1. SCIENTIFIC DISCUSSION

1. Introduction

Gliomas comprise a heterogeneous group of neoplasms that differ in location within the central nervous system (CNS), age and sex distribution, growth potential, extent of invasiveness, morphological features, tendency for progression, and response to treatments. 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. 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. 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. Even for experienced neurosurgeons, it is very difficult to define the margins of a GBM tumour during surgery. 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. 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.
Aminolevulinic acid hydrochloride (5-aminolevulinic acid HCl; 5-ALA) is a prodrug 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 - 410 nm), the PPIX, which has accumulated selectively in the malignant tissue, emits a red-violet light. This phenomenon is potentially exploitable to guide tumour resection. The therapeutic indication claimed for Gliolan was visualisation of malignant tissue during surgery for malignant glioma (WHO grade III and IV). Gliolan should only be used by experienced neurosurgeons conversant with surgery of malignant gliomas and in-depth knowledge of functional brain anatomy, that have completed a training course in fluorescence-guided surgery (see SPC section 4.2). The recommended dosage is 20 mg 5-aminolevulinic acid hydrochloride per kilogram body weight. The solution should be administered orally three hours (range 2-4 hours) before induction of anaesthesia. Use of 5-ALA under conditions other than the ones used in the clinical trials entail an undetermined risk. Gliolan is contraindicated in case of hypersensitivity to 5-aminolevulinic acid hydrochloride or porphyrins, acute or chronic types of porphyria, or pregnancy (see SPC section 4.3).

2. Part II: Chemical, pharmaceutical and biological aspects

Introduction

Gliolan contains the chemically simple active substance 5-aminolevulinic acid hydrochloride, (5-ALA.HCl) presented in the form of a powder for the subsequent preparation of an oral solution by dissolving in 50 ml water. The product is a sterile lyophilised preparation in colourless glass vials, containing 1.5 g 5-ALA HCl (corresponding to 1.17 g of 5-ALA), and sealed with bromobutyl rubber stoppers. No other ingredients are used.

Active Substance

- Manufacture

Information has been supplied in an ASMF (open & closed). Basically the commercial manufacturing process for the synthesis of 5-aminolevulinic acid hydrochloride utilizes two starting materials

(succinic acid monomethyl ester chloride and hippuric acid) and is performed in three steps: In the first reaction step succinic acid monomethyl ester chloride reacts with hippuric acid in 4-methylpyridine (γ-picoline). This results in the formation crude 2-phenyl-4-(3-carbomethoxypropionyl)-5-oxazolinone. In the second reaction step the oxazolinone is hydrolysed by hydrochloric acid treatment. This results in the formation of carbon dioxide, benzoic acid, methanol and crude 5-aminolevulinic acid hydrochloride. The third step consists of purification of the crude active substance. Discussion of critical manufacturing steps has been provided in the closed part of the ASMF, together with In-Process Controls, Control of Materials, Control of Intermediates, and Process Validation, and these are all considered to be satisfactory.

The chemical structure of 5-aminolevulinic acid hydrochloride has been elucidated by elemental analysis, IR, ^{1}H-, ^{13}C-NMR spectroscopy and mass spectrometry. All spectral data are consistent with the structure of 5-aminolevulinic acid.

- Specification

The specification contains tests by validated methods for assay, identity, impurities etc.

Process impurities originating from each of the starting material and carried over during the synthesis are adequately discussed in the dossier together with a description of degradation impurities. The content of these substances was shown to be consistently low. Batch analyses show good uniformity and compliance with the agreed specification.

- Stability

A stability report on nine batches of 5-aminolevulinic acid hydrochloride drug substance, three of which were pilot scale batches, was presented. The results for the pilot scale batches indicate that no significant change with regard to the assay, the known impurities as well as the known degradation product pyrazin-2,5-dipropionic acid occurred during 14 months storage at -20 °C and 6 months at +5 °C. The stability data support a satisfactory retest period when stored at -20 °C in the proposed container.

Medicinal Product

- Pharmaceutical Development

5-ALA.HCl is freely soluble in water and so an oral solution is a viable option, taking into account the high dose. Particle size and polymorphism are not relevant considering that 5-ALA is in solution, and the solution allows rapid absorption. The company had initially chosen to sterilise the product to accommodate two development programs in different indications. In order to produce a sterile product, filtration sterilisation is necessary as the active substance is unstable to heat. The poor stability in solution necessitated lyophilisation to the dry powder form in order to have a reasonable product shelflife.

- Manufacture of the Product

The manufacturing process briefly consists of the dissolution preparation, sterile filtration (0.45 followed by 0.22 microns) aseptic filling and assembling process and lyophilisation.

- Product Specification

The product specification is simple and includes relevant tests by validated methods for identity (IR/HPLC) , assay (HPLC), clarity, colour, purity (degradation products by HPLC), water content (PhEur), sterility (PhEur), endotoxins , pH, particulate matter, residual solvents (GC) , etc.

Batch analyses show good uniformity and compliance with the agreed specification.

- Stability of the Product

Thirty-six months of stability data are provided for lyophilised preparation batches stored at 25ºC/60%RH and six months of stability data are provided for the lyophilised preparation stored at the accelerated condition of 40ºC/75%RH. The results of the stability analyses support the shelflife and storage conditions as defined in the SPC.

Discussion on chemical, pharmaceutical and biological aspects

Gliolan is a simple formulation of a simple molecule and all quality aspects relating to manufacture and control of the active substance and product have been evaluated and found to be satisfactory.

Perhaps the most interesting feature of the product is that it is presented in a sterile form, arising from an initial development program for a different indication. This sterile form is not strictly essential for an oral solution for the diagnosis of glioma. However, it is not a disadvantage in terms of quality, and the CHMP had no objection.

3. Part III: Toxico-pharmacological aspects

Pharmacology

- Primary pharmacodynamics

5-ALA is 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 precancerous conditions. 5-ALA is also a subject of many non-clinical and clinical trials designed for development of new indications. The idea of therapeutic use of 5-ALA is related to the role of this substance as porphyrins precursor in the heme synthesis pathway. The tissue porphyrin accumulation observed in some diseases (e.g. porphyrias) and induced by excessive 5-ALA delivery from exogenous sources (e.g. medicinal products) generates conditions enabling, light-dependent activation. This activation leads to luminescence and/or porphyrins decomposition with free radicals generation with highly cytotoxic properties.Diagnostic use of 5-ALA is based on the same mechanism of action as therapeutic use. Similarly to therapeutic use, it exploits the well-known ability of some malignant tissues to accumulation porphyrins at higher concentration than observed in normal tissues. This difference gives a chance for obtaining therapeutic and/or diagnostic agent with potentially encouraging selectivity. The possibilities of therapeutic or diagnostic use of 5-ALA are conditioned by huge number of factors, including tumour sensitivity, tumour selectivity, possible dose and route of administration to be used, drug toxicity, energy and method of activation (wave length).

Prove of concept studies were not conducted by the Applicant. A literature review is included in the file and is the basis for the primary pharmacology file of Gliolan. The pharmacodynamic studies revised in the file conceptually support the use of 5-ALA for photodynamic detection of brain tumours and provide evidence that 5-ALA penetrates into brain tumour after systemic administration and that surrounding tissue and normal brain may also be affected, depending on their ability to synthesize PpIX. Studies in normal and animal models of brain tumours as well as in vitro studies in cultured glioma cells have been revised for PpIX formation from 5-ALA, tissue distribution and timings. The formation of the photosensitizer PpIX and its production in tumours or by tumour cells has been shown, in vivo and in vitro. The available information suggests that fluorescence may differ in different tumour cell types and different grade tumours. Maximum detection in tumour sections was observed from 2 to 8 h after 5-ALA administration and its almost total disappearance – after 22 h. Distribution of fluorescence was not restricted to tumour only; increased porphyrin fluorescence was also detected in normal brain tissue, in contralateral white matter, ventricle ependyma and pia mater. Porphyrins accumulation in brain tumours after 5-ALA administration is therefore preferential at the most but not selective. Data about porphyrins distribution to normal and adjacent tissues shows that the possibility of toxic effects to healthy brain exposed to light after 5-ALA administration cannot be excluded. This threat is especially evident in experiments designed for assessment of 5-ALA based photodynamic therapy with exposition to broad spectrum activating light. When used as potentially diagnostic agent under light protection ($\lambda < 635$ nm) 5-ALA seems to be more useful because of probably better safety profile with good visualization properties. The photodynamic threshold dose for 5-ALA induced PpIX after i.v. doses of 20 and 100 mg/kg to induce damage was determined in tissue of normal and tumour-bearing rabbit brain. Little white matter damage was observed. In addition, the concentration of the photosensitizers was significantly lower in white matter than in cortex and tumour. Normal brain structures lacking a blood-brain barrier showed high uptake of the sensitizer. The lack of non-clinical studies simulating faithfully proposed clinical procedure of diagnostic use of 5-ALA is a weak side of pharmacological part of this dossier. If the applicant design and performed animal studies aiming to prove diagnostic utility of 5-ALA had been performed under

conditions similar to operation theatre (the same wave length, the same exposition time for white and filtered light as recommended clinically and used in neurosurgery practice), the non-clinical knowledge about diagnostic potential of 5-ALA would be more useful for judgment about its clinical perspectives. Some of the studies do raise some questions but no answers in relation to the conditions of human use. Aspects/questions on the human use that were considered as not supported/solved by nonclinical data include the selection of the 5-ALA dose, the selection of the timing for administration before surgery and the criteria for definition of the «border» for resection, taking into consideration that normal tissues surrounding the tumour may present increased PpX levels which might have been formed in the tumour and diffused subsequently. In this aspect, the timing of PpX formation and best fluorescence balanced with diffusion into normal tissue could have been discussed. Also, no reference is given to the need to adapt the 5-ALA use to different tumour types and grades. In their response to questions put by CHMP further published information has been provided in rat and rabbit models, which reinforced the basis for a persistence of lucid contrast tumour/normal tissue for at least 6 hours. In addition, a rabbit model of glioma undergoing fluorescence assisted resection in addition to white light has presented a higher survival, and a lower volume of unresected tumour. These results provide some reassurance in relation to previously raised concerns.
IN addition it might be that several of these aspects may have been addressed and solved in the clinical context and therefore further animal studies for prove of concept are in principle, not requested. However, additional nonclinical information may provide useful to improve the efficiency of the photosensitizer use as well as its safety.
A justification for the administration schedule proposed as well as a comment in relation to the existence of possible differences in tumour fluorescence according to the type and grade, and the impact of such differences in tumour visualisation is requested to the Applicant.

