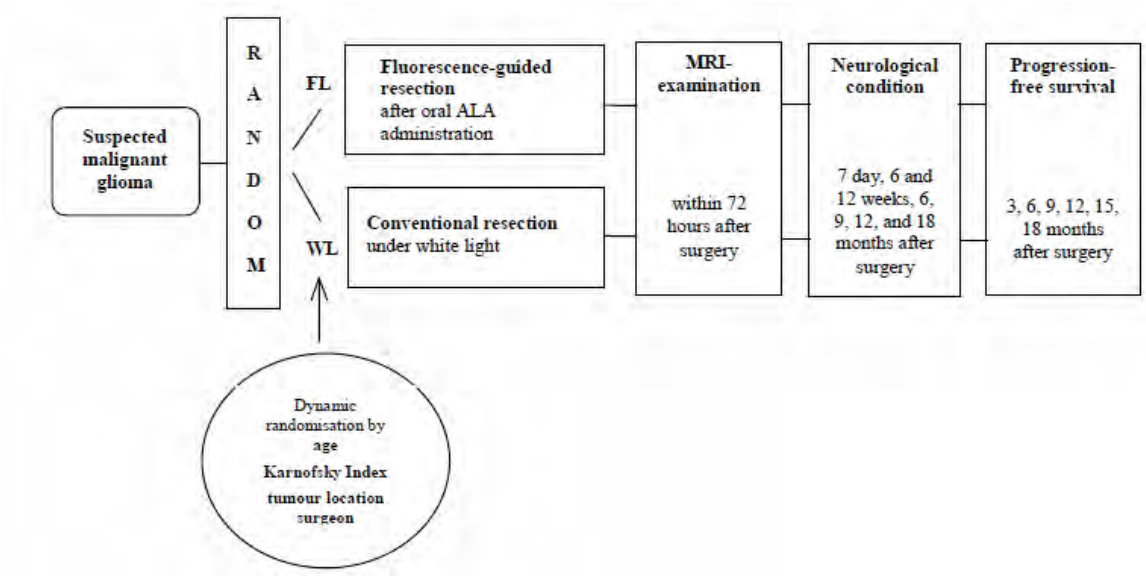
7.1.1. Pivotal efficacy study

7.1.1.1. Study MC-ALS.3/GLI

7.1.1.1.1. Study design, objectives, locations and dates

Study design: The trial was a randomised, group-sequential, blinded, balanced parallel-group, controlled multicentre phase III study to compare standard surgical resection versus fluorescence-guided resection in patients undergoing initial surgery for newly diagnosed malignant glioma. The design is shown in the following schema.

Figure 1. Schema of Pivotal Study



Objectives: The aim of this study was to determine the efficacy and safety of fluorescence-guided resection of malignant gliomas with 5-Aminolevulinic acid (5-ALA) (FL-group) compared to conventional resection (WL-group) and to assess the clinical usefulness of this method.

Locations and dates: The trial was conducted in 19 German centers from 8 October, 1999 to 19 July 2004.

7.1.1.1.2. Inclusion and exclusion criteria

Males or females aged 18 to 72 years with cranial magnetic resonance imaging (MRI) justifying diagnosis of unilocular malignant glioma (WHO grades III-IV) for whom surgical treatment was indicated were included in this study. The location of contrast enhancing tumour was to be such that should allow complete resection. Patients with tumours located in the midline, the basal ganglia, the cerebellum or brain stem and patients with more than one contrast agent-

accumulating lesion unrelated to the primary tumour or presenting with extracerebral metastases were excluded. Furthermore, patients with a Karnofsky Performance Score < 70, known porphyria or hypersensitivity to porphyrins, renal or hepatic insufficiency or other malignancies were also excluded.

7.1.1.1.3. Study treatments

Three hours (range 2-4 hours) prior to induction of anaesthesia 1.5 grams of 5-aminolevulinc hydrochloride were dissolved in 50 ml of water as ready-to-use solution and administered orally at a dose of 20 mg/kg body weight to patients randomized to the ALA arm. No further doses were administered and no control medications were administered to the reference group

7.1.1.1.4. Efficacy variables and outcomes

The **primary efficacy outcomes** were

- Percentage of patients with a histologically confirmed malignant glioma (grade III or IV – WHO) without definite residual contrast-enhancing tumour in the early post-operative control MRI (within 72 hours after surgery).
- Progression-free survival at the 6-month-visit after primary surgical treatment of a histologically confirmed malignant glioma (grade III or IV- WHO).

Comment: Note that this evaluation of PFS was as an event assessed by MRI at a fixed time of 6 months, with one month allowed before or after that time. Because of possible variation in the true assessment dates, the more usual and more accurate assessment of PFS using time-to-event Kaplan-Meier estimates was done. The second primary efficacy outcome was added to the protocol in 2002 whereas the trial had begun in 1999. At the same time dot points 2 and 3 below in Secondary efficacy outcomes were also added.

Both target criteria were to be tested in an a-priori defined hierarchical order, i.e. confirmatory testing of the second target criterion will take place when fluorescence-guided resections have been shown to be superior to the conventional resections as the first test criterion.

The **secondary efficacy outcomes** were

- Volume of residual tumour.
- Determination of progression-free survival (PFS) 9, 12, 15 and 18 months after primary surgical treatment.
- Determination of overall survival with follow up of the patients until death, but not longer than 18 months after primary surgical treatment of the last study patient.
- Toxicity after oral administration of 5-Aminolevulinic acid
- Neurological condition 7 days, 6 and 12 weeks, 6, 9, 12, and 18 months after primary surgical treatment.
- Comparison of the results of fluorescence diagnostics (intraoperative residual tumour) with those of early post-operative MRI (radiological residual tumour).

7.1.1.1.5. Randomisation and blinding methods

Randomisation: Randomisation was performed centrally by staff of the randomisation center. After patients had given their written informed consent and were found to fulfill all eligibility criteria according to the investigator, the randomisation centre was contacted to obtain patient number and treatment group. To optimise balancing of the distribution of important patient characteristics in the treatment arms a randomisation algorithm was used to stratify for the following known risk factors:

- Surgeon (1,2, 3….32)
- Age (≤55, >55)
- Karnofsky Index (≤80, >80)
- Surgeon's impression with respect to tumour location within or close to eloquent areas or structures (risk present yes/no).

After the treatment group had been generated, the next available patient number was determined by a list that was carried on continuously by the randomisation centre. Data were then communicated to the local investigators, first by phone and additionally by fax in a second step together with a treatment schedule for the individual patient. A detailed description of the randomization method was given in an appendix [to the CSR].

Blinding: Because of the differing visual impression (red fluorescence when using the blue light operating mode) between the treatment arms it was impossible to blind the study treatment to the investigators at the trial sites. Although placebo could theoretically have been given to the reference group of patients, this was not considered to be necessary since the surgeon would be immediately unblinded after start of surgery.

Care was taken in the present open label study not to focus on outcome measures that might have been influenced by the observers' or the patients' knowledge of study allocation, such as measures for clinical progression or quality of life scores. Therefore, MRI was chosen as an objective measure for outcome and this was assessed by blinded raters. Neuroradiological assessment of all study related MRIs was blinded at all times, thus totally unbiased. MRT was conducted by standardised procedures and independent radiological institutes in all centres. Within each study centre, MRI was performed within the same neuroradiological department for all patients, irrespective of treatment allocation, using the same MRI device and the same scanning protocol as defined by the study protocol. Thus, no differences in MRI quality could be expected between treatment groups.

For evaluation biopsies and MRIs central neuropathological and neuroradiological assessment were blinded to treatment at all times. Staff of neuropathological and neuroradiological departments was locally distant from any of the neurosurgical study centres and were not allowed to contact centres for information on the treatment arm. Because of this "rater-blinded design" no bias resulting from the unblinded procedure at the investigative sites was expected for the efficacy parameters. However, a certain degree of detection bias regarding safety parameters, e.g. "over-reporting" of adverse events, and SAE-reports due to a more pronounced attentiveness for symptoms in patients having received the test drug, could not be excluded.

7.1.1.1.6. Analysis populations

A special feature of this trial was that patient randomisation was to be based on pre-operative imaging (MRI), without histological confirmation of malignant glioma histology prior to study surgery. In other words, decisive diagnostic tests were not performed until after randomisation. As a consequence, the use of conventional intention-to-treat criteria for statistical analysis would have included patients without malignant glioma. Exclusion of patients without malignant glioma prior to randomisation could have only been circumvented by subjecting all patients to stereotactic biopsies before craniotomy. However, due to possible, additional complications and discomfort for the patient, stereotactic biopsy was not a prerequisite for patient inclusion. Therefore, a certain number of drop-out patients with histologies other than malignant gliomas (WHO grade III/IV) were expected to be randomised to the study. This was considered reasonable, because medical care and follow-up procedures of patients with conditions other than malignant glioma (e.g. brain abscess, metastasis) is so different from patients with malignant glioma. Similarly, a number of patients were to be excluded by the central neuroradiological review due to an assessment not in line with the inclusion criterion as defined by the study protocol.

In summary, the **Full Analysis Set** in this trial comprised all randomized patients who underwent study surgery, had a histopathological diagnosis consistent with WHO grade III/IV gliomas (as assessed centrally by an independent neuropathologist blinded to treatment), and which had a unilocular tumour with features characteristic for malignant gliomas (as independent neuroradiologist blinded to treatment). The patients within the Full Analysis Set were analysed in their initial group of randomisation.

The study report states that this strategy was unlikely to have biased the results because the decision for exclusion was based solely on the results of the central neuropathology or central neuroradiology, which were masked as to treatment allocation.

Comment: I agree that no bias would be expected.

The **Per-Protocol-Set** included all patients within the Full-Analysis-Set except those with major protocol deviations. Major protocol deviation occurred due to early withdrawal, lost-to-followup, major deviations from the planned time schedules and major deviations with respect to surgical procedures. All patients of the Per-Protocol-Set were to be analysed within their group of actual treatment.

For **safety analysis** two analysis sets were created. One population comprised all randomised patients included in the Full-Analysis-Set, with treatment groups defined according to 5-ALA administration. This patient population was created in order to allow for valid safety conclusions on the main target population. Furthermore, a Safety-Analysis-Set was created consisting of all randomised patients who received 5-ALA and all randomised patients who did not receive 5-ALA administration but qualified for Full-Analysis-Set.

7.1.1.1.7. Sample size

For sample size estimation, the following statistical assumptions were made:

- 30% of patients were expected to have their tumours resected to the extent that no residual, contrast-enhancing tumour would be visible on early postoperative MRI using conventional white light surgery, compared to 50% using fluorescence-guided resections.
- 25% were expected to have progression-free survival at the 6 months-visit using conventional white light surgery. An increase by 15 percentage points using fluorescence guided resection was assumed to be relevant to the degree that - if present – statistical significance should be attained with a high statistical certainty.
- The null hypotheses of the equality of both treatment groups with respect to the two primary endpoints should be tested against the alternative hypothesis of a difference prevailing between treatments .
- The test statistics for both primary target criteria were based on the normal distribution approximation of the difference from the treatment-specific probabilities of success.
- The two-tailed multiple significance level was set to 5%. Based on the a-priori defined hierarchical order of the decision strategy as described in section 9.1 of this report, both primary target criteria could nonetheless be tested on the local significance level of 5% to maintain the multiple significance level of 5%.
- The so-called "complete power", i.e. the probability of rejecting all false null hypotheses, was fixed at 80% based on the minimum clinically relevant treatment differences specified above.
- The second primary efficacy criterion was subjected to confirmatory analyses through a group-sequential approach. To take into account the inflation of the error level, the nominal local significance levels for the second primary target criterion were to be adjusted for multiple looks in the data.

Simulation studies were performed based on the above assumptions to achieve a complete power of at least 80%. These revealed that the first statistical analysis should be performed with 270 evaluable patients in the Full-Analysis-Set. If statistical significance was not attained, randomisation would have been continued until n=350 patients were eligible in the Full-Analysis-Set. In order to avoid any fixed a-priori defined drop-out rate, the total number of patients randomised to this trial, was decided to be increased dynamically dependent on the number of patients who do not qualify for Full-Analysis-Set.

7.1.1.1.8. Statistical methods

All statistical tests were two-sided and the multiple level of significance was fixed at 0.05. A-priori ordering of primary efficacy criteria and O'Brien-Fleming-like boundaries for progression-free survival rate at 6-month visit were applied to adjust nominal significance levels for multiple endpoints and multiple looks, respectively. This led to a nominal significance level of 0.05 for the first primary, 0.022 and 0.043 for second primary endpoint at interim and final analysis, respectively.

Chi-square tests were applied for confirmatory testing of primary efficacy parameters. Odds ratios served as effect measures. Associated 95% confidence intervals for analysis of second primary criterion adjusted for multiple looks were calculated. For exploratory purposes, Cochran-Mantel-Haenszel techniques were applied and logistic regression models were fitted to adjust for any potential covariates. Covariates were those chosen within the randomization process.

Although study surgeons formed a subgroup in patient stratification, they were not included in the final logistic regression model. The justification given in the Study Report was as follows: "Due to the fact, that 36 surgeons were involved within this study, many strata with very few patients arose when including the surgeons as an stratification factor in addition to the other covariates used within the randomisation procedure. For this reason, surgeons were not included as a fourth stratification factor within the stratified efficacy analyses. Merely for exploratory purposes, treatment effects were analyzed stratified by surgeons alone, and centres alone, respectively. Surgeons who had operated on less than four patients within the full analysis set were pooled. Centres with less than eight patients were pooled. Due to the large number of surgeons, logistic regression as well as Cox proportional hazards models could not be fitted when incorporating the effect of surgeons. Therefore, centre was used as an additional factor in these analyses for exploratory purposes".

Time-to-event parameters were analysed with Kaplan-Meier methods; comparison were made using log rank tests. Cox-regression models served for additional sensitivity analyses.

Further exploratory testing of secondary efficacy and safety parameters was conducted with Mann-Whitney-Wilcoxon tests for comparison of ordinal and continuous parameters. Chi-square and Fisher's exact tests were used for binary data analysis.

Comment: The subgroups of study surgeons and study centers are important as the study report later claims that data show the operative procedures in the study can be performed by any trained neurosurgeon in most centers. I will examine this conclusion in detail later (see results section below).

7.1.1.1.9. Participant flow

For the final analysis, 415 consecutive patients with suspected malignant glioma (according to the local investigator's findings based on the patient's preoperative MRI scan) were randomised to one of two treatment arms in a 1:1 ratio. Two hundred and seven (207) patients were randomised to treatment arm "FL" (arm A, fluorescence group, FL-group) and 208 patients were randomised to treatment arm "WL" (arm B, white light group, WL-group, control group).

The disposition of patients is shown diagrammatically in Figure 2 and the analysis groups in Table 6 below.

Figure 2. Plot of AUC of ALA vs dose by regression analysis

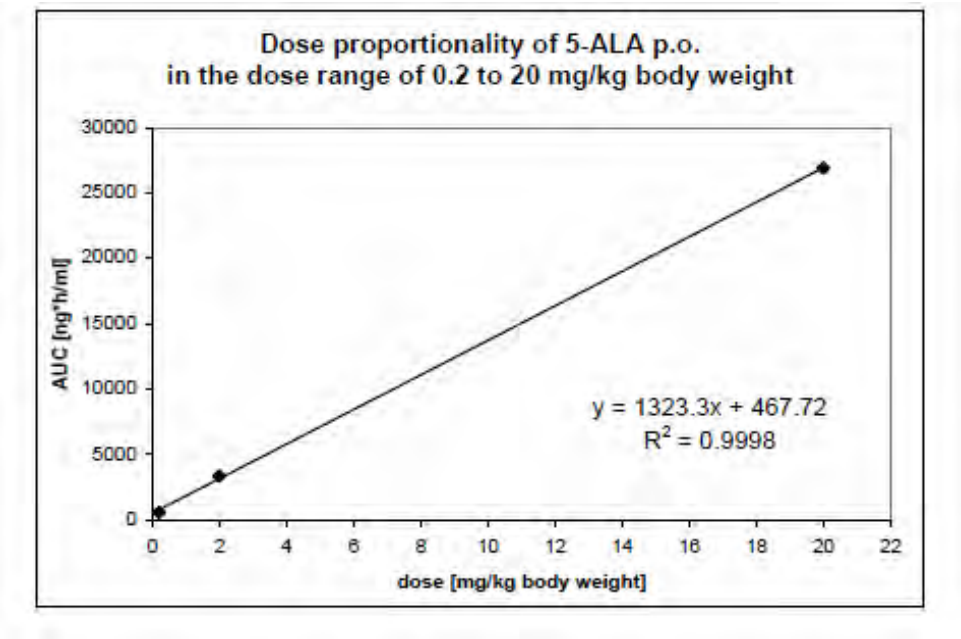


Table 6. Data Sets Analysed

	FL	WL	Overall
Safety-Analysis-Set	201	173	374
Full-Analysis-Set	176	173	349
Per-Protocol-Set	160	166	326

Full Analysis Set: All patients were analysed as randomised. Of 207 patients randomised to the FL-group, 176 (85.0%) were analysed in the FL-group. Of 208 patients randomised to the WL-group, 173 (83.2%) were analysed in the WL-group. Taken together, 349 patients were analysed in the Full-Analysis-Set. Of 207 patients randomised to the FL-group, 31 patients (15.0%) were excluded from this data set whereas 35 of 208 patients (16.8%) randomised to the WL-group were excluded from the Full-Analysis-Set. Reasons for excluding those patients were: ineligible histology (FL-group: n=21; WL-group: n=20), ineligible preoperative MRI-findings (FL-group: n=5; WL-group: n=10), and patients withdrawing their consent before start of surgery (FL-group: n=2; WL-group: n=3). Tumour resection was not performed in two patients of the FL-group and in one patient of the control group. One patient from each group was excluded from the Full-Analysis-Set for other reasons. An overview of all randomised patients excluded from the Full-Analysis-Set and the specific reason for exclusion; and more detailed information was given in the CSR.

Per-Protocol Set: Twelve additional patients in the FL-group and eleven additional patients of the WL-group were excluded from efficacy analysis in the Per-Protocol-Set. As a result, a total of 43 (20.8%) patients randomised to the FL-group and a total of 46 (22.1%) patients randomised to the WL-group were excluded from the Per-Protocol-Set. These were listed in the application CSR. Additional criteria that led to exclusion of additional patients in comparison to the Full-Analysis-Set were failure of the fluorescence device, reoperation within one week after primary surgery, or missing efficacy parameters (follow-up MRIs) due to a patient's early death.

Although randomised to the FL-group, four patients were analysed within the WL-group in the Per-Protocol-Set due to failure or absence of the fluorescence filter module of the operating microscope or missed application of study drug. Consequently, the Per-Protocol-Set comprised 160 patients in the FL-group and 166 patients in the WL-group.

Comment: The exclusions from the Full Analysis Set were acceptable and consistent with the study protocol. Two imbalances in the two arms were noted – major time deviation with respect to efficacy evaluation: 7(3.4%) FL, 2(1%) WL; and radiological diagnosis not met: 5(2.4%) FL, 10(4.8%) WL.

Safety Set: A third set of patients was analysed for safety as defined previously.

7.1.1.1.10. Major protocol violations/deviations

The most frequently occurring protocol deviations belonged to the categories "procedure deviation" (e.g. MRI not performed), "time schedule deviation", and "treatment deviation" (e.g. deviation between the actual applied dose of the study drug and the planned dose). Percentages of protocol deviations were similar between individual study centres. There were no marked differences in the frequency of protocol deviations between any of the surgeons or between treatment arms.

7.1.1.1.11. Baseline data

Table 7 summarizes the primary demographic characteristics (age, body weight, and gender) for all of the 349 patients of the Full-Analysis-Set.

Table 7. Demography of Full-Analysis Set in Pivotal Study

		FL	WL	Overall
Age	<=55 yrs	55 (31.3%)	52 (30.1%)	107 (30.7%)
	> 55 yrs	121 (68.8%)	121 (69.9%)	242 (69.3%)
	N	176	173	349
	Nmiss	0	0	0
	Mean (SD)	58.3 (10.04)	58.9 (9.27)	58.6 (9.65)
	Median (Range)	61.0 (23-73)	60.0 (30-73)	60.0 (23-73)
Body Weight (kg)	N	176	172	348
	Nmiss	0	1	1
	Mean (SD)	79.1 (14.87)	79.1 (15.05)	79.1 (14.94)
	Median (Range)	78.0 (50.0-120.0)	80.0 (43.0-119.0)	78.5 (43.0-120.0)
Gender	F	74 (42.0%)	62 (35.8%)	136 (39.0%)
	M	102 (58.0%)	111 (64.2%)	213 (61.0%)

N = number of non-missing observations, Nmiss = number of missing observations,
SD = standard deviation
Data Source: Appendix 16.2.4, Listing 16.2.4.3, Appendix 16.1.7, Listing 16.1.7.1,
Table 14.1.1A, Table 14.1.2A, Table 14.1.3A
Program: BASELINE.SAS

Overall, the median age of the whole data set of patients was 60 years (range 23-73). Patients in the FL-group were slightly older than in the control arm [(FL-group: 61 years (range 23-73 years); WL:-group: 60 years (range 30-73 years)]. In the Full-Analysis-Set, about two third of the patients (61.0%) were male and about one third (39.0%) were female without notable differences between the study arms (FL: 58% male, 42% female; WL: 64.2% male, 35.8% female). Body weights ranged from 43 to 120 kg (median 79 kg) for the whole data set of patients without meaningful differences between treatment groups.

7.1.1.1.12. Results for the primary efficacy outcome

First outcome for primary efficacy: The first primary efficacy parameter was the percentage of patients without residual tumour on early postoperative MRI. For the Full-Analysis-Set, 63.6% of all patients in the FL-group and 37.6% of all patients in the WL-group did not show residual tumour on early postoperative MRI. This difference was highly statistically significant

using the Chi-square test (p<0.0001). The crude odds ratio was calculated as 2.91 (95% CI: 1.88 - 4.49).

A very similar result was obtained for the Per-Protocol-Set. In the FL-group, 63.8% of patients were operated on without residual tumour on the early MRI versus 39.2% of patients in the control-arm. This result was statistically significant (p<0.0001) with a crude odds ratio of 2.73 (95% CI: 1.75 - 4.28). Thus, results were homogenous in both patient sets analysed.

Subgroup analyses: Similar results were obtained for all analysed subgroups of patients (age, KPS, endangerment of eloquent areas, study surgeon, and study centre). Pronounced heterogeneities between subgroups could not be detected. Logistic regression models showed that fluorescence-guided resection had the most important influence on the percentage of patients without residual tumour (P < 0.0001).

There were more patients without residual tumour on early postoperative MRI if they were young (≤55 years: FL-group: 74.5% vs WL-group: 48.1%; p=0.0049), had a better preoperative KPS (>80%: FL-group: 66.9% vs WL-group: 40.2%; p<0.0001), or had tumours without endangerment of eloquent brain areas (FL-group: 69.1% vs WL-group: 47.2%; p=0.0060). Point and interval estimates of the odd ratios did not change when adjusted for the prognostic factors age. Similar results were obtained for the Per-Protocol-Set.

For the subgroups study surgeon and study centre, the study report stated "When each surgeon and centre was analysed, superiority in the odds ratios of the FL-group was observed for the majority of surgeons and, except for two study sites, all of the study centres, indicating that the treatment effect could be achieved by most of the surgeons."

Comment: The detailed results for surgeons show that most of the 25 named surgeons and the pooled group of 11 surgeons ("others") each had small number of patients. Nineteen (19) to 21 surgeons had less than 10 patients in each of the FL and WL groups, so that convincing statistical comparisons between their results was tenuous. The 11 surgeons combined in the "others" group had a total of 16 patients in the combined treatment groups. In only two case were the p values for the differences less the 0.05, and in only one of these (Woic, below) was the lower value of the 95% CI of the Odds Ratio (O.R.) greater than 1.0. Four surgeons had more than 10 patients in each treatment group with the following results for the percentage of patients free of residual tumour in early post-operative MRI, and the corresponding OR:

Woic- FL 55.6% (10/18), WL 17.6% (3/17), p=0.0204, OR 5.83, 95% CI 1.23 to 27.63.

Lang- FL 42.9% (6/14), WL 23.1% (3/13), p=0.4197, OR 2.50, 95% CI 0.37 to 19.87.

Oppe- FL 58.8% (10/17), WL 31.3% (5/16), p=0.1119, OR 3.14, 95% CI 0.75 to 13.16.

Mayf- FL 46.2% (6/13), WL 27.3% (3/11), p=0.4225, OR 2.29, 95% CI 0.32 to 19.10.

The small numbers of patients for each surgeon raises doubts about the conclusions from such statistical analyses, so that the conclusion that "..the treatment effect could be achieved by most of the surgeons" is not convincingly supported by the data. The combined results of all surgeons on the other hand do show a significant difference in the outcome of the two treatments.

A similar criticism applies to the statistical analysis of the results for the 14 study centers [1 as "others"]. Compared to the study surgeon group, individual patient numbers were higher - 7 centres had more than 10 patients, one more

than 20 and another more than 30 in each treatment group. Of the 14 values for OR, the p value was less than 0.05 in only 3 cases (ORs 6.38, 2.96, 24.0), while the overall OR was 2.91, and the p value <0.0001. The third value of 24.0 is anomalous and clearly an outlier (8.3 times the value of the OR for the whole population.

Second Outcome for Primary Efficacy: The study goal was to increase the percentage of patients without progression at the 6-month-visit by 15% (pre-study calculation: 25% in WL-group vs 40% in FL-group) within the Full-Analysis-Set. The 6 months visit was scheduled 6 months after surgery with a tolerance of ±one month. Results are shown in Table 8. The percentage of patients without progression at the 6-months-visit was nearly doubled through the fluorescence-guided resection and the difference between both treatment groups was significant. Subgroup analysis of patients with regard to age, KPS, and endangerment of eloquent areas revealed odds ratios that always favoured the experimental treatment. Within a logistic regression model, the kind of treatment had the most important influence on the prediction of PFS rate (irrespective of ignoring or including the study centre as an additional covariate)

Comment: Note however that in the Kaplan-Meier analysis (time-to event), the study centers were not included as covariates as they did not fit the model (see results for Kaplan-Meier analysis).

Table 8. Progression-free survival rate [%] at 6-month visit

Parameter	5-ALA group	Control group	P value*	Odds ratio (95% CI)
All patients	36 (20.5%)	19 (11.0%)	P = 0.0152	2.08 (1.12-3.88)
Age ≤ 55 years	14 (25.5%)	7 (13.5%)	P = 0.1185	2.20 (0.78-6.17)
Age > 55 years	22 (18.2%)	12 (9.9%)	P = 0.0643	2.02 (0.93-4.40)
KPS ≤ 80%	6 (16.2%)	5 (12.2%)	P = 0.6104	1.39 (0.37-5.23)
KPS > 80%	30 (21.6%)	14 (10.6%)	P = 0.0143	2.32 (1.14-4.71)
No endangerment of eloquent areas	22 (27.2%)	7 (9.7%)	P = 0.0060	3.46 (1.34-8.96)
Endangerment of eloquent areas	14 (14.7%)	12 (11.9%)	P = 0.5558	1.28 (0.55-3.01)
** Chi-square test*				
7 patients in the FL- and 5 in the WL-group with missing 6-month MRI were considered as having progression				

From the above analysis based on the 6-month time point, the PFS rate was lower at 11% than the 20.5% expected in the control arm. However, PFS is usually calculated with time-to-event analysis based on the exact times of MRI examinations. With this kind of Kaplan-Meier analysis, 6 months PFS rate was calculated to be 35.2% (95% CI: 28.2; 42.3) vs 21.8% (95% CI: 15.6 – 28.0) in favour of the experimental group (log-rank: P=0.0215). Using this method of calculation, the observed result in the control arm (21.7%) approximated that predicated (25%). Median PFS was 3.6 months (95% CI: 3.20; 5.10) versus 3.5 months (3.10; 4.20), not significantly different.

The above table shows that the better Kaplan-Meier estimate of PFS rate at 6 months in the experimental arm could be observed in all analysed subgroups (Table 9 shows Kaplan Meier estimates according to several prognostic factors).