- Secondary pharmacodynamics

Secondary Pharmacology: the set of studies from literature which were classified by the Applicant as secondary pharmacology studies are limited. For example, the binding of 5-ALA to different receptor types or interaction with neurotransmitters only covers glutamate, GABA and aspartate. Other receptors relevant in the CNS, particularly in the brain should have been investigated. For photodynamic detection this might be less a problem but if a photodynamic therapy with 5-ALA will be developed, then such aspects should be studied. The studies presented do suggest a possible effect of 5-ALA on GABA, glutamate and aspartate neurotransmission as well as on neuronal glucose uptake. Neuronal effects/toxicity under 5-ALA therapy cannot therefore be excluded on the basis of these interactions. The potential for neuronal toxicity caused by PDD with 5-ALA is therefore not completely clarified. In addition, the two studies in the literature addressing the potential toxicity of photodynamic therapy on the normal brain, conducted in rats and rabbits are not consistent. While in rats (C6 tumours) 5-ALA induced damage in normal tissue has been considered not different from the one induced by photoirradiation alone, in the rabbit (VX2 tumours) a higher sensitivity of the normal vs tumour tissue was observed and neuronal damage has been identified. Though the radiation wavelength for therapy and detection is different, the damaging effect of the light to be used in the last one has not been addressed. In the rat study, animals were randomized to five groups: 1) photo irradiation of cortex (200 J/cm^2, 635 nm argon-dye laser); 2) photo irradiation of cortex (200 J/cm^2) 6 h after intravenous administration of 5-ALA (100 mg/kg); 3) cortical cold injury for edema induction; 4) cortical cold injury with simultaneous administration of 5-ALA (100 mg/kg) and photo irradiation of cortex (200 J/cm^2) 6 hour later; 5) irradiation of cortex (200 J/cm^2) 6 hours after intravenous administration of Photofrin II (5 mg/kg). Tumours were induced by cortical inoculation of C6 cells and 9 days later, MR images were obtained. On Day 10, animals were given 5-ALA (100 mg/kg) and their brains were irradiated (100 J/cm^2) 3 or 6 hours later. Irradiation of brains after administration of 5-ALA resulted in superficial cortical damage, the effects of which were not different from those of the irradiation alone. Induction of cold injury in combination with 5-ALA and irradiation slightly increased the depth of damage. In the group that received irradiation after intravenous administration of Photorfin II the depth of damage inflicted was significantly greater. The extent of damage in response to 5-ALA and irradiation in brains harboring C6 tumours corresponded to the extent of tumour determined from pretreatment MR images. Although the authors didn't use wavelength recommended by the applicant for diagnostic use of Gliolan, results obtained in experimental therapeutic use indirectly revealed relative selectivity of 5-ALA with possible diagnostic application in glioma. Rabbit study has been performed to assess effectiveness of

four different photosensitizers for intracranial photodynamic therapy (PDT) of normal brain tissues and an intracranial tumour was investigated in rabbits, using the photodynamic threshold model.. Results obtained in this study indicated that photodynamic threshold values (number of photons absorbed by the photosensitizer per unit tissue to induce tissue necrosis) were significantly lower in normal tissue than in the tumour. It was concluded, that normal brain is sensitive to porphyrin photodynamic therapy, including 5-ALA induced PpIX photodynamic therapy (5-ALA dose: 100 mg/kg). This is an important safety complain for therapeutic use of 5-ALA for brain tumours PDT. Nevertheless, as suggested above, the excitation light proposed for 5-ALA photodynamic diagnostic of brain tumours is not the same as tested in the above-mentioned study and the observed risk in therapeutic study may be considered as questionable from diagnostic point of view.

- Safety pharmacology programme

Safety pharmacology experiments have been performed by the applicant and cover testing the effects of 5-ALA on major systems such as gastro-intestinal, the central nervous, the renal, and the cardiovascular. The experiments have been conducted with light protection (the only light source didn't emit light with $\lambda < 635$ nm) to avoid possible phototoxic reactions, similarly to conditions recommended for clinical use.

Brief summary of safety pharmacology tests of 5-ALA performed by the applicant

Test type	Dose/Concentration of 5-ALA	Results
Spasmolytic and spasmogenic effects on isolated guinea-pig ileum	0.5 – 500 µg/ml bath fluid	No spasmolytic or spasmogenic effects. The reduction of agonist-induced contractions observed at the highest tested concentration was considered unspecific.
Hexobarbital induced sleeping time in mice after i.v. injection	0; 40; 100; 250 mg/kg (i.v.)	No influence after i.v. injection up to the highest dose tested.
Spontaneous motility of mice	0; 40; 100; 250 mg/kg (i.v.)	No influence of the spontaneous motility.
Diuresis and saluresis in rats	0; 40; 100; 250 mg/kg (i.v.)	No effect on the diuresis. At 250 mg/kg possible marginal saluretic effect (cumulative increase in sodium excretion) and significant increase in potassium excretion during collection period 0 – 1 h.
Cardiovascular and respiratory parameters in the dog	0, 5, 15, 45 mg/kg (i.v.)	NOEL 15 mg/kg. At 45 mg/kg slight decrease in peripheral arterial blood pressure and systolic left ventricular pressure was recorded. In addition, a significant decrease in dp/dt max was observed immediately after dosing. Five minutes after administration, the start values had been reached again. Effects observed were considered to be linked to the i.v. administration and rapidly reversible

Results of applicant-sponsored studies were supplemented by literature reference containing data about 5-ALA influence on neurotransmitters system and phototoxicity. Phototoxic damage to sebaceous glands and hair follicles of mice following systemic administration of 5-ALA was evaluated after intraperitoneal dose of 250 mg/kg. The skin rapidly developed the characteristic red fluorescence of PpIX. Light microscopy of skin taken at 12, 24 and 36 h as well as 2, 3, 4, 6, 10 and 55days after whole body exposure of 5-ALA treated mice to photoactivating light (white light from a 100-watt lamp for 6 h, 21 mW/cm^2) revealed destruction of sebaceous cells, focal epidermal necrosis with a transient acute inflammation and diffuse reactive changes in the keratinocytes. The location and severity of the

phototoxic damage correlated well with the location and intensity of the red fluorescence. 5-ALA induced PpIX was found to photosensitize the epidermis and epidermal appendages when 5-ALA was administered systemically, but does not appear to photosensitize the vascular structures or the connective tissue elements. the safety studies presented, including those conducted by the Applicant may be relevant for the indication proposed for 5-ALA and have identified the potential of tumour photodynamic detection with 5-ALA to induce some level of toxicity at relevant organ systems like the brain and CSN, the skin and intraperitoneal organs (this last due to exposure to white light in the operating room). At the cardiovascular system, the potential of 5-ALA and PpX to bind to HERG channels with the consequent arritmogenic effects have not been studied.

- Pharmacodynamic drug interactions

Pharmacodynamic interaction studies were not performed by the Applicant. From published literature L-tryptophan, reduced glutathione, N-acetylcysteine, melatonin, L-methionine, L -cysteine, mannitol and glycine were found to protect from photodamage induced by 5-ALA (incubation with 0.2 mM) and laser irradiation (4.6 J/cm2, 630 nm) in vitro. The results of pharmacodynamic drug interactions studies described in the literature didn't give an evidence that there are interactions with significant, negative influence on single, diagnostic use of 5-ALA.

Pharmacokinetics

Pharmacokinetic profile of 5-ALA was documented by the applicant by mixed documentation including his own studies and literature publications, and, in dogs, it revealed similar by both p.o. and i.v. route of administration, excluding half-life (prolongation after oral administration comparing to intravenous administration). Also the Pharmacokinetics of 5-ALA administered to dogs is similar to human pharmacokinetics after equivalent oral dose (20 mg/kg) but there is no similarity between PpIX concentrations observed after the same doses administered to dogs and humans (PpIX concentrations in humans were much higher than in dogs). The dog appeared a good animal model for 5-ALA pharmacokinetics from clinical point of view, although not for PpIX pharmacokinetics because of much higher PpIX concentrations observed in humans compared to dogs, after equivalent dose of 5-ALA.

Absorption: the in vivo studies performed by the Applicant and the publication in dogs suggest that 5-ALA is well absorbed and do support the use of the oral route proposed for Gliolan. Its absolute bioavailability in dogs is about 86% after administration dose of 20 mg/kg that is equal to proposed clinical dose. When comparing the dog and the human data it is evident that plasma PpIX reaches much higher proportion vs 5-ALA in man than in dogs. The reason is not clarified. Tmax values were similar in man and dog.

Distribution studies were not performed by the Applicant and a literature review is included in the file. Studies were conducted in (nonbrain) tumour-bearing mice, normal rats, nonbrain- and glioma-bearing rats and in dogs. In tumour bearing mice (thymus aplasia, tubulo papillary adenocarcinoma of human colon) iv treated with 50mg/Kg 5-ALA HCl porphyrin fluorescence was studied and observed in all tissues. Brightest fluorescence was found in the tumour and the maximum contrast of the fluorescence intensity between the tumour and the non-malignant organs was observed at 4h-6h post injection. The kinetics of porphyrin concentration was organ-dependent.

Also in tumour-bearing mice the distribution pattern was independent of the route (iv or oral) of administration and at 3 hours post administration 5mg 5-ALA induced maximal accumulation of porphyrins in normal skin and skin overlaying the tumour.

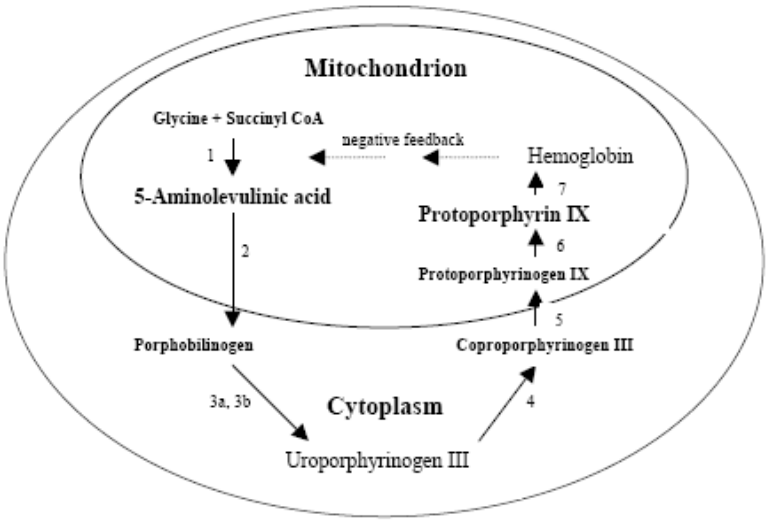
In dogs administered 100 mg/Kg porphyrin concentration in plasma increased in 1 hour and then gradually decreased though it was still markedly increased at 8 hours post administration. Maximum plasma porphyrin concentration was 50 times the baseline value (2 dogs). In most tissues porphyrin concentrations increased progressively up to 6-10 hours post 5-ALA and were higher in the liver than in other tissues. Increased porphyrin concentration were significantly increased in liver, pancreas, prostate, bladder and muscle compared to baseline. Increase in the skin was slight but not significant.