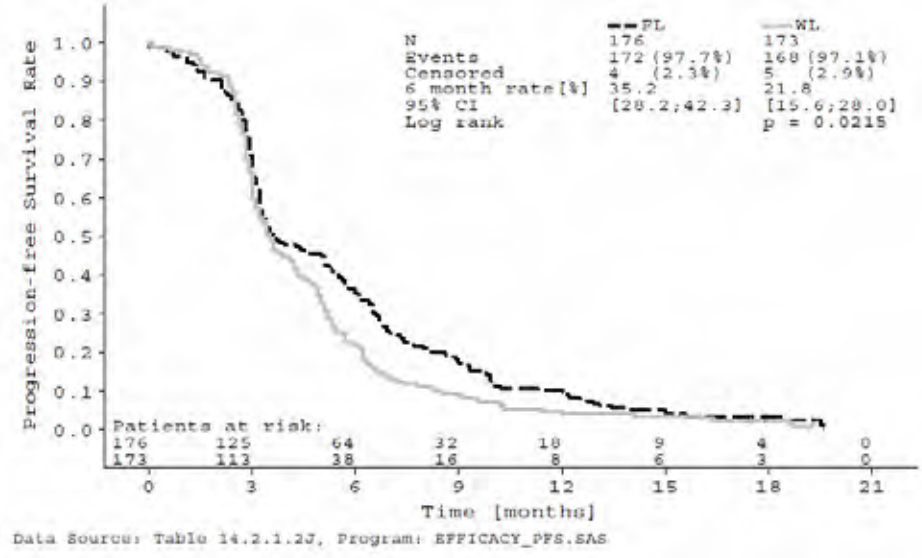
Table 9. PFS rate at 6-months (95%CI): Kaplan Meier estimates according to several prognostic factors

Parameter	5-ALA group	Control group
All patients	35.32 (28.17; 42.28)	21.76 (15.56; 27.97)
Age ≤ 55 years	38.18 (25.34; 51.02)	25.49 (13.53; 37.45)
Age > 55 years	33.88 (25.45; 42.32)	20.17 (12.96; 27.38)
KPS ≤ 80%	27.03 (12.72; 41.34)	17.95 (5.90; 29.99)
KPS > 80%	37.41 (29.37; 45.45)	22.90 (15.71; 30.10)
No endangerment of eloquent areas	39.51 (28.86; 50.15)	18.31 (9.31; 27.31)
Endangerment of eloquent areas	31.58 (22.23; 40.93)	24.24 (15.80; 32.68)

A Cox proportional hazards model was applied to estimate the simultaneous effect of the treatment and other prognostic factors on PFS while ignoring the effect of the study centre. This model showed a hazard ratio of 0.792 (95% CI: 0.638-0.983) for the experimental treatment indicating a 21% reduction in the risk of radiologic progression (P = 0.0341)

The following figure shows the Kaplan-Meier estimates for PFS in the Full-Analysis-Set:

Figure 3. Progression-free survival, Kaplan-Meier estimates



Comment: In my later risk-analysis assessment, the benefit to the patients will be considered. It is important in considering the above results to appreciate that the hazard ratio (0.79%) indicates that there is 21% lower probability of radiological progression for any patient treated with ALA compared to a patient not treated with ALA. No time effects are involved here. These would be shown from the median time to progression, which shows no significant difference in the two groups.

An inspection of the above curve shows this, with the two curves approximately together at the 0.5 ordinate point (median) and only separating after that, explaining why the median values are the same but the 6 month values are significantly different.

7.1.1.1.13. Results for other efficacy outcomes

The results for the following secondary endpoints will be given in the safety section:

· toxicity after oral administration of 5-ALA*HCl

· neurological condition 7 days, 6 and 12 weeks, 6, 9, 12, and 18 months after surgery

The results for the various secondary efficacy endpoints were as follows:

Residual Volume of tumour: The median residual tumour volume in the early postoperative MRI was smaller in the experimental arm than in the control group (0.0 cm^3 [range: 0-45.1 cm^3] vs 0.5 cm^3 [range 0-32.6 cm3]. 75% of patients in the experimental arm had a residual volume of ≤0.7 cm^3 whereas in the control arm 75% of patients had a residual volume of ≤2.1 cm^3 (P < 0.0001; Wilcoxon-Mann-Whitney test). Except for patients with poor KPS (≤80%), there was significantly less residual tumour volume in all subgroups when stratifying by age, KPS, and endangerment of eloquent areas.

A maximum of 80.7% reduction in tumour volume could be achieved in 95% of patients of the experimental arm, compared to a maximum of 70.1% tumour reduction in 95% of patients in the control group (P < 0.0001; Wilcoxon-Mann-Whitney).

Comment: In this analysis, 6 comparisons were made with single variables (those above) and 12 combining the variables. In the combined comparisons, the number of patients with a positive outcome was small in both FL and WL groups (0 to 2) so the comparisons are unreliable. On the other hand those of the single variables are more convincing.

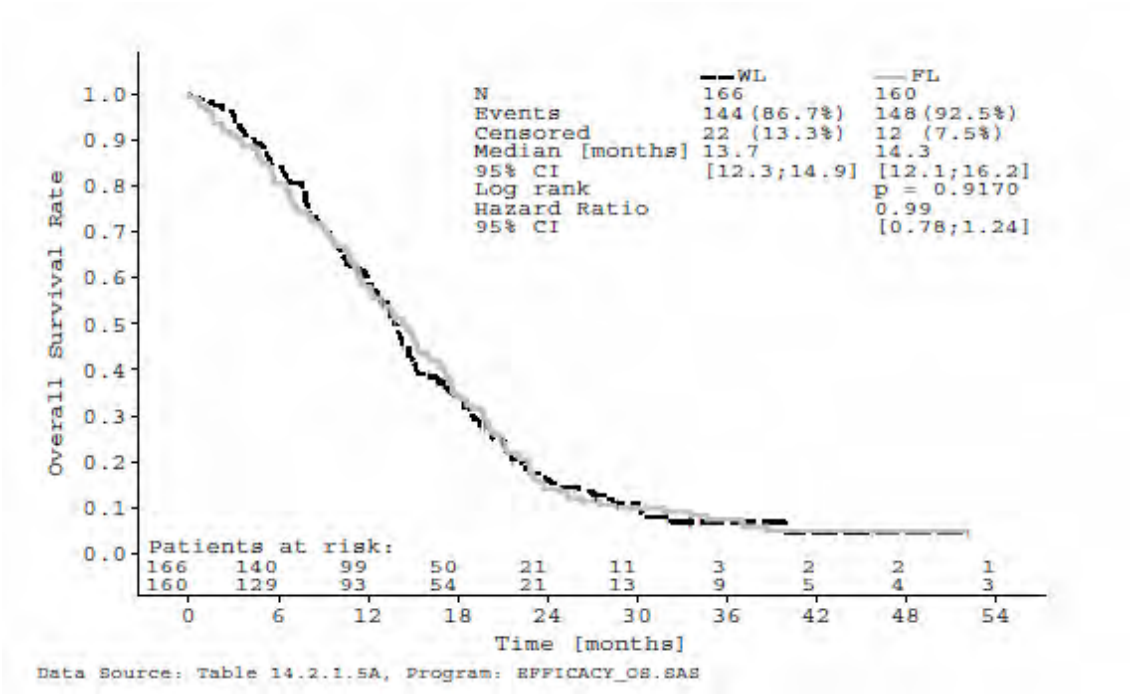
Progression-free survival at 9, 12, 15, and 18 months: Although the PFS rates at 9, 12, 15, and 18 months favoured the experimental arm with odds ratios clearly above 1, the differences did not reach the level of statistical significance. This is shown in Table 10 below. Similar results were shown with the Kaplan-Meier method.

Table 10. Progression-free survival at 9,12,15 and 18months after surgery [%].

Parameter	5-ALA group	Control group	P value	Odds ratio (95% CI)
Number of pts.	176	173		
9 months visit	18 (10.2%)	9 (5.2%)	0.0790*	2.08 (0.91-4.76)
12 months visit	11 (6.3%)	6 (3.5%)	0.2274*	1.86 (0.67-5.13)
15 months visit	6 (3.4%)	3 (1.7%)	0.5022**	2.00 (0.42-12.53)
18 months visit	4 (2.3%)	2 (1.2%)	0.6849**	1.99 (0.28-22.21)
** Chi-square test; ** Fisher's exact test*				

Overall Survival: Analysis of overall survival in this study was of explorative nature because second-line treatment after tumour progression was not standardised. Overall survival was primarily analysed in the Per-Protocol-Set in order to focus on such patients who sufficiently adhered to the fluorescence-guided resection procedure. The survival curve is shown in Figure 4 below. Median overall survival was comparable in both treatment arms (FL vs WL: 14.3 vs 13.7 months; P = 0.9170, log-rank test) and the crude hazards ratio was 0.99 (95% CI: 0.78 - 1.24).

Figure 4. Overall Survival – Kaplan Meier estimates from pivotal trial



Survival rate one year after study surgery was 58% in both study groups. With a Cox proportional hazards model, patient's age (followed by KPS) was the most important factor with a statistically significant influence on overall survival.

Supplemental analyses: Exploratory analyses were also undertaken as well as supplemental detailed efficacy analyses required by EMEA during the Marketing Authorization procedures.

Exploratory analyses:

1. *Median Overall Survival in patients with and without residual disease*

To further investigate any potential impact of the extent of surgery on overall survival, patients of the Per-Protocol-Set were assigned to two different groups according to the results of the early postoperative MRI: patients with residual enhancement and patients without residual enhancement. Median overall survival was 16.7 months (95% CI: 14.3 - 18.6 months) in patients with complete resections according to early post-OP MRI compared to 12.0 months (95% CI: 10.7 - 13.9 months) in patients with residual tumour with a crude hazards ratio for death of 0.62 (95% CI: 0.49 - 0.78). Differences were statistically significant using the log-rank test ($p<0.0001$).

Comment: This analysis is similar to those comparing survival of patients who respond to chemotherapy with those who do not respond. Positive outcomes for responders is not accepted as a measure of the efficacy of treatment, because the responding tumours, or in the present case tumours that can be totally resected, may be more favourable per se in their clinical course, independent of treatment.

2. *Effects of post-surgical therapies*

At the time of planning of this pivotal study, adjuvant radiotherapy was standard treatment for patients with malignant gliomas. Approximately 90% of patients in both study arms received adjuvant postoperative radiotherapy. Post-surgical therapies were not specified. Seventy percent (70%) of patients received either chemotherapy, most commonly temozolomide, or repeat surgery.

Combining the censoring criteria of re-operation and/or start of (any) chemotherapy, median overall survival was 18.2 months for patients in the FL-group, compared to 12.7 months for patients in the control group (log-rank test, p=0.294). The time to reintervention after radiological progression (chemotherapy and/or reoperation) was also compared in the FL and WL groups. The time favoured patients in the FL group but was not statistically significant (FL-group: 9.0 months vs WL-group: 7.1 months; log-rank-test p=0.0863).

Supplemental detailed efficacy analyses required because of concerns raised by EMEA during the Marketing Authorization procedures:

1. Evaluation of clinical effects of treatment

The EMEA noted that in assessing progression free-survival (PFS), no data had been presented of neurological or other clinical progression, and no clinically significant effects had been observed or presented in terms of other efficacy endpoints, such as overall survival, health-related quality of life, neurological progression, or other important clinical efficacy endpoints to support a clinical benefit of fluorescence-guided resection of malignant gliomas with 5-ALA. In response the sponsor carried out time to event analyses for tumour progression defined according to the Macdonald (3) or Stupp (4) criteria. The former assessed efficacy of neuro-oncological therapies based on major changes in tumour size on the MRI scan. These scan changes were interpreted in light of steroid use (stable or increased) and neurologic findings. Progressive disease was defined by a 25% or more increase in size of enhancing tumour or any new tumour on MRI scans in case of stable or increased steroids, or neurologically worsening in case of stable or increased steroids.

In a study on the efficacy and safety of radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma, Stupp et al. (4) published data on tumour progression defined according to the modified WHO criteria as an (radiologically determined) increase in tumour size by 25 percent, the (radiological) appearance of new lesions, or an increased need for corticosteroids. Data from the present pivotal trial were reassessed with tumour progression defined according to Stupp et al., that is, time from study surgery to radiological progression or steroid increase or death, whatever occurred first. Again, an advantage for patients in the FL-group was observed. In the FL-group, 27.3% of patients were event free six months after surgery compared to 15.5% in the control-group (P = 0.0122).

"Neurologically worse" was defined as a deterioration in the NIH stroke score by at least 1 point relative to the preceding visit. Using this clinically meaningful combined endpoint, patients treated in the experimental arm had a clear clinical benefit (46% of patients event-free six months after surgery) compared to the control arm (29.3% event-free six months after surgery) in a statistically significant manner (P = 0.0331). In addition, "neurologically worse" was defined as a deterioration in the NIH stroke score by at least 2 points relative to the preceding visit. Still, a clear clinically significant benefit (P =0.0316) resulted from this analysis.

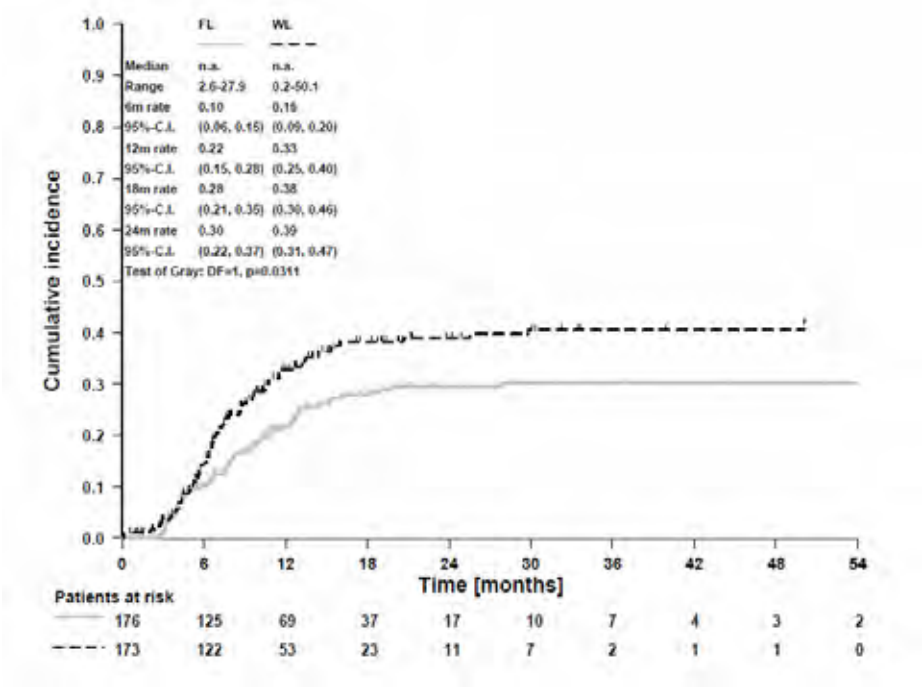
2. Re-evaluation of the time to re-intervention (re-operation or chemotherapy)

Comment: In an exploratory analysis of the time of post-surgical therapies (point 2 *Effects of post-surgical therapies*), the time to re-intervention after radiological progression was analysed. In this analysis, it is not clear how the information on patients who died without any re-intervention was handled. The EMEA seems to have requested a similar analysis of the time to re-intervention after surgery (rather than after the diagnosis of progressive disease) with censoring of patients who died prior to any intervention because the inclusion of such patients would give misleading incidence of re-intervention. This analysis was described in the study report as applying more correct and sophisticated cumulative incidence methods.

Cumulative incidence of re-operation after study surgery (Full-Analysis-Set): The study report claims that the time to re-operation after surgery was significantly shorter in the FL

group compared to the time in the WL group. The results were as follows, using the more correct and sophisticated cumulative incidence methods requested by the EMEA. There was a significant benefit for the FL group with 46% vs 29.3% event-free at 6 months (p=0.033) [Figure 5, below]

Figure 5. Cumulative incidence of re-operation after surgery vs time



Comment: I am not familiar with the "Test of Gray". Although this test shows significance for the whole time sequence (p=0.0311), each pair of 95%CI values for the different times in both groups overlap.

Neither cumulative incidence of chemotherapy after study surgery (Full-Analysis-Set) nor cumulative incidence of chemotherapy with temozolomide after study surgery (Full-Analysis-Set) were significantly different in the FL and WL groups (p=0.092 and 0.064 respectively).

3. Other statistical analyses requested by EMEA:

A further analysis was requested to examine the treatment effect on neurological progression-free survival (time from randomization to radiological progression or neurological progression or death, whichever first) for the ITT population, log rank and multivariate Cox proportional hazards model adjusted for important prognostic factors. A similar analysis of clinical and radiological PFS (time from randomization to radiological progression in the presence of clinical progression or death) was also requested. The former analyses were carried out with similar results to those previously, but similar statistical analyses for clinical and radiological PFS (time from randomisation to radiological progression in the presence of clinical progression or death) were not conducted due to study design which required patients to be taken from study as soon as radiological progression was detected - irrespective of any clinical progression. Since radiological progression can be expected to occur prior to clinical signs of disease progression such an analysis would be completely underpowered to yield any meaningless interpretation.

7.1.2. Other efficacy studies

7.1.2.1. Studies MC-ALS. 28/GLI and MC-ALS.30/GLI

The primary objective of both studies was to determine the positive predictive value of tissue fluorescence, defined as the percentage of patients showing positive tumour cell identification in all biopsies taken from areas of weak and strong fluorescence. A biopsy was termed "positive

tumour cell identification" if the reference neuropathological institute observed a tumour cell content greater than 0%. The secondary objective was to determine the positive predictive value of tissue fluorescence at the biopsy level, defined as the percentage of tumour positive biopsies among all biopsies taken from areas of weak and strong fluorescence. In both studies, a sample size of 33 patients was required to estimate the positive predictive value with a precision of ± 20% expressed as the half length of the associated two-sided 90% confidence interval with a power of 80%.

In the first study (28/GLI), 39 patients (males or females aged 18 to 75 years with cranial magnetic resonance imaging (MRI) justifying diagnosis of malignant glioma [WHO grades III-IV] for whom first surgical treatment was indicated were included into this study and assigned to undergo fluorescence-guided resection which yielded 33 patients qualifying for the Full-Analysis-Set and 36 patients for the Safety-Analysis-Set.

In the second study (30/GLI), males or females aged 18 to 75 years with diagnosis of recurrent malignant glioma (WHO grades III-IV), for whom repeat-surgery was indicated were included into this study. Patients must have undergone previous surgery including open craniotomy for newly diagnosed glioma plus standard radiotherapy; biopsy-only would not have been sufficient. Patient's Karnofsky Performance Scale (KPS) was required to be ≥60%. In the second study a total of 40 patients were assigned to undergo fluorescence-guided resection to yield 36 patients qualifying for the Full-Analysis-Set within the final analysis.

Results: Patients in 28/GLI had an average of 6 biopsies each, taken from different areas of fluorescence. The primary endpoint, the positive predictive value showing tumor cells on biopsy, was 100% (90% CI: 91.1 -100.0%) for patients whose areas of strong fluorescence had been biopsied, and for areas of weak fluorescence, 83.3% (90% CI: 68.1% - 93.2%). The number of tumour positive biopsies among all biopsies taken from areas of any fluorescence (weak and strong fluorescence), was 96.2% (90% CI: 93.0% - 98.2%). For biopsies from strongly fluorescent areas, 100.0% (90% CI: 96.9% - 00.0%) were tumour positive compared to 92.2% (90% CI: 85.9% - 96.3%) of biopsies from weakly fluorescent areas. An additional assessment in this study was to correlate residual fluorescence/tumour observed intraoperatively and residual tumour on early postoperative MRI. In 65.2% of patients with residual tumour/fluorescence recorded intraoperatively, reference neuroradiology was able to detect residual contrast enhancement. On the other hand, in two of ten patients (20%) without residual tumour/fluorescence according to intraoperative assessment, reference neuroradiology detected residual contrast enhancement. In these patients, residually enhancing tumour was located at a distance to the resection cavity and was not expected to be detectable during surgery from within the cavity. 76.2% of sites with intraoperatively suspected residual tumour were visible only under fluorescence conditions (but not using standard white light) whereas 23.8% of sites with suspected tumor were detected both under fluorescence conditions and using standard white light.

In study 30/GLI, the positive predictive value of strong tissue fluorescence was higher (91.7%; 95% CI: 77.5% - 98.2%) compared to that of weak fluorescence (83.3%; 95% CI: 67.2% - 93.6%). In total, there were 28/36 patients with positive tumour cell identification in all weak or strong fluorescing biopsies, resulting in a positive predictive value of 77.8% (95% CI: 60.8% - 89.9%). The number of tumour positive biopsies among all biopsies taken from areas of any fluorescence was 96.6% (95% CI: 94.2% - 98.2%). The positive predictive value was higher if only strong fluorescent biopsies were taken into account (98.2%; 95% CI: 94.8% - 99.6%) compared to weak fluorescent biopsies only (95.3%; 95% CI: 91.2% - 97.8%).

False positive and false negative results: The results of Study 30/GLI are not considered here, as the patient population in that study had repeat surgical resections, an indication not requested in the application. The following only refers to Study 28/GLI and patients with initial surgical resections.

False positives: Intraoperatively detected areas of weak or strong fluorescence were tumour-cell positive in 84.8% of patients (90% CI: 70.7% - 93.8%)], so that in 15.2% of patients, the results were false positives, ie tumor cell negative. For all biopsies taken, a smaller figure of 7 biopsies (3.8%) were tumour cell negative ie false positives.

Comment: For these data it was not possible to calculate the sensitivity and the specificity of the test as the number of patients with true and false negative results was not determined. This could be done however for the biopsies (see following section). Such data and calculations are regarded as Standard of Truth, as defined in the EMEA guidelines for diagnostic agents.

False negatives: These data were available from biopsy data (Table 11). Seventy percent (70%) of non fluorescent biopsies taken from normal appearing adjacent tissue were tumour-cell positive. In 2 of 10 patients (20%), 5-ALA-induced fluorescence detection failed to identify areas that show contrast enhancement in postoperative MR, especially in those cases where the tissue was not accessible to the blue light source.

Table 11. Biopsy based frequency distribution of tumour histology stratified by fluorescence quality (Full analysis Set)

Predominant type of tissue in evaluated section of biopsy	strong n (%)	weak n (%)	none, adjacent tissue n (%)	none, distant cortex n (%)
Total	96 (100.0)	90 (100.0)	66 (100.0)	48 (100.0)
missing	1 (1.0)		1 (1.5)	1 (2.1)
vital, solid, proliferating tumor	79 (82.3)	12 (13.3)	3 (4.5)	2 (4.2)
infiltrative tumor	14 (14.6)	63 (70.0)	42 (63.6)	20 (41.7)
necrosis	2 (2.1)			
normal tissue		15 (16.7)	20 (30.3)	25 (52.1)

Evaluator's calculation of sensitivity and specificity of fluorescence guided biopsies of tumours and adjacent tissue (based on Table 11)

Disease Present: True Positive (a) + False Negative (b);

Disease absent: False Positive (c) + True Negative (d).

For all fluorescent tissue (strong and weak) censoring missing data and data from necrotic tissue:

True positives (a) n= 183-15+168; False negatives (b) n= 45

Sensitivity a/a+b = 168/168+45 = 168/213 = 0.79 of 79% (probability).

For all non-fluorescent tissue adjacent to tumour:

False positives (c) n= 15; True negatives (d) n= 20

Specificity d/c+d = 20/15+20 = 20/35 = 0.57 or 57% (probability).

Comment: I have used these data (Table 11 above) to calculate a sensitivity of 79% and a specificity of 57% for ALA induced fluorescence in tissue adjacent to the tumour. The adjacent tissue is of major importance since the aim of the treatment with ALA and resection is to remove more of the tumour than without ALA treatment, without removing excessive amounts of normal brain tissue.

These results shows that while the sensitivity of 79% is acceptable in identifying tumour by the presence of strong or weak fluorescence, the low specificity of 57% is not acceptable in identifying brain tissue that is free of tumour by the

absence of fluorescence. The acceptable sensitivity is similar to the high positive predictive value above (78% of all biopsies).

The EU evaluator had similar concerns about the low specificity and states "The results showed that the strong fluorescent area has a much higher cellularity than the comparators areas and this makes the positive predictive value of strong fluorescence 100%. However none of the drugs clearly discriminate because the confidence intervals are overlapping. It seems that if an area is strong fluorescent it is tumour but if it is not it can be tumour also. This is an important drawback for the clinical utility of the method. In the answers provided by the applicant this aspect was clarified although not completely solved because the data available is not sufficient. Yet, it satisfactorily shown that the fluorescence is highly specific for tumour. Since the major problem is to remove healthy tissue taken as tumour (false positives) the high specificity (sic) is reassuring. The issue of real sensitivity (sic) remains but it less critical for the clinical use."

In practical terms then, the use of ALA induced fluorescence allows the resection of fluorescence tissue that has a high probability of being malignant, but does not help define residual normal tissue. Because of the need to limit possible neurological damage, this means that in most cases residual cancer is left with an eventual fatal outcome.

7.2. Evaluator's conclusions on clinical efficacy for the requested indication

Does improved tumour visualisation under fluorescence light enable the surgeon to resect the tumour more completely?

Contrast-enhancing tumour was resected in significantly more patients (64 %) in the experimental group in the pivotal trial compared to 38 % in the control group ($p<0.001$).

Does more radical tumour surgery improve the outcome for patients with GBM with respect to progression-free (PFS) and overall survival (OS)?

At the visit 6 months after tumour resection, based on the time-to event Kaplan-Meier analysis, 35.3 % of 5-ALA-treated-patients and 21.8% % of patients who underwent standard surgery were alive at the 6 month visit without progression. The difference was statistically significant (log rank $p=0.0215$). Although this is a convincing result, it is worrying that the median values of the PFS rates were not significantly different. The trial was not powered to detect a difference in OS and none was demonstrated.

What clinical benefits if any were achieved by the use of fluorescence guided resection of tumour?

Supplemental time to event analyses, based on events defined as radiologic progression or deterioration of NIH stroke score by at least one point relative to the preceding visit or death showed that patients treated in the experimental arm had a clinical benefit compared to the control arm (46 vs 29.3% event-free six months after surgery) in a statistically significant manner ($P = 0.0331$). As well, the time from study surgery to radiological progression or steroid increase or death, whatever occurred was significantly longer in the FL-group [at 6 months: 27.3 vs 15.5%; $P = 0.0122$).

What was the sensitivity and specificity of fluorescence detection of tumor with ALA?

The probability that the test result was positive when the disease was present (sensitivity) was 79%.

The positive predictive value or the probability that the disease is present when the test is positive was 96.2% ie when only fluorescent areas were biopsied. The probability that the test result was negative when the disease was not present (specificity) was 57%.

8. Clinical safety

8.1. Studies providing evaluable safety data

The following studies provided evaluable safety data:

8.1.1. Pivotal efficacy study MC-ALS.3/GLI

In the pivotal efficacy study, the following safety data were collected:

- General adverse events (AEs) assessed by documentation of adverse events (AEs) and collecting blood samples for laboratory tests.
- AEs of particular interest, including those more clearly related to the surgical procedure than to the study drug, were to be evaluated by documentation of the NIH stroke score, the Karnofsky Performance Scale, and adverse events.

Coding and grading of adverse events (AEs) was by NCI Common Toxicity Criteria, version 1. Both the safety analysis and the full analysis sets were analysed for incidence of AEs.

NIH Stroke Score: One major disadvantage of a more complete brain tumour resection might be that the patient suffers from more neurological deficits postoperatively. No validated scales were available for assessing acute neurological deficits from surgery of brain tumours, so within the three trials in glioma patients, the NIH Stroke Scale (NIH-SS)6 was used for assessment of the neurologic outcome and degree of recovery from surgery. The NIH-SS was originally developed for characterising cerebral vascular strokes, which – in analogy to surgery –result in acute neurological deficits. A modified form of the NIH-SS was used. This score covered 16 single neurologically relevant items with a theoretically maximum (worst) score of 36 points. Absolute change in NIH-SS relative to baseline was tabulated and the number/percentages of patients with deterioration, no change or improvement presented.