The studies presented (namely the rat studies) have highlighted the ability of 5-ALA-induced porphyrins to distribute in normal and tumour tissues, namely brain tumours, though in normal brain the penetration of 5-ALA is very low due to the blood brain protection. The high levels of 5-ALA derived PpIX in tumours, including brain tumours are related to the metabolising capacity of tumour vs normal cells. The evidence that PpIX fluorescence in the surrounding tissue of the brain tumour do

raise a concern related to the risk of resection of normal tissue and further induction of neuronal damage when Gliolan is used for photodynamic detection.
Studies on the metabolic profile of 5-ALA have not been performed by the applicant but are described in the literature though not extensively. Either *in vivo* or *in vitro* it is evident that PpXI is a major metabolite, which is the basis for the use of exogenous 5-ALA for PDD of tumours, since these will uptake 5-ALA and will metabolise it into the photosensitizer. The uptake of 5-ALA in the normal brain is low, but the presence of the photosensitizer has been identified outside the tumour, possibly due to diffusion from the tumoural tissue. The metabolic pathway of 5-ALA is related to haemoglobin synthesis and is described in the figure below.

Figure 1: Hemoglobin biosynthesis

1: 5-ALA synthase; 2: porphobilinogen synthase; 3a: porphobilinogen deaminase; 3b: uroporphyrinogen III synthase; 4: uroporphyrinogen decarboxylase; 5: coproporphyrinogen oxidase; 6: protoporphyrinogen oxidase; 7: ferrochelatase.



Excretion: after oral and i.v. administration of 5-ALA, PpIX and further metabolites are excreted via the kidneys in the rat and dog, through reabsorption and excretion mechanisms. In man 5-ALA seem to be excreted renally and prophyrins formed in the liver are excreted via urine and bile and partially reabsorbed enterally.

The pharmacokinetic part of the dossier prepared by the applicant contained misleading information concerning values of some non-clinical and clinical pharmacokinetic parameters. Adequate correction and clarification has been provided.

Toxicology

- Single dose toxicity

In single dose toxicity studies on 5-ALA HCl in the rat (p.o.) and mouse (i.p.), no clinical signs, macroscopic abnormalities at necropsy or mortalities were observed at any of the tested dose levels. The LD50 of 5-ALA HCl was determined to be above the highest dose tested (2500 mg/kg).

Single dose i.v. toxicity studies in the mouse and rat revealed intolerance reactions consisting of reduced motility, ataxia, dyspnoea, lateral position and muscular hypertonia. No macroscopic findings were noted at necropsy. The NOEL was 500 mg/kg in mice and 250 mg/kg in rats. The LD50 was calculated to be 1064 mg/kg for male mice, 949 mg/kg for female mice, 949 mg/kg for male rats and 1064 mg/kg for female rats. There was no indication of delayed toxicity. The acute i.v. toxicity thus proved to be similar in both rodent species.

- Repeat dose toxicity (with toxicokinetics)

Oral administration of 5-ALA 2mg/Ml in the drinking water to SWR mice for 5 and 8 weeks and to DBA/2 mice for 8 weeks led to a significant increase in liver porphyrin levels. Decarboxylase insufficiency was also observed in SWR mice after 8 weeks treatment. (*Constantin et al, Biochemical Pharmacology, 52:1407-13, 1996*).

GLP repeated dose toxicity studies were performed in rats (oral and iv) and dogs (iv). The experiments were performed in the dark with a light source which did not produce UV light to avoid photosensitization of animals due to accumulation of PpIX.

In rat using the oral route, 10 animals/sex/dose were treated with 5-ALA HCL for 14 days orally by gavage with 0, 30, 100 and 300 mg/Kg. Additional 5 animals/sex/group were used for recovery (14 days). 6 satellite/sex were treated with the low and the high dose for toxicokinetics (determined at days 1 and 14 of the study, through the evaluation of 5-ALA and PpIX). No mortality has occurred. Body weight was slightly decreased in high dose animals. Body weight gain of males was less 9% than in controls. Total bilirubin activity was dose-dependently increased vs controls in male rats of all dose groups and in female rats of the medium and high-dose groups. ASAT activity was slightly increased at 30 (+6%) and 300 mg/kg (+19%) in male animals. Urinary 5-ALA and urobilinogen were dose-dependently increased in all main group animals. Relative liver weights were dose-dependently increased in both sexes at the medium and high-doses. Microscopy: dose-related bile duct changes were noted in main group males at the medium and high-dose, which were not reversible within the 14 day recovery period. All other afore-mentioned changes had subsided at the end of the 14 day recovery period. The NOEL in this study was below 30 mg/kg.

Using the intravenous route, 10 animals/sex/dose were treated with 0, 125, 250 and 500 mg/Kg 5-ALA HCl for 14 days. A recovery group of 5 animals/sex/dose and a TK group of 6 animals/sex/dose (for low and high dose only) were also included in the study. There were no premature deaths. No effects at the injection sites were reported. High dose animals: from Day 5 onwards all presented ataxia, dyspnoea and a slow gait after injection with 1 minute duration. Slight decreased body weight vs controls was also observed.

The following changes were observed:

-Dose-dependent increased of bilirrubin (significant at high dose M,F)

-increase of cholesterol (dose dependent in M but not in F).

-increased creatinine in all groups

-increased blood urea in mid and high dose groups

-increased total protein for all dose groups

-increased calcium levels at the high dose animals

-dose-dependent increase of ALAT and ASAT activity in mid and high dose groups, M,F.

-increased LDH activity for all male groups and for mid dose female group.

Miniamal to moderate bile duct changes consisting of bile duct proliferation, enlargement of the biliary epithelium, peribiliar fibrosis and inflammatory infiltrates , intraductal plugs and peribiliar bile pigment accumulation, were observed in all males and in 8,9 and 7 females from low, mid and high dose group. These changes were not reversibilized in the 14 Day period. The NOEL in this study was determined as below 125mg/Kg.

There was no accumulation of 5-ALA or PpIX after 14 days of i.v. 5-ALA HCl administration to rats. Further, there was no difference in toxicokinetics between sexes.

In dog, 1 animal/sex/dose were treated with 25 or 75mg/Kg 5-ALA HCl for 7 days by iv route. Vomiting, reduced body weight and food consumption and dose-related changes in clinical and biochemical parameters (increased bile acid, total bilirrubina nd total blood urea, ALAT (GPT), aP, ASAT (GOT) an LDH and decreased potassium) were observed. ECG recording did not reveal abnormalities. On day 7 both animals presented ataxia, reduced motor activity, tremor, reduced body temperature and dispnoea, gelatinous feces and/or yellowish discoloured. The high dose female died

1 day after study termination, and the high dose male was sacrificed 2 days after study termination. Both dogs presented around 20 reddish foci in the left ventricle in the heart. A NOEL was not determined.

3 animals/sex/dose were treated with 5-ALA HCl at the doses of 0, 3, 9 or 27 mg/Kg for 14 days by iv route. Additional 2 animals/sex/dose served as recovery for 14 days period. The study observations included ECG and heart rate recordings at the end of week 2 and at the end of recovery period. Blood pressure was measured in conscious animals at days 1, 14 and 28 in all animals, including the recovery group. No deaths were observed. Vomiting was observed among animals once or repeatedly between 20min and 6 hour after injection.

Slight decrease of body weight was observed in the high dose animals during weeks 1 and 2.

Food consumption was not influenced in animals of the low dose group but was decreased in animals of the other dose groups. Biochemical changes were observed in the animals of the intermediate and the high dose groups. Total bilirubin was increased in males at 9 and 27 mg/kg and significantly increased in females at 27 mg/kg. LAT activity was dose-dependently increased in both sexes at the intermediate and high-doses. SAT activity was increased in males and females at 9 and 27 mg/kg. LDH activity was increased at the high-dose in both sexes. These findings were substance-related and correlated with the hepatic changes noted at histopathology. Macroscopic inspection revealed a black discoloured liver in high-dose females. The finding was considered substance-related and correlated with the hepatic changes noted at histopathology. Decreased liver weights were noted at the high-dose in both sexes. An intrahepatic cholestasis was noted in animals of all tested dose levels, which was dose-dependent in severity, and was characterised by bile plugs in the extended bile canaliculi and was considered treatment-related. There were no left ventricle changes of the heart, as seen in the preliminary i.v. dose-range-finding study in high-dose animals (75 mg/kg).

Body weight reduction, increased ALAT activity and bile duct lesions were not completely reversible within the 14 day recovery period at the intermediate and high-dose. All other afore-mentioned changes had subsided at the end of a 14 day recovery period. The NOEL was determined to be below 3 mg/kg. There was no accumulation of 5-ALA or PpIX after 14 days of i.v. 5-ALA HCl administration to rats. There was no difference in toxicokinetics between sexes.

- Genotoxicity

The genotoxic potential of 5-ALA has been studied by the Applicant through the battery as requested by ICH guidelines. In experiments performed in the dark negative results were obtained and it has been concluded that 5-ALA per se, in absence of light is nongenotoxic. However, in presence of light, white or UV, DNA damage has been observe in L1210 mouse leukemia cells and AS52 CHO cells and formation of the mutagenic marker of oxidative damage 8-OxodGuo has been observed in other cell types in culture and from organs of rats treated with 5-ALA. In parallel, in most experiments porphyrin levels were determined.

- Carcinogenicity

Carcinogenicity studies on 5-ALA or 5-ALA HCl have neither been performed by the applicant nor been reported in literature.

- Reproduction Toxicity

Reproductive toxicity studies of 5-ALA were not performed by the Applicant, but some information exists in the literature and has been included in the dossier. 5-ALA exhibited embryotoxicity in chick embryos in the light, but not dark at concentrations ≥ 3 μg.

- Local tolerance

Local tolerance of aqueous solutions of 5-ALA was evaluated in the rabbit. The experiments were performed in the dark. Local tolerance was very good following intravenous and intraarterial administration. Paravenous, subcutaneous and intramuscular administration caused local irritant effects, which were believed to be linked to the low pH of the test solution (pH=2.4). Also in repeated dose studies in rats and dogs by the iv route no signs of local effects were identified.