The frequency of monitoring is shown in the following table

Table 12. Safety parameters monitored and frequency (pivotal trial)

MC-ALS.3/GLI	(Serious) adverse events	Up to 18 months after surgery*
	Laboratory tests	
	Haematology (blood counts, haematocrit, haemoglobin)	Baseline, day 1/7, week 6 after surgery*
	Serum chemistry (AST, ALT, AP, γ-GT, bilirubin, LDH, PT, PTT, electrolytes, creatinine, urea, uric acid)	Baseline, day 1/7, week 6 after surgery*
	Karnofsky Performance Scale	Baseline, after 6 weeks, after 3/6/9/12/15/18 months*
	NIH Stroke Scale	Baseline, after 2/7 days, after 6 weeks, after 3/6/9/12/15/18 months*
	(Serious) adverse events	Up to 18 months after surgery*
** monitoring ended ahead of schedule in case of progression or death, whatever occurred first*		

8.1.2. Pivotal studies that assessed safety as a primary outcome

Not applicable

8.1.3. Dose-response and non-pivotal efficacy studies

The dose-response and non-pivotal efficacy studies provided safety data, as shown in Table 13 below [see exposure section]. Studies 20/BV, 8-I/GLI, 28/GLI and 32/GLI used the same drug dosage in each for similar patient populations. In Study 30/GLI, the patient population was different, with patients operated on a second time. However the patient numbers were small (40), so all these studies will be considered together. In a number of tables, the pivotal trial, 3/GLI, is included for comparison.

8.1.4. Other studies evaluable for safety only

8.1.4.1. Study MC-ALS.32/GLI:

Trial MC-ALS.32/GLI was launched in September 2004 after recruitment to the phase III trial MC-ALS.3/GLI had been terminated. The primary objective was to determine the incidence of adverse events after 5-ALA-supported fluorescence-guided resection of newly diagnosed patients with malignant gliomas. This trial was performed instead of a "Compassionate Use" programme because the legal base for "Compassionate Use" in Germany had not yet been defined at that time. The inclusion criterion for radiological diagnosis was less rigorous than that in the pivotal trial, 3/GLI.

In the pivotal trial the criterion was "Radiological suspicion of a malignant glioma with distinct ring- or garland-shaped, contrast agent-enhancing tumour structures and a core area of reduced intensity in the MRI (tumour necrosis)" and in study 32/GLI "First radiological suspected diagnosis (MRI) of a malignant glioma."

8.2. Pivotal studies that assessed safety as a primary outcome

Not applicable

8.3. Patient exposure

All patients were administered a single dose of ALA except in the pharmacological study ALS-MC.20/BV, when the same dose was administered on two days, days 1 and 4. The studies and dosing is shown in the following Table:

Table 13. Exposure to ALA in clinical studies

Study ID	Subjects	N*	5-ALA dose regimen	Route
MC-ALS.20/BV	Healthy male volunteers	12	1 x 20 mg/kg; day 1 1 x 2 mg/kg; day 4	PO IV
		9	1 x 20 mg/kg	PO
MC-ALS.8-I/GLI	Patients with newly diagnosed malignant glioma	7 7 7	1 x 0.2 mg/kg 1 x 2.0 mg/kg 1 x 20 mg/kg	PO PO PO
MC-ALS.28/GLI	Patients with newly diagnosed malignant glioma	36	1 x 20 mg/kg	PO
MC-ALS.3/GLI	Patients with newly diagnosed malignant glioma	201	1 x 20 mg/kg	PO
		173	Control group	-
MC-ALS.32/GLI	Patients with newly diagnosed malignant glioma	243	1 x 20 mg/kg	PO
MC-ALS.30/GLI	Patients with relapsed malignant glioma	40	1 x 20 mg/kg	PO
**Safety-Analysis-Set*				

The total number of patients administered the requested dose of 20 mg/kg was 548. The imbalance in the number of patients in the Safety-Analysis-Set of the two treatment arms of study MC-ALS.3/GLI is due to the fact that those patients of the control group who were excluded from the efficacy analysis (mainly because histology did not reveal a glioblastoma) were not further followed up whereas the same patients of the 5-ALA group were followed up for AEs for 6 weeks.

8.4. Adverse events

8.4.1. All adverse events (irrespective of relationship to study treatment)

8.4.1.1. Pivotal studies

Most frequently observed adverse events in both treatment groups were of neurological origin followed by "sensory organs" (Table 14 below). Although the total incidence in each treatment group was similar (43% cf 45%), there was a significant difference with respect to adverse events related to the sensory organs worse in the ALA treatment arm, FL, (17%cf 8.7%; p=0.020), mostly related to impaired vision (investigator's AE term: hemianopsia)with incidence values of 14.8% cf 7.5%, p=0.04). Most of these events were of CTC grade I (FL group, grade I/II/III: n = 15/7/5 patients; WL group: n = 8/3/2 patients).

The incidence of specific AEs (>3%) in patients in each trial group is shown in Table 15. Early after brain tumour surgery (up to day 7), most reported AEs were related to the neuro-/sensory system. Nearly half of all reported AEs related to the neuro-/sensory system occurred during this first week after brain tumour surgery.

Table 14. Adverse events summarised by CTC category and treatment group (excluding SAEs) – sorted by incidence

CTC category of AE	**FL group** Safety Analysis Set	**FL group** Full Analysis Set	**WL group** Safety = Full Analysis Set	**p-value** Safety Analysis Set	**p-value** Full Analysis Set
Data source (FSR table)	*14.3.1.1A*	*14.3.1.2A*	*14.3.1.1A*	*14.3.1.1A*	*14.3.1.2A*
Number of patients	201 (100%)	176 (100%)	173 (100%)	201 vs 173	176 vs 173
Number of pts. with any event	118 (58.7%)	109 (61.9%)	100 (57.8%)	0.860	0.431
Neurologic	86 (42.8%)	80 (45.5%)	77 (44.5%)	0.738	0.859
Sensory organs *- impaired vision*	31 (15.4%) *27 (13.4%)*	30 (17.0%) *26 (14.8%)*	15 (8.7%) *13 (7.5%)*	0.047 *0.068*	0.020 *0.041*
General condition	24 (11.9%)	23 (13.1%)	34 (19.7%)	0.040	0.096
Fever/infection/flu-like sympt.	16 (8.0%)	16 (9.1%)	10 (5.8%)	0.409	0.239
Cardiac	10 (5.0%)	10 (5.7%)	8 (4.6%)	0.874	0.655
Gastrointestinal	9 (4.5%)	8 (4.5%)	11 (6.4%)	0.420	0.455
Dermatologic / allergic	6 (3.0%)	6 (3.4%)	3 (1.7%)	0.431	0.324
General symptoms	5 (2.5%)	4 (2.3%)	2 (1.2%)	0.343	0.422
Pulmonary	4 (2.0%)	4 (2.3%)	1 (0.6%)	0.236	0.183

Table 15. Frequently (>3%) reported AEs (excl SAEs) up to 7 days after surgery

CTC category of AE	FL group Safety Analysis Set	FL group Full Analysis Set	WL group Safety = Full Analysis Set	p-value Safety Analysis Set	p-value Full Analysis Set
Data source (FSR table ...)	*14.3.1.1D*	*14.3.1.2D*	*14.3.1.1D*	*14.3.1.1D*	*14.3.1.2D*
Number of patients	201 (100%)	176 (100%)	173 (100%)	201 vs 173	176 vs 173
Vision impaired	16 (8.0%)	15 (8.5%)	7 (4.0%)	0.134	0.122
Neuro-motor	14 (7.0%)	14 (8.0%)	5 (2.9%)	0.098	0.057
Speech impairment	14 (7.0%)	13 (7.4%)	9 (5.2%)	0.524	0.510
Personality change	9 (4.5%)	8 (4.5%)	6 (3.5%)	0.793	0.786
Neuro-cortical	7 (3.5%)	7 (4.0%)	5 (2.9%)	0.779	0.771
Neuro-headache	7 (3.5%)	6 (3.4%)	7 (4.0%)	0.792	0.785
Ataxia	6 (3.0%)	6 (3.4%)	1 (0.6%)	0.129	0.121

8.4.1.2. Other studies

Treatment-related AEs and all SAEs are presented for the supporting studies (see next sections).

8.4.2. Treatment-related adverse events (adverse drug reactions)

8.4.2.1. Pivotal study

In the pivotal study, two patients experienced drug-related AEs – one, mild vomiting 48 hours after surgery, and the second mild photosensitivity 48 hours after surgery.

Comment: Not mentioned in this section of the study report is the report of a probably drug-related Serious Adverse Event (SAE) of respiratory insufficiency in a patient who received 3000mg of ALA instead of 1580mg pre-surgery. The reaction resolved.

8.4.2.2. Other studies

Drug-related adverse events: A total of 9 subjects/patients experienced adverse events (1.7% of all 541 subjects/patients treated with 1 x 20 mg/kg 5-ALA*HCl), which were related by the investigator as at least possibly related to the study drug (see Table 16).

Table 16. Drug-related AEs in studies other than the pivotal trial

Study	Patient Identifier	Description of adverse event
MC-ALS.20/BV	-	-
MC-ALS.8-I/GLI *(Data source: 12.2.3.3)*	11	Mild dermatitis solaris, mild photosensitivity, moderate fever
MC-ALS.28/GLI *(Data source: 12.2.3.3)*	001	Chills CTC grade II
	005	Neuro-sensory CTC grade II (hypaesthesia)
	036	Diarrhoea CTC grade I
MC-ALS.30/GLI	-	-
MC-ALS.32/GLI *(Data source: 12.2.2D and Listing 16.2.7.1)*	095	Brain oedema grade III, procedural hypotension grade III
	162	Deep vein thrombosis grade I
	243	Hepatic enzyme increased grade I
MC-ALS.3/GLI *(Data source: 12.2.3.5)*	232	Mild vomiting 48 hours after surgery
	350	Mild photosensitivity 48 hours after surgery

8.4.3. Deaths and other serious adverse events

8.4.3.1. Pivotal studies

Deaths: All deaths were considered as consequences of brain tumour surgery or pre-existing cardiac disease. With 30 days of follow-up post surgery, 5 patients of 158(2.5%) in the FL(ALA) arm and 3 of 131(1.7%) in the WL (control) arm died. The frequency was similar in each group for both the Safety and the Full Analysis Sets.

The following reasons for death within 30 days were reported for patients in the FL-group: Three patients died of suspected pulmonary embolism, one patient died of transtentorial herniation, another patient's death was of cardiac cause (ventricular fibrillation). For patients in WL-group the following reasons for death were documented: One patient died due to pulmonary embolism, one patient died due to sepsis and circulatory failure, and for another patient sudden cardiac death was diagnosed after suspected pulmonary embolism.

With follow-up to 180 days post-surgery, 17 of 201 (8.5%) patients in the FL-group and 11 of 173 (6.4%) patients in the WL-group died as an outcome of a SAE. No particular type of SAE predominantly occurring in one of both treatment groups could be identified except for pulmonary embolism (FL-group: 4.0% vs WL-group: 0.6%), however, the difference was not statistically significant and most of the embolisms occurred late during follow-up. Results did not differ in the Full-Analysis-Set.

Other Serious Adverse Events (SAEs): A summary of SAEs by system organ class is given in Table 17.

Table 17. Frequency distribution of all serious adverse events reported up to 180 days after study surgery summarised by SOC and treatment group (Safety Analysis Set)

System Organ Class	FL	WL	Total
Number of patients	201 (100.0%)	173 (100.0%)	374 (100.0%)
Any event	60 (29.9%)	40 (23.1%)	100 (26.7%)
Body As A Whole - General Disorders	5 (2.5%)	3 (1.7%)	8 (2.1%)
Cardiovascular Disorders, General	2 (1.0%)	2 (1.2%)	4 (1.1%)
Centr & Periph Nerv Syst Disorders	25 (12.4%)	20 (11.6%)	45 (12.0%)
Endocrine Disorders	0 (0.0%)	1 (0.6%)	1 (0.3%)
Gastro-intestinal System Disorders	2 (1.0%)	1 (0.6%)	3 (0.8%)
Heart Rate And Rhythm Disorders	4 (2.0%)	1 (0.6%)	5 (1.3%)
Liver And Biliary System Disorders	1 (0.5%)	1 (0.6%)	2 (0.5%)
Metabolic And Nutritional Disorders	0 (0.0%)	1 (0.6%)	1 (0.3%)
Neoplasms	0 (0.0%)	1 (0.6%)	1 (0.3%)
Nervous System Disorders	1 (0.5%)	0 (0.0%)	1 (0.3%)
Platelet,bleeding & Clotting Disorders	19 (9.5%)	7 (4.0%)	26 (7.0%)
Psychiatric Disorders	3 (1.5%)	2 (1.2%)	5 (1.3%)
Resistance Mechanism Disorders	4 (2.0%)	4 (2.3%)	8 (2.1%)
Respiratory System Disorders	7 (3.5%)	7 (4.0%)	14 (3.7%)
Secondary Terms - Events	2 (1.0%)	1 (0.6%)	3 (0.8%)
Skin And Appendages Disorders	3 (1.5%)	0 (0.0%)	3 (0.8%)
Urinary System Disorders	0 (0.0%)	1 (0.6%)	1 (0.3%)
Vascular (extracardiac) Disorders	3 (1.5%)	2 (1.2%)	5 (1.3%)
Vascular Disorders	0 (0.0%)	1 (0.6%)	1 (0.3%)
Vision Disorders	1 (0.5%)	0 (0.0%)	1 (0.3%)

The overall incidence of patients with any SAE reported within the Safety-Analysis-Set was similar for both treatment groups, with at least one SAE reported in 60/201 (29.9%) of patients in the FL-group and 40/173 (23.1%) of patients in the WL-group. For both treatment groups, the most frequently reported SAEs were those assigned to the WHO system organ class "central & peripheral nervous system disorders" (FL-group: 12.4% vs WL-group:11.6 %), followed by "platelet, bleeding & clotting disorders" (FL-group: 9.5% vs WLgroup:4.0%) and "respiratory system disorders" (FL-group: 3.5% vs WL-group: 4.0%). Overall, there were few observable differences between the treatment groups in the frequency of patients with SAEs of a particular system organ class, except for the category "platelet, bleeding & clotting disorders", mainly due to different numbers of patients with reported pulmonary embolism.

Comment: Another difference is in the frequency of “heart rate and rhythm disorders” with 4 (2.0%) and 1 (0.6%) in the FL and WL groups respectively. However the numbers are small and may be random and will be compared in other studies on safety.