- Other toxicity studies

Induction of phototoxicity was investigated in mice treated with 5-ALA HCl i.v. (250 and 750 mg/Kg) and subsequent 1h UV irradiation 4h or 24h after administration, in order to estimate the photosensitizing potential of 5-ALA induced PpIX. I.v.

Administration of 5-ALA HCl followed by an UV irradiation after 4 and 24 h produced phototoxic reactions, the effect was observed at both doses when 1h UV irradiation was performed 4 hours post 5-ALA administration, and in high dose animals only when UV irradiation occurred 24h post 5-ALA administration. After UV irradiation at 4 hours post injection 3/5 low dose animals survived and revealed pronounced phototoxic reaction of edema grade 2. All 5 high dose animals died withing 24h after UV radiation. Necropsy revealed inflammatory reactions of the shoulder skin and eyelids consisting of granulocytic dermatitis, ulceration and epithelial necrosis. No inflammatory or degenerative lesions of the eyeball were observed. UV irradiation 24 hours post 5-ALA injection caused erythema (grade 1 and 2) in all high dose animals at investigation 24h after irradiation and 3 animals at investigation 48h after irradiation. 72h post irradiation 2 hih dose animals presented moderate to severe erythema (grade 3). Low dose animals did not show signs of phototoxicity.

The fluorescence emission kinetics and phototoxic properties of ALA were assessed in C6 glioma cells. No dark toxicity was observed for 5-ALA. Light applied after incubation with ALA led to a reduction in cell survival. Increasing the incubation time increased the phototoxicity of ALA. ALA induced phototoxicity in C6 glioma cells in vitro after short incubation times and the effect was believed to be linked to an increase of the amount of PpIX synthesis. (*Eléouet et al, Photochemistry and photobiology , 71:447-454, 2000*)

In a skin sensitization test in guinea-pigs, no skin sensitizing properties for 5-ALA have been identified. 2% Benzocaine (positive control) promoted a sensitizing reaction in the animals.

Ecotoxicity/environmental risk assessment

The risk posed to the environment by Gliolan has been assessed according to the draft guideline on ERA of Medicinal Products for Human Use CHMP/SWP/4447/00. The $PEC_{surfacewater}$ was calculated as 0.0002µg/L not requiring further studies.

Discussion on the non-clinical aspects

The acute toxicity of 5-ALA has been shown to be very low in rats and mice treated through different routes of administration, including the oral route and ip and iv routes. The experiments were performed in the absence of UV light, in the dark to avoid the production of PpIX. This seems to be a disadvantage since patients situation will not be the same. Patients are expected to be exposed to white light during operation and therefore at least acute toxicity studies mimicking the intended clinical situation would have been meaningful. In this perspective, the information provided by these single dose studies only concerns 5-ALA HCL and not the activated photosensitizer that will be formed in the clinical setting. Again, this information should have been collected before the administration in man and therefore, since clinical data is available currently, no additional studies are requested. GLP repeated dose toxicity studies of up to 2 weeks duration were performed in rats (oral and iv) and dogs (iv). In rats 5-ALA orally administered for 2 weeks increased total bilirubin (dose-dependent), ASAT activity. Urinary 5-ALA and urobilinogen were dose-dependently increased in all main group animals. Relative liver weights were dose-dependently increased in both sexes at the medium and high-doses. Microscopic observations included dose-related bile duct changes noted in males at the medium and high-dose, which were not reversible within the 14 day recovery period. The NOEL in this study was below 30 mg/kg corresponding to AUC levels similar or below those obtained in man with the proposed clinical dose. In the iv 2 weeks rat study dose-dependent increased of bilirrubin, creatinine, blood urea, total protein, calcium levels, ALAT and ASAT activity and LDH activity were observed. Soft dark masses were seen in the caecum with dose-dependent incidence with no histological correlation. The meaning of the observation has not been clarified. Microscopic evaluation revealed minimal to moderate bile duct changes consisting of bile duct proliferation, enlargement of the biliary epithelium, peribiliar fibrosis and inflammatory infiltrates, intraductal plugs and peribiliar bile pigment accumulation. These changes were not reversibilized in the 14 Day period. The NOEL in this study was determined as below 125mg/Kg, for which dose a systemic exposure to 5-ALA more than 20 times higher than the one observed in man was obtained. The exposure to PpIX was lower than in man. The safety margin could therefore not be determined. In dogs 14-day toxicity study by i.v. route of administration vomiting was observed among animals once or repeatedly between 20min and 6

hour after injection. Clinical Biochemistry changes were observed in the animals of the intermediate and the high dose groups. Total Increased bilirubin, LAT activity, SAT activity, LDH activity, which were considered substance-related and correlated with the hepatic changes were noted at histopathology. Also black discoloured liver in high-dose females was considered substance-related and correlated with the hepatic changes noted at histopathology which included intra-hepatic cholestasis in animals of all tested dose levels, which was dose-dependent in severity, and was characterised by bile plugs in the extended bile canaliculi. There were no left ventricle changes of the heart, as seen in the preliminary i.v. dose-range-finding study in high-dose animals (75 mg/kg). Body weight reduction, increased ALAT activity and bile duct lesions were not completely reversible within the 14 day recovery period at the intermediate and high-dose. The NOEL was determined to be below 3 mg/kg which induced a systemic exposure of 5-ALA and PpIX well below (less than twice) the AUC levels to be expected in the clinic. Also repeated dose studies were performed in the absence of UV light, in the dark to avoid the production of PpIX. This does not allow determining the potential toxicity of photosensitised PpX which may be a gap on these studies.

The genotoxic potential of 5-ALA has been studied in an appropriate battery of studies according to ICH. Negative results were obtained in the dark. Together, the information available suggests that 5-ALA may cause photodynamic oxidative damage of DNA and that porphyrins are involved. The effect is observed either in presence of UV or white light and melatonin seem to display protective effect. The oxidative damage of tissues exposed to 5-ALA and PpIX and light, even white light of the operation room may be a possibility. Thus, photogenotoxic potential has been identified under irradiation conditions which may be related to PpIX. This is the relevant aspect and therefore the Gliolan use may have to be considered as potential photogenotoxicant.

Carcinogenicity studies were not performed and are not requested. Reproductive toxicity studies were also not performed but available studies in the literature do suggest embryotoxic, fetotoxic and teratogenic potential.

The published studies sugest 5-ALA to display reproductive toxicity potential, may affect pregnancy and be embriotoxic and teratogenic. The use of 5-ALA is contraindicated during pregnancy.

A revision of studies addressing male and female fertility do not suggest a relevant effect. If less severe indication will be considered later, the reproductive toxicity of Gliolan may have to be revisited and studies may be requested. Also phototoxic properties have been identified for 5-ALA in vitro and in vivo, correlated to the PpIX formation in irradiated cells and tissues, where destruction of sebaceous cells, focal epidermal necrosis, inflammation and keratynocyte changes were observed. Skin lesions were reversible with exception of the reduction of hair follicles.

No effects were reported that could suggest oral intolerance or local effects after oral administration. Local intolerance is therefore not considered as a potential issue for Gliolan.

5-ALA revealed to be phototoxic in vivo and in vitro, in a time-dependent way. In animals, when UV light is applied 4h post 5-ALA administration the phototoxicity is higher, which may be related to the PpIX production kinetics. The same conclusion has been reached in vitro with C6 glioma cells.

A risk to the environment is not anticipated.

In conclusion, if clinical use in the indication requested in the current application is considered beneficial, additional nonclinical studies are not considered as needed.

4. Part IV: Clinical aspects

Introduction

GCP

The Clinical trials were performed in accordance with GCP as claimed by the applicant. The applicant has stated that no clinical trials were conducted outside the community.

Pharmacokinetics

The pharmacokinetics/pharmacodynamics of 5-ALA was studied within the following two trials:

Listing of clinical pharmacology studies

Study ID	Study title	Objectives
MC-ALS.20/BV	Single dose study on the absolute bio-availability of oral doses of 20 mg/kg_{BW} 5-aminolevulinic acid in comparison to 2 mg/kg b.w. intravenous administration in healthy male subjects	Absolute bioavailability IV vs PO; Duration of photosensitisation
MC-ALS.8-I/GLI	Clinical phase I/II study on 5-amino-levulinic acid hydrochloride (5-ALA) for the fluorescence-guided resection of malignant gliomas	Detection of a dose-efficacy relationship between the dose levels and quality of fluorescence in the tumour score

The primary objective of trial MC-ALS.20/BV was the calculation of bioavailability after oral administration.

The study provided the following pharmacokinetic parameters for 5-ALA:

Pharmacokinetic data of 5-ALA*HCl in human volunteers

Parameter	**IV Administration** [Dose: 1 x 2 mg/kg IV]	**PO Administration** [Dose: 1 x 20 mg/kg PO]
	Median (Range)	Median (Range)
$AUC_{0-\infty}$ [mg x h/L]	3.46 (17.94 - 41.17)	34.15 (17.94 – 41.17)
C_{max} [mg/L]	6.77 (2.9 - 8.49)	20.76 (11.65 – 27.67)
t_{max} [h]	0.17 (0.15 – 0.17)	0.76 (0.5 – 1.0)
$t_{1/2}$ [h]	0.71 (0.41 – 1.08)	0.88 (0.79 – 1.34)
$A_{e,ur}$ [mg]	43.73 (15.50 – 73.27)	398.68 300.83 – 673.03)
$A_{e,ur}$ [% of dose]	32.30 (15.72 – 61.62)	30.58 (27.08 –56.61)

Pharmacokinetic parameters of 5-ALA after oral administration of 1 x 20 mg/kg 5-ALA*HCl

Parameter		**Study**	
		MC-ALS.20/BV	**MC-ALS.8/GLI**
Number of patients:		12	7
$t_{1/2}$ [h]	Geo Mean (DF)	0.92 (1.17)	3.05 (2.09)
	Median (range)	0.88 (0.79 - 1.34)	1.94 (1.60 – 10.04)
t_{max} [h]	Geo Mean (DF)	n.a.	0.94 (1.51)
	Median (range)	0.76 (0.50 - 1.00)	1.00 (0.52 – 2.00)
c_{max} [mg/L]	Geo Mean (DF)	20.90 (1.25)	8.27 (1.11)
	Median (range)	20.76 (11.65 - 27.67)	8.24 (7.42 – 9.70)
AUC_{inf} [mg x h/L]	Geo Mean (DF)	33.13 (1.26)	26.91 (1.91)
	Median (range)	34.15 (17.94 - 41.17)	27.14 (20.41 – 34.63)

$A_{e,ur}$ [mg]	Ari Mean (DF) Median (range)	420.62 (108.16) 398.68 (300.83 - 673.03)	Not determined
$A_{e,ur}$ [% of dose]	Ari Mean (DF) Median (range)	33.62 (7.93) 30.58 (27.08 – 56.61)	Not determined
Geo Mean = geometric mean; DF = dispersion factors; Ari Mean = arithmetic means; SDEV = standard deviation			

Pharmacokinetic parameters of PPIX after oral administration of 1 x 20 mg/kg 5-ALA*HCl

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

- Absorption

Absorption of 5-ALA occurs rapidly with maximum plasma concentrations reached 0.623 h (in dogs) and 0.88 h (in healthy volunteers) after oral administration of 20 mg/kg b.w. 5-ALA*HCl. Within the preclinical development programme of 5-ALA*HCl, bioavailability after oral administration of 20 mg/kg 5-ALA*HCl in dogs was found to be 86%. In healthy volunteers, an absolute bioavailability of 100% was calculated based on the $AUC_{0-\infty}$ data. This result was confirmed by comparing the cumulated amount excreted in the urine (Ae,ur) during 12 h following oral or IV administration of 5-ALA*HCl.

- Distribution

A study on the plasma protein binding of 5-ALA showed that protein binding was 12%. Previously published findings indicate that orally administered 5-ALA*HCl is able to penetrate into brain tumour tissue in sufficient amounts to form intracellularly enough PPIX which is necessary to visualise the tumour.

- Elimination

The metabolic fate of endogenous 5-ALA is well known. Aminolevulinic acid dehydratase (ALAD) condenses two molecules of 5-ALA to form the monopyrrole porphobilinogen (PBG). PBG deaminase catalyses the polymerisation of four molecules of PBG to hydroxymethylbilane. Hydroxymethylbilane is further metabolised to uroporphyrinogen I and III (by Uroporphyrinogen cosynthase). Uroporphyrinogen decarboxylase sequentially removes a carboxylic group from the acetic side chains of each of the pyrrole rings to yield coproporphyrinogen. Coproporphyrinogen oxidase removes a carboxyl group from the propionic groups on 2 of the pyrrole rings to yield protoporphyrinogen IX. Protoporphyrinogen oxidase forms protoporphyrin IX (PPIX) by removing 6 hydrogen atoms from protoporphyrinogen IX. Finally, ferrochelatase mediates the insertion of ferrous iron into the porphyrin macrocycle, forming heme. PPIX is the last step before incorporation of ferrous iron. Furthermore, this species is responsible for the fluorescence occurring in tumour tissue after treatment with 5-ALA*HCl. Pharmacokinetics of PPIX were studied within trial MC-ALS.20/BV. Maximum plasma PPIX levels were reached at 4 h after administration. In the next 20 hours, PPIX plasma levels rapidly declined and at 48 hours they were no more detectable.

Following administration of a single 20 mg/kg oral dose of 5-ALA, approximately 30% of the dose has been recovered unchanged in the urine within 12 hours. For the IV dose the value is ca. 32%, confirming the absolute bioavailability result obtained from plasma determinations.

- Dose proportionality and time dependencies

Pharmacokinetic parameters after oral administration of 0.2, 2.0, and 20 mg/kg b.w. 5-ALA*HCl were calculated in a total of 21 patients using non-compartmental procedures.

- Special populations

Special pharmacokinetic studies with 5-ALA*HCl related to intrinsic (e.g., age, sex, race, renal and hepatic impairment) or extrinsic factors (e.g., smoking, concomitant drugs, diet) have not been performed by the applicant. Patients with renal or hepatic impairment as well as patients with known porphyria or hypersensitivity to porphyrins had been excluded from all clinical trials with 5-ALA*HCl.

- Pharmacokinetic interaction studies

The applicant did not perform PK interaction studies.

- Pharmacokinetics using human biomaterials

Pharmacodynamics

- Mechanism of action

Exogenous administration of 5-ALA leads to a selective accumulation of its metabolite PPIX in tumour cells. Upon excitation of PPIX by blue light, red fluorescence is emitted which allows the detection of a malignant lesion. The mechanisms explaining the selective accumulation of PPIX in neoplastic tissue upon systemic 5-ALA administration are not well understood. Possible mechanisms include an increased activity of the PPIX-producing enzyme porphobilinogen deaminase and/or a decreased activity of the PPIX-converting enzyme ferrochelatase in tumour cells compared to normal cells, as well as the reduced availability of iron in tumour cells.

- Primary and Secondary pharmacology

In MC-ALS.20/BV the extent and duration of skin photosensitisation after oral administration of 5-ALA*HCl was investigated within this trial in a total of 21 patients. This was done by measuring the Minimal Erythema Dose (MED) and the corresponding PPIX plasma concentrations. These results will be shown in this section. The trial has been performed in healthy male Caucasian subjects aged 18-55 years. A total of 21 healthy volunteers were included into this part of the study. A test described by *Filbeck et al.* was used to evaluate the photosensitisation of the skin.5 In brief, small skin areas on the dorsum and the gluteal region (which were not usually exposed to sun light) were exposed to a progressively graded series of defined UVA-light doses (n = 8; 5-56 J/cm²; light intensity: approximately 60 mW/cm²; wave length 330-450 nm) one day before (baseline) as well as 12, 24 and 48 h ± 30 min after oral administration of 20 mg/kg 5-ALA*HCl. The dose applied to the skin depended on the distance between the UV light source and the skin, was auto-regulated by the light source device and checked by a sensor placed on the skin. Immediate and late reactions were determined 16 min and 24 ± 0.5 h after starting the application of the light. 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 Minimal Erythema Dose (MED) was defined as the dose of irradiation after which a minimal skin reaction was visible in the respective area. The skin reaction was observed by two distinct sub-investigators (one for the immediate reaction and another for the late reaction after 24 hours). The late skin reaction (24 hrs after irradiation) was observed by a physician who was blinded for the result of the immediate reaction directly after irradiation.

Furthermore, PPIX levels in plasma were determined at baseline (one day before administration of 5-ALA) and 12, 24 and 48 h ± 30 min after oral administration of 5-ALA*HCl.

All subjects who received medication and who had at least one evaluation of MED were included into the analysis. Individual MEDs were compared to baseline and described statistically by arithmetic means, standard deviation, coefficient of variation, ranges, and medians.

12 and 24 hours after administration of 5-ALA*HCl, MED measured shortly after end of irradiation (immediate reaction) was significantly reduced compared to baseline ($P < 0.0001$ [RM-ANOVA

test*]). 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 could only be observed 12 hours after administration of 5- ALA*HCl.

Photosensitisation of the skin, immediate and late reaction (arithmetic mean ± SD) together with PPIX plasma concentrations (arithmetic mean) in 21 healthy volunteers after oral administration of 20 mg/kg b.w. 5-ALA*HCl

Time-point of irradiation	**PPIX plasma level**	**Immediate reaction**		**Late reaction**	
	[µg/L]	MED (J/cm²)	x/x_0	MED (J/cm²)	x/x_0
Baseline (x_0)	< LLQ	18.19 ± 4.38	1.00 ± 0.00	23.81 ± 7.59	1.00 ± 0.00
12 hrs a.a.	104.44	7.38 ± 3.41	0.42 ± 0.19	6.05 ± 2.22	0.28 ± 0.14
24 hrs a.a.	10.12	8.52 ± 3.39	0.50 ± 0.25	21.71 ± 7.16	1.03 ± 0.54
48 hrs a.a.	< LLQ	17.33 ± 5.49	0.98 ± 0.32	28.00 ± 12.87	1.32 ± 0.83
*MED = minimal erythema dose; LLQ = lower limit of quantification; a.a. = after administration of 5-ALA*HCl*					

Correlation of PPIX plasma concentrations and MED values (immediate and late skin reaction) were analysed for the time point 12 h after administration of 5-ALA*HCl with both parametric (Pearson) as well as nonparametric (Spearman) measures.
No correlation could be found with both methods

Discussion on Clinical Pharmacology

5-aminolevulinic acid (5-ALA), the active substance of Gliolan, is a natural biochemical precursor of heme that is metabolised in a series of enzymatic reactions to fluorescent porphyrins, particularly protoporphyrin IX (PPIX). 5-ALA synthesis is regulated by an intracellular pool of free heme via a negative feedback mechanism.

Systemic administration of 5-ALA results in an overload of the cellular porphyrin metabolism and accumulation of PPIX in various epithelia and cancer tissues. Malignant glioma tissue (WHO-grade III and IV, e.g. glioblastoma multiforme, gliosarcoma or anaplastic astrocytoma) has also been demonstrated to synthesise 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, oligodendroglioma) 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 overexpression of membrane transporters in glioma tissue.

After excitation with blue light (λ=400-410 nm), PPIX is strongly fluorescent (peak at λ=635 nm) and can be visualised after appropriate modifications to a standard neurosurgical microscope.

Fluorescence emission can be classified as intense (solid) red fluorescence (corresponds to vital, solid tumour tissue) and vague pink fluorescence (corresponds to infiltrating tumour cells), whereas normal brain tissue lacking enhanced PPIX levels reflects the violet-blue light and appears blue.

In a phase I/II-trial including 21 patients, a dose-efficacy relationship between the dose levels and the extent and quality of fluorescence in the tumour core was detected: Higher doses of 5-ALA HCl

enhanced the fluorescence quality and the fluorescence extent of the tumour core compared to demarcation of the tumour core under standard white illumination in a monotone, non-falling fashion. The highest dose (20 mg/kg body weight) was determined to be the most efficient.

A positive predictive value of tissue fluorescence of 84.8 % (90 % CI: 70.7 %-93.8 %) was found. This value was defined as the percentage of patients with positive tumour cell identification in all biopsies taken from areas of weak and strong fluorescence. The positive predictive value of strong fluorescence was higher (100.0 %; 90 % CI: 91.1 %-100.0 %) than of weak fluorescence (83.3 %; 90 % CI: 68.1 %-93.2 %). Results were based on a phase-II trial including 33 patients receiving 5-ALA HCl in a dose of 20 mg/kg body weight. The resulting fluorescence was used as an intraoperative marker for malignant glioma tissue with the aim of improving the surgical resection of these tumours.

The main PK characteristics have been determined in a very scant way. Only two studies have been performed: one absolute bioavailability study at 0.2 mg/kg i.v. and 20mg/kg p.o.. This study helped characterise 5-ALA and PPIX pharmacokinetics. However a complete mass balance study has not been performed. The other study tried to establish dose proportionality and characterise the PK of 5-ALA and PPIX in patients. Together these studies provide minimal information for the safe use of 5-ALA in the proposed indication. No studies concerning intrinsic and extrinsic factors have been performed.