Neurological: The most common SAE (experienced by at least two patients in either treatment group) reported within a follow-up period of 180 days after study surgery was convulsions. Adverse events coded as convulsions were reported as SAEs in 12 patients (6.0%) in the FL-group and in 5 patients (2.9%) in the WL-group. The other most frequently reported neurological SAEs were convulsions coded as Grand Mal (FL-group: 3.5% vs WL-group: 2.9%), hemiparesis (FL-group: 4.0% vs WL-group: 2.3%), aphasia (FL-group: 3.5% vs WL-group: 0.6%), stupor (FL-group: 0.5% vs WL-group: 1.7%), and hypertension intracranial (FL-group: 0% vs WL-group: 2.3%).

Comment: It is assumed that the figures given for the incidence of Grand Mal was included in the incidence figure for convulsions and that the combined frequency of convulsions (convulsions plus Grand Mal episodes) was not 6.0%+3.5%, ie 9.5%.

Non-neurological: The most frequently reported non-neurological SAEs comprised pulmonary embolism (FLgroup:6.5% vs WL-group: 1.2%), thrombosis (FL-group: 1.5% vs WL-group: 1.7%), pneumonia (FL-group: 2.0% vs WL-group: 2.9%), condition aggravated (FL-group: 1.5% vs WL-group:1.2%), arrhythmia (FL-group: 1.5% vs WL-group: 0.6%), and psychosis (FL-group: 1.5% vs WL-group: 0.6%). Serious adverse events coded as cardiac failure, haematoma, abscess, cyst NOS, and hygroma cystic, were reported in two or less patients in either treatment group.

Comment: The number of patients with convulsions, hemiparesis, aphasia, and pulmonary embolism reported were higher in the FL-group. Of these, only the difference for pulmonary embolism was statistically significant (p=0.015; Fisher's exact test). However the number s of neurological AEs were small, and the higher incidence was always in the FL arm suggesting that in a study powered to detect a statistical significance, these events may have achieved that significance. The median time of onset of pulmonary embolism after study surgery was 35 days (range 1-150 days). Except for two patients with PE 108 days and 20 days post-surgery, none of the patients with PE demonstrated signs of immobility. PE was known in medical history of a single patient in the FL-group, whereas PE was not documented in other patients of either treatment group before study surgery. PE occurred two days post-OP in one patient and 99 days after study surgery in another patient in the WL-group.

Early (up to 7 days post-surgery) SAEs: This analysis was to distinguish early SAEs (post-surgical) from those occurring later during follow-up. The analysis showed the most frequent early SAEs in the Safety-Analysis-Set were (with percentage of patients in the FL-group vs WL-group, respectively): hemiparesis (3.0% vs 1.2%), convulsions (2.5% vs 1.2%), and aphasia (2.5% vs 0%). Early SAEs reported in two or less patients comprised arrhythmia, pulmonary embolism, haematoma, psychosis and cyst NOS. None of these differences were of statistical

significance using the Fisher's exact test. No major differences were observed in the frequencies of early serious adverse events between the Safety-Analysis-Set and the Full-Analysis-Set.

SAEs and Previous History of Convulsions, Hemiparesis or Aphasia: Some patients with reported convulsions, hemiparesis, or aphasia as SAEs suffered from these symptoms already before study-surgery, presumably due to interference of the tumour lesion with the normal brain. Data was also provided on the number of patients who developed convulsions, hemiparesis or aphasia only after study but did not demonstrate these symptoms before study surgery.

Of the 17 patients (12 FL; 5WL) with post-surgical convulsions, only 2 had existing symptoms before surgery. By contrast, of the 8 patients (7 FL; 1WL) with aphasia post-surgery, 5 had a history of aphasia before surgery. Five of the 8 cases occurred within 7 days of surgery. Half of patients with reported hemiparesis in both groups had hemiparesis before study surgery. Of the 8 patients in the FL-group, 6 of 8 with hemiparesis developed the condition within 7 days post-surgery; in the WL group the numbers were 2 of 4. Similar results were obtained for the Full-Analysis-Set.

Comment: Although the study report claims that none of the differences between treatment groups in the rates of patients with these SAEs that had their first onset only after study surgery (within 7 days post-surgery) were of statistical significance, in all cases (convulsions, aphasia and hemiparesis) the numbers in the FL-group were consistently higher than in the WL-groups, often markedly so. The small numbers meant the study was underpowered to show a statistical difference, and the descriptive difference is clinical significant.

Drug-related SAE: see Section 8.4.2.1 above.

8.4.3.2. Other studies

Deaths: None of the 10 deaths observed up to 28 (30) days after 5-ALA*HCl treatment could be related to the administration of the study drug. All deaths were considered as consequences of brain tumour surgery or pre-existing cardiac disease.

Serious Adverse Events (SAEs) leading to death: Seventeen patients (8.5%) in the 5-ALA*HCl-treated group and 11 patients (6.4%) in the control group died from a serious adverse event within 180 days after surgery. No particular type of serious adverse event leading to death could be identified to be predominant in one of the treatment groups except for pulmonary embolism (FL vs WL: 8 [4.0%] vs 1 [0.6%]). Most of the embolisms occurred late during follow-up. None of the death in the FL-group was related by the investigator to the administration of the study drug.

Serious Adverse Events (SAEs): The SAEs were reported in two groups. Although NCI CTC criteria were used as well, the SAEs for the four supporting studies and the pivotal study were listed by System Organ Class. In study MC-ALS.32/GLI, the SAEs were classified only by the MedDRA scheme.

In the 3 trials with 28 days of follow-up, the incidence of SAEs was 19%, 22% and 11%. One case of pulmonary embolism was reported (Study 8-I/GLI).

Comment: The longer follow-up period of 180 days in the pivotal trial (3/GLI) showed that the median time of onset of pulmonary embolism after study surgery was 35 days (range 1-150 days), so that 28 days of follow-up as used in the supporting trials did not give a reliable figure for the incidence of pulmonary embolism.

Forty-nine (20.2%) patients in study 32/GLI experienced serious adverse events. Most SAEs were nervous system disorders (12.8%).Twenty-four (9.9%) patients experienced SAEs within 48 hours after surgery. One case of pulmonary embolism (0.4%) was reported.

Neurological SAEs in Study MC-ALS.32/GLI: The most frequent neurological SAEs up to 6 weeks were hemiparesis (15/243, 6.2%), hemiplagia (sic) (7/243, 2.9%), aphasia (3/243, 1.2%). The frequency of epilepsy was 1 (0.4%).

Comment: For comparison, in study MC-ALS.3/GLI, as described above, adverse events coded as convulsions were reported as SAEs in 12 patients (6.0%) in the FL-group and in 5 patients (2.9%) in the WL-group. The other most frequently reported neurological SAEs were convulsions coded as Grand Mal (FL-group: 3.5% vs WL-group: 2.9%), hemiparesis (FL-group: 4.0% vs WL-group: 2.3%), aphasia (FL-group: 3.5% vs WL-group: 0.6%).

The comparison of the two studies shows that the frequency of convulsions was higher in the pivotal study (9.5% cf 0.4%), hemiparesis plus hemiplagia (sic) was higher in Study 32/GLI (8.1% cf 4.0%) and aphasia higher in the pivotal study (3.5% cf 1.2%).

It is noted that the duration of follow up in the two trials with the largest numbers of patients (3/GLI and 32/GLI) were different, up to 180 days and 42 days respectively. Therefore a comparison of the frequency of SAEs may not be strictly valid, and the higher frequency of SAEs overall in study 3/GLI for patients in the FL arm (29.9%) may reflect the longer follow-up compared to those in study 32/GLI (20.2%). However the higher frequency of convulsions and of aphasia in the pivotal study is not explained. The higher frequency of hemiparesis/hemiplegia in Study 32/GLI are also unexplained but may have resulted from the surgery of less skilled surgeons in that community study compared to the pivotal study that was in major neurological centers.

8.4.4. Discontinuation due to adverse events

8.4.4.1. Pivotal studies

Since the test drug was administered as a single dose, discontinuation does not apply.

8.4.4.2. Other studies

Since the test drug was administered as a single dose, discontinuation does not apply.

8.5. Laboratory tests

Laboratory values were standardised across patients with regard to their units. The laboratory normal ranges (LLN = lower limit of normal and ULN = upper limit of normal) used for all laboratory parameters and all patients are those provided by the laboratory facilities at each study centre. For applicable parameters, the NCI Common Toxicity Criteria (version 1.0) were incorporated.

In the pivotal study, parameters were determined at baseline (before surgery) and 24 hours, 7 days, and 6 weeks after surgery. The average number of missing values was < 5% at baseline, 5-15% at 24 hours and 7 days, and 20-40% at 6 weeks. In Study MC-ALS.32/GLI in which a significant numbers of patients (245) were to be assessed for safety, the report of Study 32/GLI, Section 12.4.2, "No laboratory values were routinely measured within the observation period."

Comment: Study 32/GLI was briefly outlined on p38, this evaluation. The sponsor commented on the less rigorous safety follow-up in the study in its response to an enquiry, saying that "A few laboratory values were measured before treatment to decide whether the patient fulfilled the inclusion/exclusion criteria", and that "After treatment, laboratory values were not measured routinely but could be measured if clinically indicated. Laboratory values out of normal range were reported as adverse event". This procedure was justified by

the results of the lack of significant drug-related abnormalities in the pivotal study.

8.5.1. Liver function

8.5.1.1. Pivotal studies

There was a slight imbalance in the percentage of patients with normal ALT serum levels at baseline in favour of the 5-ALA group (74.1% vs 61.8%). Twenty four (24) hours after surgery, serum transaminase levels (ALT; AST), gamma-GT as well as total bilirubin increased in more patients in the experimental arm than in the control group. This difference in total bilirubin disappeared within 1 week after surgery. There were more patients with elevated liver enzymes CTC grade 1/2 as well as grade 3/4 in the experimental arm 24 hours (and 7 days) after surgery. These differences had disappeared within the 6 week observation period.

8.5.1.2. Other studies

Study MC-ALS.20/BV: Two patients had high values for ALT outside the normal range and one a low value for GGT. Another patient had an elevated bilirubin concentration of 1.82mg/DL). None were considered clinically relevant.

Study MC-ALS.8-I/GLI: After surgery, there was a clear increase of ALT, AST and gamma-GT compared to baseline in all dose groups but most prominent after administration of the highest 5-ALA*HCl dose of 20mg/kg. The mean value for the ALT at the highest dose increased 3.8-fold on day 7 and returned to normal on day 28. THe value for AST rose to 2.4-fold times normal and also was normal by day 28. Total bilirubin did not change markedly over time in all dose groups and increased above the normal range in only a few patients. Most severe deviations of laboratory parameters were graded as not drug-related by the investigator with the exception of ALT/ AST/ gamma-GT/ amylase (14-57% considered as drug-related), and bilirubin and CK (14-29%).

Study MC-ALS.28/GLI: Of the 36 patients in the safety analysis, half had ALT and gamma-GT values above ULN at baseline. AST was normal at baseline in the majority of patients (83.3%) but increased in many patients post surgery. The mean values for ALT, AST and gamma-GT increased after surgery, reaching peak levels (1.3 to 3.5-fold ULN) after one week. Mean values did not return to baseline within the observation period of 28 days.

Study MC-ALS.30/GLI: At screening, 80.0% of the 40 patients in the safety analysis had normal values for ALT/GPT, and 17.5% of patients had elevated values. The fraction of patients with high values for ALT/GPT increased and peaked at day 7 after surgery (40.0% of patients revealing high values). Results should be interpreted with caution for the 6 weeks (45.7% of patients with normal values) visits because the number of patients with missing values was relatively high (42.9%). One patient (2.5%) showed grade III/IV values.

8.5.2. Electrolytes and kidney function

8.5.2.1. Pivotal studies

Approximately 90% of patients in both groups had normal creatinine serum levels at baseline. Values did not change significantly during the follow up visits. Furthermore, there were no noteworthy differences between study arms or changes in electrolytes (sodium, potassium) throughout the observation period of 6 weeks post surgery.

8.5.2.2. Other studies

Study MC-ALS.8-I/GLI: No clinical meaningful changes in serum creatinine, uric acid and electrolytes were found. Blood urea was above the normal range in 29-43% of patients and values remained high until end of follow-up. No differences could be detected between the three dose level groups.

Study MC-ALS.28/GLI: There was no worsening of renal function (as indicated by increased creatinine/urea) after administration of 5-ALA*HCl throughout the 4-week observation period.

Study MC-ALS.30/GLI: Creatinine was within the normal range for most patients (97.5%) at screening without major differences in the number of patients with out of range values for this parameter during follow-up visits apart from a 30.0% and 42.9% missing values 7 days and six weeks after study surgery, respectively. Fraction of patients with normal urea was 92.5% at screening and this did not change the day after study surgery. Results should be interpreted with caution for the following visits because the number of patients with missing values was relatively high (up to 48.6%).

8.5.3. Other clinical chemistry

8.5.3.1. Pivotal study

There were no major differences between treatment groups in the number of patients with CTC grade 1/2 or grade 3/4 toxicity with respect to the following serum parameters: alkaline phosphatase (AP), amylase, creatine kinase (CK), glucose. CTC grade 3/4 toxicity was only observed for the parameter amylase. At baseline, the incidence of grade3/4 abnormalities in the FL and WL groups were 2.5% and 1.7% at baseline, 13.9% and 10.4% 24 hours after surgery, 2.5% and 2.9% 7days after surgery and 0% 6 weeks after surgery. The incidence of Grade 1 or 2 abnormalities at 6 weeks was the same in each arm.

8.5.3.2. Other studies

Study MC-ALS.8-I/GLI: Alkaline Phosphatase (AP) was within normal ranges for all patients in all groups at baseline. Between day 7 and 14, values elevated to upper out of range values in between 14% and 71% of patients. At these timepoints, patients of the highest dose group revealed more patients with elevated values in comparison to the other treatment groups. After 28 days, only 14% of patients had high AP levels in both the moderate and high dose treatment groups.

Amylase was within the normal range for most patients (between 86% and 100%) at baseline. Two days after treatment, between 14% and 43% of patients had high values of amylase. Two weeks after treatment, between 14% and 29% had elevated values. At day 28, values declined to normal ranges for all patients of the lowest and moderate dose group, 14% of patients of the highest dose group showed high values, but there were 43% of patients with missing values at this visit.