General characteristics

This medicinal product shows good solubility in aqueous solutions. After ingestion, 5-ALA itself is not fluorescent but is taken up by tumour tissue (see section 5.1) and is intracellularily metabolised to fluorescent porphyrins, predominantly protoporphyrin IX (PPIX).

Absorption

5-ALA HCl as drinking solution is rapidly and completely absorbed and peak plasma levels of 5-ALA are reached 0.5–2 hours after oral administration of 20 mg/kg body weight. Plasma levels return to baseline values 24 hours after administration of an oral dose of 20 mg/kg body weight. The influence of food has not been investigated because this medicinal product is generally given on empty stomach prior to induction of anaesthesia.

Distribution and Biotransformation

5-ALA is preferentially taken up by the liver, kidney, endothelials and skin as well as by malignant gliomas (WHO grade III and IV) and metabolised to fluorescent PPIX. Four hours after oral administration of 20 mg/kg body weight 5-ALA HCl, the maximum PPIX plasma level is reached. PPIX plasma levels rapidly decline during the subsequent 20 hours and are not detectable anymore 48 hours after administration. At the recommended oral dose of 20 mg/kg body weight, tumour to normal brain fluorescence ratios are usually high and offer lucid contrast for visual perception of tumour tissue under violet-blue light for at least 9 hours.

Besides tumour tissue, faint fluorescence of the choroid plexus was reported. 5-ALA is also taken up and metabolised to PPIX by other tissues, e.g. liver, kidneys or skin (see section 4.4). Plasma protein binding of 5-ALA is unknown.

Elimination

5-ALA is eliminated quickly with a terminal half-life of 1-3 hours. Approximately 30 % of an orally administered dose of 20 mg/kg body weight are excreted unchanged in urine within 12 hours.

Linearity/non-linearity

There is dose proportionality between $AUC_{0\text{-inf.}}$ of 5-ALA values and different oral doses of this medicinal product.

Patients with renal or hepatic impairment

Pharmacokinetics of 5-ALA in patients with renal or liver impairment has not been investigated.

Patients with renal or hepatic impairment

No studies have been performed in patients with clinically relevant hepatic or renal impairment. Therefore, this medicinal product should be used with caution in such patients.

Paediatric population

The active substance has not been investigated in children and adolescents. Therefore, this medicinal product is not recommended in the paediatric age group until further data become available.

Elderly patients

There are no special instructions for use in elderly patients with regular organ function.

Clinical efficacy

- Dose response study(ies)

Study MC-ALS.8-I/GLI
Primary objective of this trial was the detection of a relationship between the orally administered 5-ALA*HCl dose and the efficacy of treatment as measured by the extent and quality of fluorescence in the tumour core. Within this study, pharmacokinetic parameters were determined, too.

Pharmacodynamic results
Dose-efficacy relationship was assessed by determining the global fluorescence extent and fluorescence quality of the tumour core at the end of operation after the tumour had been resected. This was done in the following way:
During tumour resection, global fluorescence extent and quality was assessed continuously by two surgeons by repeated switching between the white and fluorescence light mode of the operating microscope. Both surgeons were blinded with respect to the administered 5-ALA*HCl dose. After complete resection of the tumour, the visual impressions of tumour fluorescence during surgery were summarised and assessed in two ways:
- fluorescence extent: estimation, how much of the tumour core identified under white light conditions was fluorescent (0, one-third, two-thirds, 100%)
- fluorescence quality: estimation, if the tumour core was strongly, weakly or not fluorescing under blue light excitation
In case of different assessments by the two surgeons, always the assessment of the first surgeon (which was in all cases the principal investigator Dr. Stummer) was used. The result was judged as successful, if statistical significance was reached with respect to both variables.

Global fluorescence extent and quality in tumour core

Dose level of 5-ALA*HCl	**0.2 mg/kg b.w.**	**2 mg/kg b.w.**	**20 mg/kg b.w.**
Number of patients	7	7	7
Fluorescence quality:			
Strong	0	0	7 (100%)*
Weak	0	6 (86%)	0
None	7 (100%)	1 (14%)	0
Extent of fluorescence:			
0 / 3	7 (100%)	1 (14%)	0
1 / 3	0	5 (71%)	0
2 / 3	0	1 (14%)	0
3 / 3	0	0	7 (100%)

Fluorescence intensity (a.u.) of tumour core; Mean [SD]

Fluorescence quality	0.2 mg/kg b.w.	2 mg/kg b.w.	20 mg/kg b.w.
Strong	-	-	3.0836 (2.1410)
Weak	-	0.1983 (0.1347)	2.3000 (n=1)
None	0.0307 (0.0124)	0.0700 (0.0631)	-

In summary, the visual impression of a stronger fluorescence quality of the tumour tissue was paralleled (and therefore confirmed) by a higher spectrophotometrically measured fluorescence intensity. This correlation was statistically significant for both tumour areas (Jonckheere- Terpstra test: $P < 0.0001$).
Furthermore, pairwise comparisons of fluorescence intensity of the tumour core between the different treatment groups revealed significant differences (Wilcoxon-Mann-Whitney test, $P < 0.001$). Additionally, biopsies were taken from the selected areas of tumour core and margin and inspected histologically for tumour cellularity by the reference pathologist who was also blinded with respect to the treatment group.

This "dose-finding" study allows a clear identification of a target dose – 20mg/kg. However one must note that between 2mg which shows some effect and 20 mg which is consistently picked as the best effect there is a wide range of values. Therefore it is unlikely that 20 mg/kg is the optimal dose.

Study MC-ALS.28/GLI

The aim of this prospective, single-arm, uncontrolled multicentre (n = 4) phase II study 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 total of 39 patients were assigned to undergo fluorescence-guided resection which yielded 33 patients qualifying for the Full-Analysis-Set and 36 patients for the Safety-Analysis-Set.

Treatment

All patients received a single dose (20 mg/kg) of 5-ALA*HCl (MC 506/1) orally 3 hours prior to induction of anaesthesia for surgery of newly diagnosed malignant glioma.

Study endpoints

The primary endpoint was 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.
Diagnosis and main criteria for inclusion (extract):
Males or females aged 18 to 75 years with cranial magnetic resonance imaging (MRI) justifying diagnosis of malignant glioma (WHO grades III-IV), that is, showing distinct ring- or garland-shaped, contrast agent enhancing tumour structures and a core area of reduced intensity on the MRI (central tumour necrosis), but lacking significant non-enhancing tumour tissue (exclusion of a secondary malignant glioma), for whom primary surgical treatment was indicated. Histological sections were assessed by external reviewers blinded to treatment at all times. Patient's had to Karnofsky Performance Scale (KPS) was required to be ≥60% and prior or tumour specific pre treatments were not allowed.
Of 39 patients included into the study, six patients (15.4%) were excluded from efficacy analysis. Reasons for excluding those patients were: ineligible histology (n=2), study drug not administered (n=3), and study surgery not performed (n=1).

The primary efficacy parameter was the positive predictive value of tissue fluorescence, defined as the percentage of patients with positive tumour cell identification in all biopsies taken from areas with 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 mean tumour cellularity per patient (i.e. mean area on a section occupied by tumour cells) in strongly fluorescing biopsies was 79.1% ± 20.1%, whereas mean tumour cellularity in weakly fluorescing biopsies was 30.78% ± 27.88% per patient. A minimum tumour cellularity of 4.5% was

detectable by virtue of its (weak) fluorescence by the surgeon. Taking all biopsies together, mean tumour cellularity with strong or weak fluorescence was 79.1% ± 19.8%.

The number of truly-positive specimens (biopsies) was higher if the quality of fluorescence was strong (n=32) rather than weak (n=25). Consequently, the positive predictive value of strong fluorescence was higher (100.0%; 90% CI: 91.1% - 100.0%) than that of weak fluorescence (83.3%; 90% CI: 68.1% - 93.2%). In total, there were 28 patients where all fluorescing biopsies showed tumour in all biopsies taken from areas of any fluorescence (strong or weak fluorescence) resulting in a positive predictive value of 84.8% (90% CI: 70.7% - 93.8%).

Biopsy-based tumour cellularities stratified by fluorescence quality (Full-Analysis-Set)

Fluorescence quality of biopsies	Nmiss	0% n (%)	1-25% n (%)	26-50% n (%)	51-75% n (%)	76-100% n (%)	Total n (%)
strong	1		7 (7.4)	4 (4.2)	6 (6.3)	78 (82.1)	95 (100.0)
weak	0	7 (7.8)	53 (58.9)	7 (7.8)	8 (8.9)	15 (16.7)	90 (100.0)
any fluorescence	1	7 (3.8)	60 (32.4)	11 (5.9)	14 (7.6)	93 (50.3)	185 (100.0)

Among 185 evaluated biopsies, seven sections did not reveal any tumour cells (false-positive specimens). In all false-positive specimens, the quality of fluorescence in was weak. Strongly fluorescing false-positive biopsies did not occur in this study.

The positive predictive value of tissue fluorescence at the biopsy level, defined as 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%). The positive predictive value was higher, if only strong fluorescent biopsies were taken into account (100.0%; 90% CI: 96.9% - 100.0%) compared to weak fluorescent biopsies only (92.2%; 90% CI: 85.9% - 96.3%).

This study only provides data for the positive predictive value. Nothing was obtained for the negative predictive value. This is understandable on ethical grounds. One would not resect non fluorescent tissue that could be normal just for the propose of study. However the samples were collected in the bulk of the tumour when the areas of clinical interest are the margins. 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 is reassuring. The issue of real sensitivity remains but it less critical for the clinical use.

- Main study(ies)

MC-ALS.3/GLI

METHODS

Study Participants
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 into this study. The location of contrast enhancing tumour should have allowed 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 had to be excluded. Furthermore,

patients with a Karnofsky Performance Score < 70, known porphyria or hypersensitivity to porphyrins, renal or hepatic insufficiency or other malignomas had to be excluded.

Treatments

Patients were randomised to receive or not receive 20 mg/kg 5-ALA*HCl orally, 3 ± 1 hours before induction of anaesthesia. Before launching the study, each participating centre had to treat three patients according to the 5-ALA arm of the study protocol to learn the method of fluorescence-guided tumour resection.

With the intention to exclude any bias with respect to the amount of accumulating contrast agent in the early postoperative MRI (primary endpoint) due to different doses of corticosteroids, according to the study protocol patients were planned to receive at least 12 mg dexamethasone two days prior to surgery and prior to performance of the early postoperative MRI. Postoperatively, conventional external beam radiotherapy commencing within 14 days after surgery was mandatory according to the protocol. 90.9% of patients in the experimental arm and 93.6% of patients in the control group received that treatment. Reasons for not applying radiotherapy were patients refusal, missing data, poor condition, early death. According to the protocol, no postoperative adjuvant chemotherapy was allowed because at the time of protocol development this was not a standard treatment option.