Creatinine-kinase (CK) values were normal for all patients in all groups at baseline and this did not change noticeably over the time of study. There were no significant differences between treatment groups.

Between 57% and 86% of patients had elevated values for blood glucose at baseline and this did not change remarkably over the time of the study. LDH values were high at baseline between 14% and 43% of patients and there was no significant change during the period of the study.

Study MC-ALS.28/GLI: Among the out-of range biochemistry parameters, amylase was related to the study drug in between 4.0% (day 14 after study surgery) and 21.4% (day 2 after study surgery) of patients, whereas blood glucose deviations were related to ingestion of 5-ALA only in a single patient.

Study MC-ALS.30/GLI: Amylase was within normal ranges for most patients (87.5%) at screening. The fraction of patients with high amylase increased from 7.5% at screening to 15.0% the day after study surgery. Seven days and six weeks after surgery there were 37.5% and 57.1% of missing values, so interpretation was difficult.

Twenty five (25) % of patients demonstrated elevated values for blood glucose at screening with one patient presenting with grade III/IV toxicity and 70 % of patients showing normal values. The day after surgery, 57.5% of patients revealed high values (32.5% of patients with

normal values). There were too many missings 7 days and six weeks after study surgery to draw definite conclusions at these time points.

8.5.4. Haematology

8.5.4.1. Pivotal studies

At baseline, 87.3% of patients in the 5-ALA group (FL) and 80.9% in the control group (WL) had a normal erythrocyte count that decreased after surgery significantly. Values returned to normal within 6 weeks in about two thirds of patients with no difference between both groups. Similar results were obtained for haematocrit and haemoglobin. About 60% of patients in both groups had high baseline leukocyte counts (approximately 12G/L). Values returned to normal within 6 weeks in approximately 50% of patients only with no significant differences between both treatment groups. Platelet counts were normal in the majority of patients at baseline (<90%). Postoperatively, there was no clear effect with some patients showing a decline of counts while others presented an increase.

Comment: The table presenting these data in the Summary of Clinical Safety shows that after 6 weeks the erythrocyte count, hematocrit and hemoglobin values were normal in only about 50% of patients, not the two-thirds claimed above in the study report.

8.5.4.2. Other studies

Study MC-ALS.8-I/GLI: Erythrocyte, haemoglobin, and haematocrit declined in most patients after surgery and had not returned to normal ranges at the day of last visit (28) in many patients. Leukocyte counts were high at baseline in the majority of patients and declined only slowly after surgery. Platelet count was normal in the majority of patients at baseline, declined after surgery, but returned to normal ranges within 2-4 weeks in the majority of patients. Overall, there were no significant differences between the different dosage groups with respect to haematological parameters. The Summary of Clinical Safety considered that the changes observed over time were most probably due to the blood loss and stress caused by brain surgery.

Study MC-ALS.28/GLI: At baseline, the majority of patients (72.2%) had normal erythrocyte (72.2%) and platelet (83.3%) counts, haematocrit (75%) and haemoglobin (75%) values. All 3 parameters dropped down significantly after operation and did not return in all patients back to normal values within the observation period of 28 days. Leukocytes were high at baseline in half of the patients (52.8%), increased further after operation, and returned to normal values at the end of the observation period in 2/3 of patients.

Study MC-ALS.30/GLI: Fifty five percent (55.0%) of patients had a normal erythrocyte count at screening, and in 45.0% of patients erythrocytes were low. After study surgery, the fraction of patients with low erythrocyte count increased to 82.5% (day 1). There were 57.1% of patients with low erythrocytes 6 weeks after study surgery. A similar pattern of out of range laboratory values was found for hemoglobin and hematocrit. Leucocytes were high in 37.5% at screening. The fraction of patients with leucocytosis increased further until 24 hours after study surgery (65.0% of patients with leucocytosis) with 5.0% of patients showing grade III/IV leucocytosis. The fraction of patients with abnormal values decreased over time and 6 weeks after study surgery, 45.7% of patients had normal values (each 11.4% of patients showed high and low values, respectively).

The majority of patients had normal platelet counts at screening (85.0%). After surgery, the percentage of patients with normal platelet counts decreased to 77.0% (day 1), 60.0% (day 7), and 57.1% (week six). Between 5.0% and 20% of patients had low platelet counts after study surgery. Platelet deviations were of mild/moderate nature. Rates of missing values for hematology parameters were relatively high seven days (35.0%) and six weeks (31.4%) after surgery, so results should be interpreted with caution.

8.5.5. Coagulation tests

8.5.5.1. Pivotal studies

There were neither major differences between treatment groups nor changes during 6-week follow-up nor CTC grade 3/4 toxicity of coagulation parameters, prothrombin time (PT) or partial thromboplastin time (PTT).

8.5.5.2. Other studies

Study MC-ALS.8-I/GLI: Coagulation parameters (PT, PTT) did not reveal any clinically significant pattern of change.

Study MC-ALS.28/GLI: Coagulation parameters (PT, PTT) did not change noticeably during the 4-week observation period.

Study MC-ALS.30/GLI: Prothrombin time (PT) was normal for most patients (95%) at screening without relevant changes at day 1 after study surgery. High values were observed in 5% or less of patients throughout all visits. There were many missing values at 7 days (37.5%) and six weeks (57.1%) after study surgery. Partial Prothrombin time (PTT) was within the normal range in 62.5% of patients at screening. Thirty five percent (35%) of patients presented with low PTT-values. Except for a high rate of missing values 7 days and six weeks after study surgery, there were no significant changes during follow-up compared to the screening distribution.

8.5.6. Electrocardiograph

8.5.6.1. Pivotal study

ECGs were not monitored in studies MC-ALS.3/GLI

8.5.6.2. Other studies

ECG was not monitored in studies MC-ALS.30/GLI and MC-ALS.32/GLI.

Study MC-ALS.8-I/GLI: In each treatment group, 5/7 patients showed abnormal ECG signs at baseline (day -1). However, none of these abnormalities required baseline medication. After administration of the study drug, ECGs taken 30 min, 60 min, and 120 min after administration of the study drug were compared with an ECG taken immediately before administration, whereas the latter ECG and ECGs taken at the following visit days (day 1, 2, and 7) were compared to that of baseline. In each treatment group 6/7 patients revealed changes of the electrocardiogram compared to baseline. These changes were of unspecific nature (e.g. changes of the heart axis, mild bradycardia) and therapeutic intervention was not necessary. No clinical significant differences between the treatment groups could be identified.

Study MC-ALS.28/GLI: No obvious deviations were registered in this study concerning the parameters blood pressure, pulse, oxygen-saturation, blood gas analysis, and ECG.

8.5.7. Vital signs

8.5.7.1. Pivotal studies

Performance Score: To further characterise the impact of 5-ALA-guided (and therefore more complete) brain tumour surgery on the quality of life of patients, the Karnofsky Performance Score (KPS) as well as the NIH-Stroke Score was assessed before and during the study. KPS was assessed 6 weeks after surgery, at 3 months and every three months thereafter (Table 18) Score was assessed before and during the study. The proportion of patients with deteriorated KPS remained nearly constant during the observation period in the FL-group (approx. 35%). In the WL-group the KPS progressively increased from 28.8% to 49.1%. The observed differences were not statistically significant. The study concluded that the more complete tumour surgery in the 5-ALA-group did not result in a worse Karnofsky Performance Score. There was a trend

for less deterioration (35.7 vs 49.1%) and more improvement (25.7 vs 17.5%) of KPS 6 months after surgery in the experimental arm.

Table 18. Categorized changes in KPS relative to baseline (Full Analysis Set)

Visit		FL group	WL group	P value
6 weeks	deteriorated	50 (32.9%)	44 (28.8%)	1.000
	no change	67 (44.1%)	81 (52.9%)	
	improved	35 (23.0%)	28 (18.3%)	
	N	152 (100%)	153 (100%)	
3 months	deteriorated	50 (36.2%)	53 (37.9)	0.9406
	no change	61 (44.2%)	58 (41.4%)	
	improved	27 (19.6%)	29 (20.7%)	
	N	138 (100%)	140 (100%)	
6 months	deteriorated	25 (35.7%)	28 (49.1%)	0.1163
	no change	27 (38.6%)	19 (33.3%)	
	improved	18 (25.7%)	10 (17.5%)	
	N	70 (100%)	57 (100%)	
** Exact Wilcoxon-Mann-Whitney-Test; n = number of non-missing observations*				

NIH-Stroke Score: The score was assessed 48 hours, 7 days, 6 weeks, 3 months, and each additional 3 months after surgery. This score covered 16 single neurologically relevant items with a theoretically maximum (worst) score of 36 points. Table 19 below shows that the percentage of patients with deterioration was slightly higher in the experimental arm during the first three visits (significant at the 48 hours visit). This difference disappeared progressively during the further follow-up. The majority of patients had an NIH-sum score of 0 – 1 only and the change from baseline during follow-up was very small (mostly 1-3 points). Therefore it is questionable whether this score is appropriate for the characterisation of neurological deteriorations after surgery. However, it can be concluded that the more complete tumour surgery in the 5-ALA-group did not result in a meaningful worsening of the NIH-SS.

Table 19. Categorized changes in NIH Sum Score relative to baseline (Full Analysis Set)

Visit		FL group	WL group	P value*
48 hours	deteriorated	45 (26.2%)	25 (14.5%)	0.0183
	no change	85 (49.4%)	94 (54.7%)	
	improved	42 (24.4%)	53 (30.8%)	
	N	172 (100%)	172 (100%)	
7 days	deteriorated	35 (20.5%)	18 (10.7%)	0.0876
	no change	81 (47.4%)	90 (53.3%)	
	improved	55 (32.2%)	61 (36.1%)	
	N	171 (100%)	169 (100%)	
6 weeks	deteriorated	26 (17.1%)	17 (11.3%)	0.2883
	no change	65 (42.8%)	68 (45.0%)	
	improved	61 (40.1%)	66 (43.7%)	
	N	152 (100%)	151 (100%)	
3 months	deteriorated	27 (19.6%)	26 (18.6%)	0.7747
	no change	60 (43.5%)	60 (42.9%)	
	improved	51 (37.0%)	54 (38.6%)	
	N	138 (100%)	140 (100%)	
6 months	deteriorated	16 (22.9%)	13 (22.8%)	0.4862
	no change	32 (45.7%)	21 (36.8%)	
	improved	22 (31.4%)	23 (40.4%)	
	N	70 (100%)	57 (100%)	
** Exact Wilcoxon-Mann-Whitney-Test; n = number of non-missing observations*				

8.5.7.1.1. Other studies

Study MC-ALS.8-I/GLI: Performance Score - All treatment groups had a median KPS of 90 before operation. After a postoperative deterioration (median KPS 80%- 70% -80% 3 days after surgery in the lowest, moderate, and highest dose group, respectively) lasting for approximately five days, the median KPS of all treatment groups increased again to median baseline values.

NIH Stroke Score: All treatment groups had a median score of 1 before operation and this did not change systematically after treatment and throughout follow up. No specific pattern could be identified.

Study MC-ALS.28/GLI: Performance Score - At baseline (day -1), median KPS was 90% (range 70% - 100%). Three days after study surgery, median KPS dropped to 80% with a wide range of values (10% -100%). Median KPS did not change at visits 7 and 14 days after surgery. Twenty-eight (28) days after study surgery, median KPS reached the baseline value again (90%, range 20% -100%). The KPS deteriorated in most patients (72.7%) within 28 days of follow-up. The fraction of patients who improved in KPS was around 9% during the first two weeks after surgery, but increased to 25% at the last study visit (28 days).

NIH Stroke Score: Absolute changes in the NIH score relative to baseline (day -1) and beginning with the second day after surgery, showed an improvement by a maximum of 1 point in the NIH score in 25% of patients. Ten percent of patients improved by a maximum of 2 points during follow-up. On the other hand, 75% of patients deteriorated by a maximum of 1 point within the first week after study surgery but did not change relative to baseline during rest of follow-up.

Study MC-ALS.30/GLI: Performance score - At screening, median KPS was 80% (range 60% - 100%). Seven days after study surgery, median KPS slightly dropped to 75% (range 50% - 90%). Median KPS again slightly decreased to 70% six weeks after surgery with a wide range of values (40% - 100%). This did not change 3 months after surgery. Six months after surgery, median KPS reached baseline levels again (80%), but with a wider range (40% - 100%) compared to baseline. When patients' disease progressed again (radiologically and/or clinically) after study surgery, median KPS was 60% (range 40% - 100%).

NIH Stroke Score: Absolute changes during follow-up in the NIH score for all patients of the Full-Analysis-Set relative to screening showed ten percent of patients improved by a maximum of 2 points, whereas 50% of patients did not change at all. On the other hand, 25% of patients deteriorated by a maximum of 3 points. When progressive disease was diagnosed radiologically or clinically, 25% of patients had a decline in the score by a maximum of 5 points.

Study MC-ALS.32/GLI: Performance score - Seven days after surgery, deteriorations in score were observed in 50.5% of the patients, no changes in 39% and improvements in 10.1% (data missing in 0.5%). Six weeks after surgery, deteriorations in score were observed in 46.3% of the patients, no changes in 34.9% and improvements in 11.0% (data missing in 7.8%). Median score values decreased from 90 at baseline to 80 seven days and 6 weeks after surgery.

NIH Stroke Score: This assessment was not done.

8.6. Post-marketing experience

One Periodic Safety Update Report (PSUR) for the 6 months from 8/3/2008 to 7/9/2008 was submitted. Its conclusion was "There were no reports of suspected adverse drug reactions received by medac during the relevant interval. Therefore no new safety concerns arose from ADR reports during the time period covered by this report. The known safety profile of 5-aminolevulinic acid containing medac products has been confirmed."

The Summary of Clinical Safety states "After marketing authorisation, from January 2008 to December 2008, a total of 1,275 patients have been treated with Gliolan outside of clinical trials (Risk Management Plan for Gliolan Version 7 [Update 16.03.2009]). No case reports of adverse events with Gliolan were received." The SCS does not refer to the Australian Specific Annex (Version 1.0,26 September 2012) to the EU-Risk Management Plan for GLIOLAN (Version 9.0, updated 08 October 2010). In this document, The Risk Management Plan (EU) referred to lists ongoing safety concerns that are included in the following section, 8.7.

Comment: No explanation was given for the absence of PSURs after December 2008. The sponsor should be asked to comment on this is the formal questions.

8.7. Safety issues with the potential for major regulatory impact

Safety issues for the proposed use of GLIOLAN arise from the drug alone and from the associated anaesthesia and surgical procedure. Identification of the safety issue for ALA alone depended on a comparison between the two treatment groups in the pivotal trial, MC-ALS.3/GLI, in which both groups had similar anaesthesia and surgery but only one group received ALA. In the following section, and in the safety conclusions in Section 8.9, the distinction stated is based on this comparison. The following safety issues are also considered in the Product Information section of this evaluation.

8.7.1. Liver toxicity

A dose-dependent mild to moderate increase of the transaminases (ASAT/ALAT) was frequently observed with a higher incidence in the 5-ALA arm of study MC-ALS.3/GLI than in the control group. These laboratory changes were not accompanied by clinical symptoms and are attributed to the anaesthesia and surgery.

Comment: The liver toxicity was also observed in the supporting studies that were not randomized. Recovery occurred in a short time without clinical symptoma.

8.7.2. Haematological toxicity

Anaemia, thrombocytopenia, and leukocytosis were related to the anaesthesia and surgical procedure.

8.7.3. Serious skin reactions

Although photosensitisation is a potentially serious skin reaction of porphyrins, including ALA, this effect was summarised in the pharmacology section and not in the Summary of Clinical Safety. The basis was the results of Study MC-ALS.20/BV (see section 5). The immediate reaction is thought to be due to an irritation of the skin pigments that depends on the 5-ALA dose in the tissue and the applied UVA dose. The late reaction is interpreted as a bronzing effect over time following irritation and not related to any concentration of 5-ALA or PPIX in the skin. The immediate reaction was significantly reduced at 12 and 24 hrs after administration of ALA and had returned to normal at 48hrs. The late reaction could only be measured 12 hrs after administration of ALA and was normal at 24 and 48 hrs. The Product Information provides advice on managing this problem.

8.7.4. Cardiovascular safety

In the studies in this application, cardiovascular events related to ALA administration were uncommon. In a tabulation that present AEs other than SAEs for all trials, only two drug-related events were mentioned, one procedural hypotension and the other deep vein thrombosis, both in trial 32/GLI. SAEs in the pivotal trial included 4 cases (2%) of heart rate or rhythm disorders in the FL arm and 1 in the WL arm, and additionally 2 cases of CVS disorders (1.0%) in each arm (FL and WL).

Comment: The SPC [Australian PI equivalent] has warnings about hypotension, especially in patients with pre-existing heart disease. While hypotension was not a problem in the studies submitted here, the selection criteria of those studies may have reduced the patients at risk. The advice is therefore appropriate. Hypotension was considered in the SPC as a possible effect of both ALA and the procedures involved.

8.8. Other safety issues

8.8.1. Safety in special populations

Intrinsic and Extrinsic Factors: No specific studies or subgroup analyses of patients with special intrinsic factors were performed.

8.8.2. Safety related to drug-drug interactions and other interactions

No studies of specific drug-drug interactions were performed. In all clinical studies, co-administration of 5-ALA*HCl with other potentially phototoxic agents was not allowed. No specific drug interaction was identified within the clinical development programme.

8.8.3. Neurologic adverse events

No evidence was found in the studies submitted that ALA itself caused significant neurological events. However the greater excision of brain tissue during removal when ALA was used was a procedural risk factor, inseparable from the requested use of the drug. Such effects were assessed in two ways, initially from the standard assessment of adverse events; and secondly by assessing the clinical effects of such events as requested by the EMEA and measured by the NIH-Stroke Scale (NIHSS).

Neurological adverse events: Neurological AEs are best assessed in the pivotal trial (3/GLI) in which the control arm (WL) did not use ALA to define the tumour to be resected. Other than SAEs, the most frequent overall neurological AEs in each treatment arms were of similar incidence whereas effects on sensory organs were significantly higher (17%) in the ALA (FL) arm compared to 8.7% in the control (WL) arm. Of these, impaired vision (14.8% cf 7.5%) was significantly more frequent in the ALA (FL arm).

Of SAEs, The most common SAE (experienced by at least two patients in either treatment group) reported within a follow-up period of 180 days after study surgery was convulsions. Adverse events coded as convulsions were reported as SAEs in 12 patients (6.0%) in the FL-group and in 5 patients (2.9%) in the WL-group. The other most frequently reported neurological SAEs were convulsions coded as Grand Mal (FL-group: 3.5% vs WL-group: 2.9%), so that the frequency of convulsions plus Grand Mal episodes was 6.0%+3.5%, ie 9.5% in the FL-group; hemiparesis (FL-group: 4.0% vs WL-group: 2.3%), aphasia (FL-group: 3.5% vs WL-group: 0.6%), stupor (FL-group: 0.5% vs WL-group: 1.7%), and hypertension intracranial (FL-group: 0% vs WL-group: 2.3%)[see comment under *Deaths and other serious adverse events* above].

8.8.4. Training of neurosurgeons in the use of ALA for brain tumour excision

Training of neurosurgeons in the use of ALA to facilitate excision of glioblastoma multiforme is required for efficacy and safety, and was a requirement in the clinical studies submitted.

In the EU such training has been by training courses organised under the auspices of the EANS neuro-oncology committee. Gliolan was to be used only by neurosurgeons who have received training in accordance with [defined] standards.

In Australia, ALA has been authorized for supply by the TGA under the Special Access Scheme for a similar indication. The Sponsor has implemented a training and accreditation procedure for qualified neurosurgeons, which is aimed at risk minimisation and to support safe and effective use of the product. According to the RMP (Australian Annex), this program will continue after commercial launch of Gliolan in Australia. The requirements and training performed to date in Australia have been described.

Comment: Note that the above Australian requirements replace the “hands on” training in the EU with instructions from a DVD and training manual for the reasons given in the submission. Presumably this has been acceptable to the TGA in the Special Access Scheme.

8.9. Evaluator’s overall conclusions on clinical safety

Based on a total of 548 patients given the proposed single dose of 20 mg/kg of ALA in the clinical studies submitted, safety outcomes were determined by two factors, the drug itself and the associated anaesthetic and surgical procedures.

Clinical Safety of ALA: When given as a single dose of 20mg/kg, ALA was a safe drug. The reported drug-related adverse events were of minimal clinical significance except for pulmonary embolism, and easily managed or prevented with appropriate care. They included the following:

Photosensitivity: This occurred during the 24 hours after administration, and was managed by avoiding exposure to strong light for that period.

Pulmonary embolism: Pulmonary embolism might be considered a complication of surgery that was performed in both arms of the trial, but in the pivotal study there was a statistically significant increase (6.5% cf 1.2%; p=0.015) in the incidence of pulmonary embolism in the FL group in which ALA was administered. The reason is unknown. No difference was found in coagulation profiles between the FL and WL groups, and the incidence of thrombosis was similar in each group (FL 1.5%; WL 1.7%).

Comment: As described in Section 8.4.3.2, of this evaluation, the supporting trials, except for 32/GLI, did not provide reliable incidence of pulmonary embolism because the follow-up time of 28 days was too short. In study 32/GLI (follow-up 6 weeks), the incidence of pulmonary embolism was much lower (0.4%) than in the FL arm of the pivotal trial. The reason for the difference is unknown.

Cardiac events: Although hypotension is listed as a risk in the RMP, and in the proposed PI, especially for patients with pre-existing cardiac conditions, cardiac adverse events were not significant safety problems in the pivotal study with no significant difference in incidence in the treatment arm, nor in study 32/GLI, in which they were of infrequent occurrence.

Liver function: Elevation of liver enzymes and bilirubin concentration was seen in the ALA group of the pivotal trial. The former returned to normal 6 weeks after surgery and the latter in one week. No clinical conditions were associated with these abnormalities.

Serum amylase: Abnormally high concentrations of serum amylase (Grade 3 or 4) occurred more frequently in the ALA arm of the pivotal trial , peaking 24hrs after surgery (13.9% of patients affected cf 10.4%), returning to normal in 7 days.

Neurological outcomes associated with the use of ALA, and the anaesthetic and surgical procedures: These procedures were an integral part of the use of ALA for the requested indication. The safety issues that arose were not because of the procedures per se but because ALA allowed greater excision of tumor with consequent risk to normal brain tissue and function. The effect therefore was best measured in the pivotal trial, in which a comparison of neurological outcomes was performed.

Neurological SAEs: The incidence of convulsions, hemiparesis, and aphasia was higher in the ALA group of the pivotal trial. The numbers were too small (underpowered) for statistical comparison but the higher incidence in the treatment arm was consistent. Pre-existing hemiparesis and aphasia predisposed to a similar post-surgical event, but this was not the case for convulsions. Unlike pulmonary embolism, these events largely occurred up to 7 days after surgery.

Clinical neurological assessments after surgery: Two assessments were carried out at the request of the EMEA - the first assessed performance scores as a measure of quality of life; the second determined the NIH-Stroke Score (see Section 8.5.7.1). The more complete tumour surgery in the 5-ALA-group did not result in a worse Karnofsky Performance Score. There was a trend for less deterioration (35.7 vs 49.1%) and more improvement (25.7 vs 17.5%) of KPS 6 months after surgery in the experimental arm. The percentage of patients with deterioration was slightly higher in the experimental arm during the first three visits (up to 6 weeks post-surgery and significant at the 48 hours visit). This difference disappeared progressively during the further 3 monthly follow-up. Results from the NIH stroke score showed that a benefit to patients in the FL group was a reduced “neurological deterioration” (46% vs 29.3% at six months)

Necessary surgical expertise: The need for special training of neurosurgeons to perform the procedures is acknowledged in the Australian Annex for the Risk Management Plan submitted in the application. The claims in the study report of the pivotal trial that no differences were found in the surgical outcomes of participating surgeons and trial centers is disputed (see comment under section 7.1.1.1.12 of this evaluation) so that implementation of the training required is an important safety issue.

9. First round benefit-risk assessment

9.1. First round assessment of benefits

The benefits of the proposed usage of ALA are:

- a statistically significant increase in PFS at 6 months. Of patients treated with ALA , the PFS rate by Kaplan Meier analysis was 35.2% and 21.8% (p=0.02) favouring the treatment arm. No Overall Survival (OS) benefit was shown. However there are reservations about accepting the difference in the number of patients without progression (PFS) at 6 months in the treatment arm as a significant clinical benefit for the following reasons:
  - the PFS was assessed mainly on imaging criteria alone,
  - the difference in the PFS was not significant at 9,12,15 and 18 months although the trend favoured the ALA treatment group,
  - the significant difference in PFS at 6 months was largely due to the maximum separation of the two curves at at this time point
  - the median PFS was the same in both groups.
- of patients in the control group, while 29.3% were "neurologically worse" 6 months after surgery, in the ALA group the number was increased to 46% (p=0.033). This analysis was not part of the study, but was carried out later at the request of EMEA.
- a benefit was originally claimed for patients in the ALA arm in the time to further surgery (re-intervention) following progressive disease. This comparison had problems and the EMEA asked for an assessment of the time to re-intervention after initial surgery. This was done and a benefit was claimed based on a complex statistical method as described.

Conclusion: The clinical benefits in the pivotal study were marginal. Although the usage allowed the surgeon to visualize and excise more tumour, the clinical benefit for the use of ALA as requested was not convincingly shown. A possible benefit was that the requested treatment reduced the number of patients who were neurologically worse after surgery.

9.2. First round assessment of risks

The risks of the proposed usage of ALA are:

- those associated with the drug itself, namely pulmonary embolism, photosensitivity, hypotension, and increased liver enzyme and serum amylase concentrations. Except for pulmonary embolism, they were not serious and were readily managed.
- those associated with the drug and the surgical procedure. The study lacked the power to demonstrate a significant difference between the treatment arms with respect to post-surgical convulsions, hemiparesis and aphasia. While pre-surgical hemiparesis and aphasia predisposed to their occurrence after surgery, this was not the case for convulsions. These adverse effects mainly resolved on follow-up.
- a risk related to the preceding risk was that the extra excision of tissue when ALA was used might result in new or additional neurological deficits. This was shown not to be the case except at 38 hrs post surgery.
- an additional risk was the possibility that the operating surgeon may be inadequately trained or inexperienced in this method of excision, possibly leading to excessive removal of normal tissue in sensitive areas of the brain. The "hands-on" training of neurosurgeons in this procedure in Europe has been replaced by training by manuals and video presentations in Australia. This risk was addressed in the RPM Australian Annex.

9.3. First round assessment of benefit-risk balance

The application with only one Phase III trial would not be approvable if the disease to be treated was less serious and if the patient population was larger because the risks to the patients would outweigh the clinical benefits observed.

However the following circumstances need to be considered: glioblastoma multiforme is a fatal disease for which no curative treatment is available; the disease affects a relatively small numbers of patients; the nature of the treatment with ALA includes complex surgery and is difficult to study in a comparative way; the additional excision of tumour tissue is desirable for a number of reasons provided neurological damage does not result, and this was shown in the pivotal study; the clinical benefits of the treatment, although minor, were consistent in most analyses and subgroups and may represent an underestimation in this difficult study; the treatment was relatively safe.

From a consideration of the above circumstances, the benefit-risk balance is favourable.

10. First round recommendation regarding authorisation

[From a consideration of the above circumstances, the benefit-risk balance is favourable.

The indication (as stated in the PI) should be:

"Gliolan is indicated in adult patients for visualisation of malignant tissue during surgery for malignant gliomas that are glioblastoma multiforme (GBM), and intended for gross macroscopic resection of all visible tumour."[2]]

11. Clinical questions

11.1. Pharmacokinetics

NA

11.2. Pharmacodynamics

NA

11.3. Efficacy

NA

11.4. Safety

The sponsor should comment on the lack of recent PSURs. The latest of those submitted was 7/9/2008 (see Section 8.6).

2 With the exception of comments on the proposed indication, the clinical evaluator's assessment of statements in the draft product literature including the product information are not included in this Extract from the CER.

12. Second round evaluation of clinical data submitted in response to questions

[The sponsor's response to the clinical question was taken into account by the Delegate when preparing the Delegate's Overview (see AusPAR section on *Overall conclusion and risk/benefit assessment*).]

13. References

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