Objectives

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

Outcomes/endpoints

In order to determine how completely tumour can be removed in both treatment arms and if this influences the outcome of patients, the following two primary endpoints were chosen:

- percentage of patients without definite residual contrast-enhancing tumour seen on early (within 72 hours after surgery) postoperative MRI,
- progression-free survival at the 6-month-visit after surgery.

The second primary endpoint was implemented 28 months after study start as a major content of amendment 3 of February 07, 2002. This was done because according to a new CPMP directive "Points to consider on the evaluation of diagnostic agents; CPMP, 2001) "the assessment of the clinical usefulness of a diagnostic agent is essential for establishing a claim for that agent". Radiological progression was defined as the occurrence of a new tumour lesion (volume > 0.175 cm^3) or an increase of residual tumour volume by more than 25% compared to baseline (within 72 hours after surgery) MRI. According to the protocol, the six-month visit was defined as the visit scheduled six months after study surgery with a tolerated time deviation of ±1 month. Progression was defined as radiological progression according to the criteria outlined in the study protocol.

Secondary study endpoints were progression-free survival 9, 12, 15, and 18 months after surgery, volume of residual tumour, overall survival, toxicity of 5-ALA, neurological condition up to 18 months after surgery, and a comparison of fluorescence diagnostics (intraoperative residual tumour) with those of early postoperative MRI (radiological residual tumour).

For evaluating the primary endpoints, MRI scans were evaluated within 72 hours after surgery (for measuring residual tumour) and 3, 6, 9, 12, 15, and 18 months after surgery (for detection of tumour progression). Radiological progression was defined as the occurrence of a new tumour lesion (volume > 0.175 cm^3) or an increase of residual tumour by more than 25% compared to baseline (within 72 hours after surgery) MRI. MRI scans and histological sections of resected tumours were assessed by external reviewers blinded to treatment at all times.

Sample size

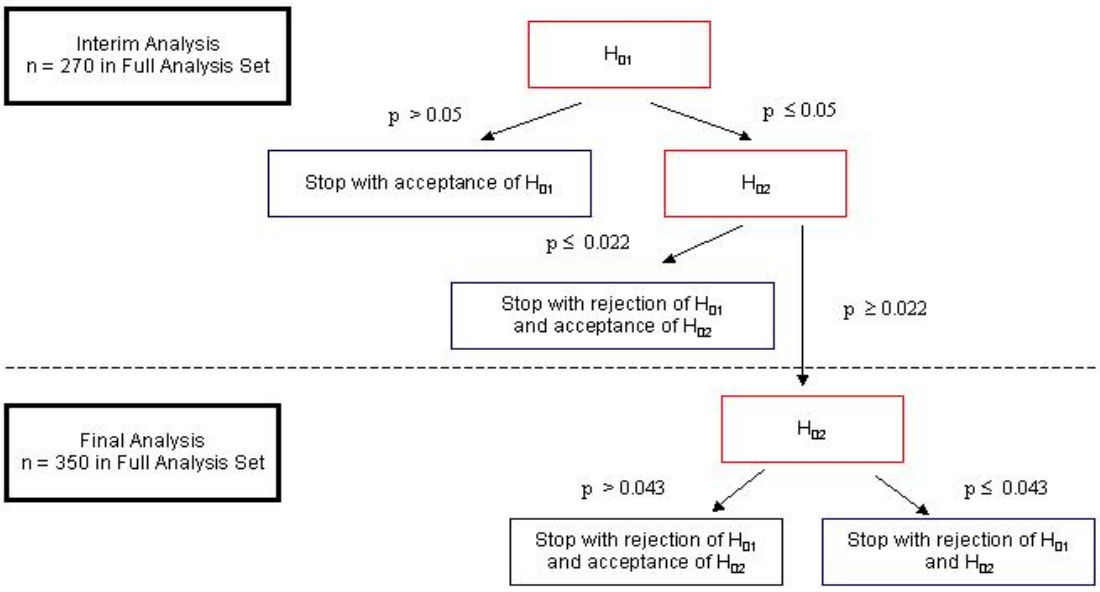
Sample size was determined by assuming a 20% point increase in tumour-free resection (30% in WL-group vs 50% in FL-group) and 15% point increase in progression-free survival rate at the 6-month-visit (25% in WL-group vs 40% in FL-group) within the Full-Analysis-Set. 350 patients were required in the Full-Analysis-Set to provide 80% power within experiment-wise type I error of 0.05. To allow for early stopping, interim analysis for second primary efficacy criterion was foreseen with 270 patients in the Full-Analysis-Set.

Randomisation

Randomisation was done centrally. A dynamic randomisation algorithm was used with respect

for the following 4 factors: surgeon, patient's age (≤ 55, > 55 years), Karnofsky Performance Score (≤ 80, > 80), surgeon's impression with respect to tumour location within or close to eloquent areas or structures (risk present yes or no).

Blinding (masking)
It was impossible to blind the investigator (surgeon) for the two treatments because they differ in their visual effects (fluorescing tissue after 5-ALA treatment versus no fluorescence).
Additionally, the use of a placebo did not make any sense within such a study. Therefore, the control group received no 5-ALA and was operated under conventional white light conditions.
All MRT images taken for analysing the two primary endpoints of the study were evaluated by a central reference radiologist who was blinded with respect to the randomisation of the patient to one of the two treatment arms (rater-blinded study design). Additionally, the reference pathologist who evaluated all tumour specimens was also blinded with respect to the study arm.
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. Both primary efficacy parameters had to be statistically tested in a predefined hierarchical order. H01 denotes the first null hypothesis of equality of the probabilities of tumour-free resection, H02 denotes the second null hypothesis of equality of the probabilities of progression-free survival at 6-month-visit. The nominal two-sided significance levels needed for rejection of the individual null hypotheses are annotated.



RESULTS

Participant flow
At the time of final study analysis, a total of 415 patients were randomly assigned to undergo either fluorescence-guided resection (FL-group) or standard white light surgery (WL-group)
Recruitment

Conduct of the study
19 patients (10.8%) in the experimental arm had a treatment deviation, mostly with respect to the planned dose (n = 5) and the time difference between 5-ALA*HCl administration and induction of anaesthesia (n = 8). One patient in the control group accidentally received 5-ALA*HCl, however was operated under white light conditions only.

90.9% of patients in the experimental arm and 93.6% of patients in the control group received postoperative conventional external beam radiotherapy. 12.5% of patients in the experimental arm and 13.3% of patients in the control arm received adjuvant chemotherapy before radiological proof of tumour progression. The most common type of chemotherapy administered in these patients was temozolomide.

After radiologically proven progression, approximately half of the patients in both groups received chemotherapy (54.5% vs 57.2%). Again, temozolomide was the most common type of chemotherapy applied. 30.1% of patients in the experimental arm and 39.3% in the control arm underwent one reoperation, and further 5.1% and 12.1% two re-operations or did receive other tumour-specific therapies.

Baseline data
Treatment groups were well balanced for the parameters age, KPS, and endangerment of eloquent areas within all data sets analysed as indicated by Table 11.1F. There was no particular surgeon who operated on significantly more patients in a particular treatment arm in any of the data sets analysed.
Overall, patients in the two treatment arms were comparable with respect to age, body weight, and gender.

Parameter	**Study group**		
	Fluorescence light N = 176	**White light** N = 173	**Overall** N = 349
Age (years)			
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)
≤55 years	55 (31.3%)	52 (30.1%)	107 (30.7%)
> 55 years	121 (68.8%)	121 (69.9%)	242 (69.3%)
Gender n (%)			
Female	74 (42.0%)	62 (35.8%)	136 (39.0%)
Male	102 (58.0%)	111 (64.2%)	213 (61.0%)
Body weight (kg)			
Mean (SD)	79.1 (14.87)	79.1 (15.05)	79.1 (14.94)
Median (Range)	78 (50-120)	80.0 (43-119)	78.5 (43-120)

Summary of tumour histology (Full-Analysis-Set)

Tumour histology	**Study**			
	ALS.3/GLI		**ALS.8-I/GLI**	**ALS.28/GLI**
Treatment group	*FL*	*WL*	*All*	*All*
Number of patients	*176*	*173*	*21*	*33*
Missing	0	1 (0.6%)	0	0
WHO grade III tumours	4 (2.3%)	6 (3.5%)	1 (4.8%)	4 (12.1%)
AOA	0	1 (0.6%)	0	0
AO	0	2 (1.2%)	0	0
AA	4 (2.3%)	3 (1.7%)	1 (4.8%)	4 (12.1%)

Tumour histology	**Study**		
	ALS.3/GLI	**ALS.8-I/GLI**	**ALS.28/GLI**

Treatment group	*FL*	*WL*	*All*	*All*
Number of patients	*176*	*173*	*21*	*33*
WHO grade IV tumours	171 (97.2%)	166 (96.0%)	20 (95.2%)	29 (87.9%)
Glioblastoma gliosarcoma	10 (5.7%)	11 (6.4%)	2 (9.5%)	0
Gliobastoma gigantocellulare	5 (2.8%)	2 (1.2%)	0	1 (3.0%)
Glioblastoma multiforme	156 (88.6%)	153 (88.4%)	18 (85.7%)	28 (84.8%)
Other	1 (0.6%)	0	0	0
Astroblastoma	1 (0.6%)	0	0	0
AOA=anaplastic oligo-astrocytoma; AO= anaplastic oligodendroglioma; AA=anaplastic astrocytoma				

Karnofsky Performance Score	**Study**			
	ALS.3/GLI		**ALS.8-I/GLI**	**ALS.28/GLI**
Treatment group	*FL*	*WL*	*All*	*All*
Number of patients	*176*	*173*	*21*	*33*
60	1 (0.6%)	0	0	0
70	15 (8.5%)	19 (11.0%)	2 (9.5%)	5 (15.2%)
80	21 (11.9%)	22 (12.7%)	4 (19%)	9 (27.3%)
90	87 (49.4%)	77 (44.5%)	15 (71.5%)	11 (33.3%)
100	52 (29.5%)	55 (31.8%)	0	8 (24.2%)
Median (range)	90 (60-100)	90 (70-100)	90 (70-90)	90 (70-100)

Numbers analysed

Of 415 patients randomised, 349 patients qualified for the Full-Analysis-Set. 31 patients in the experimental arm (FL) and 35 patients in the control group (WL) were excluded from efficacy analysis mostly because of ineligible histology (other reasons: ineligible preoperative MRI-findings, withdrawal of consent before surgery, no tumour resection performed). Additional three patients in the control group withdraw their consent after surgery or were lost during follow-up. These three patients were included in the Full-Analysis-Set and considered as non-responders. Of those patients in the control group who did not reveal eligible histology or preoperative MRI findings, in contrast to the experimental arm follow up was terminated with respect to safety analysis. This caused an imbalance in the Safety-Evaluation-Set between both treatment arms (201 vs 173 patients).

Data sets analysed

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

4 patients randomized to the FL-group were analysed as WL-patients in the Per-Protocol-Set due to failure of fluorescence mode (#38, #225, #295) or missed application of study drug (#31)

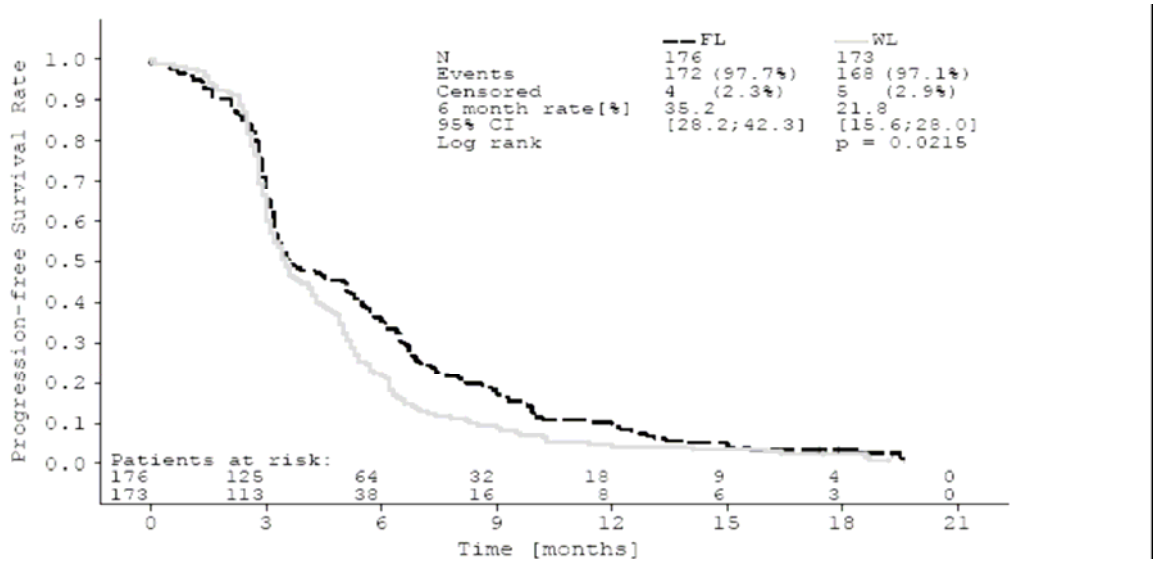
Outcomes and estimation

Significantly more patients in the experimental arm were tumour-free on early postoperative MRI than in the control group (Full-Analysis-Set: 63.6% vs 37.6%; $P < 0.0001$). 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.
Classification of subgroups were chosen according to the criteria for randomisation, i.e. age (≤55 vs >55 years), KPS (≤80% vs >80%), and endangerment of eloquent areas (no vs yes). 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).
Odds ratios of almost all analysed subgroups indicated a higher percentage of patients without residual tumour in the FL-group compared to the WL-group. Pronounced heterogeneities between subgroups could not be observed.
In the Full-Analysis-Set, 20.5% of patients in the FL-group and 11.0% of patients in the WL-group were alive at the six-month visit without progression. This difference was statistically significant using the Chi-square test (p=0.0152) with a crude odds ratio of 2.08 (95% CI: 1.12 - 3.88). In the Per-Protocol-Set, the progression-free survival rate after at the six-month visit was 22.5% in the FL-group and 10.8% in the WL-group. This result was statistically significant (p=0.0047) with a crude odds ratio of 2.39 (95% CI: 1.27 - 4.50).

The progression-free survival rate at six-month visit was further analysed by stratifying for the prognostic factors age (≤55 vs >55 years), KPS (≤80% vs >80%), endangerment of eloquent areas (no vs yes), and combinations thereof.
More patients were alive and progression-free at the six-month visit if they were young (≤55 years: FL-group: 25.5% vs WL-group: 13.5%; p=0.1185), had a better preoperative KPS (>80%: FL-group: 21.6% vs WL-group: 10.6%; p=0.0143), or tumours without endangerment of eloquent brain areas (FL-group: 27.2% vs WL-group: 9.7%; p=0.0060).

Odds ratios of almost all analysed subgroups indicated superiority of the FL-group compared to the WL-group. The beneficial treatment effect was equally present within the subgroups. Odd ratios and their confidence intervals did not change when adjusted for the prognostic factors age, KPS, endangerment of eloquent areas, and combinations thereof, but were all of about the same size as the crude odds ratio. P-values adjusted for the prognostic factors using the Cochran- Mantel-Haenszel test were statistically significant with respect to the treatment effect.
Similar results were obtained for the Per-Protocol-Set.



Ancillary analyses

– *Prognostic factors*

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)

– *Volume of residual tumour*

The median residual tumour volume in the early postoperative MRI was smaller in the E perimental arm than in the control group (0.0 cm³ [range: 0-45.1 cm³] vs 0.5 cm³ [range 0-32.6 cm³]. 75% of patients in the experimental arm had a residual volume of ≤ 0.7 cm³ whereas in the control arm 75% of patients had a residual volume of ≤ 2.1 cm³ (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).

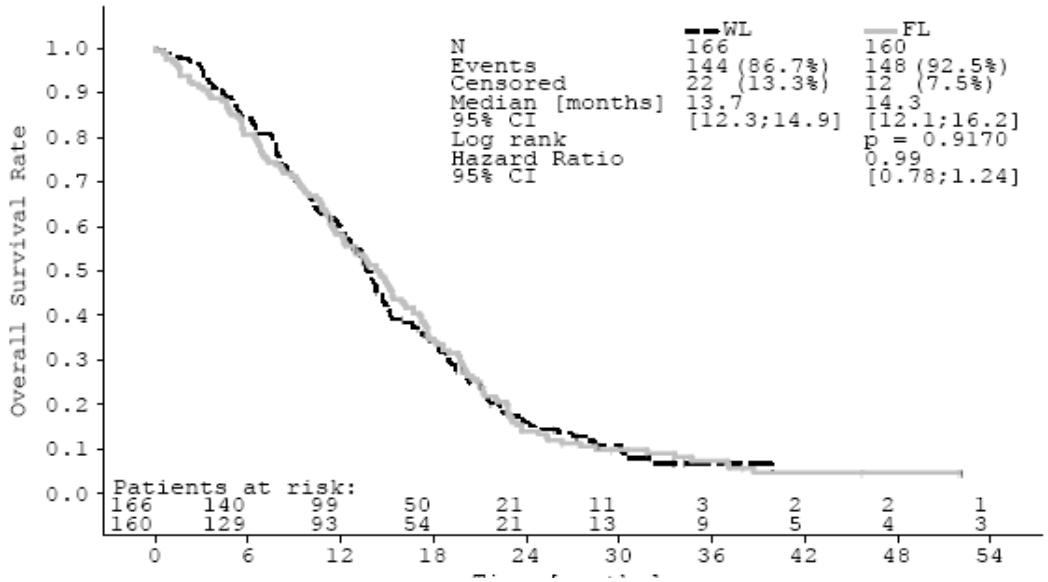
– *Progression-free survival at 9, 12, 15, and 18 months*

PFS rates at 9, 12, 15, and 18 months always favoured the experimental arm with odds ratios clearly above 1, however, the differences did not reach the level of statistical significance.

– *Overall survival*

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). Survival rate one year after study surgery was 58% in both study groups.

Overall Survival – Kaplan Meier estimates



- Clinical studies in special populations

No studies have been performed in the paediatric population. Subgroup analysis of efficacy in patients with special demographic or intrinsic/extrinsic factors
(age; Karnofsky Performance Score, endangerment of eloquent areas) have been performed.

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)

- Supportive study(ies)

- Discussion on clinical efficacy

5-aminolevulinic acid (5-ALA), the active substance of Gliolan, is a natural biochemical precursor of heme that is metabolised in a series of enzymatic reactions to fluorescent porphyrins, particularly protoporphyrin IX (PPIX). 5-ALA synthesis is regulated by an intracellular pool of free heme via a negative feedback mechanism.

Systemic administration of 5-ALA results in an overload of the cellular porphyrin metabolism and accumulation of PPIX in various epithelia and cancer tissues. Malignant glioma tissue (WHO-grade III and IV, e.g. glioblastoma multiforme, gliosarcoma or anaplastic astrocytoma) has also been demonstrated to synthesise 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, oligodendroglioma) 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 overexpression of membrane transporters in glioma tissue.

After excitation with blue light (λ=400-410 nm), PPIX is strongly fluorescent (peak at λ=635 nm) and can be visualised after appropriate modifications to a standard neurosurgical microscope.

Fluorescence emission can be classified as intense (solid) red fluorescence (corresponds to vital, solid tumour tissue) and vague pink fluorescence (corresponds to infiltrating tumour cells), whereas normal brain tissue lacking enhanced PPIX levels reflects the violet-blue light and appears blue.

In a phase I/II-trial including 21 patients, a dose-efficacy relationship between the dose levels and the extent and quality of fluorescence in the tumour core was detected: Higher doses of 5-ALA HCl enhanced the fluorescence quality and the fluorescence extent of the tumour core compared to demarcation of the tumour core under standard white illumination in a monotone, non-falling fashion. The highest dose (20 mg/kg body weight) was determined to be the most efficient.

A positive predictive value of tissue fluorescence of 84.8 % (90 % CI: 70.7 %-93.8 %) was found. This value was defined as the percentage of patients with positive tumour cell identification in all biopsies taken from areas of weak and strong fluorescence. The positive predictive value of strong fluorescence was higher (100.0 %; 90 % CI: 91.1 %-100.0 %) than of weak fluorescence (83.3 %; 90 % CI: 68.1 %-93.2 %). Results were based on a phase-II trial including 33 patients receiving 5-ALA HCl in a dose of 20 mg/kg body weight. The resulting fluorescence was used as an intraoperative marker for malignant glioma tissue with the aim of improving the surgical resection of these tumours.

Overall the trial was properly conducted with a well defined statistical plan that considered an interim analysis and an hierarchical evaluations of the 2 primary endpoints – absence of residual tumour in the post surgical NMR and PFS at 6 months. The advantage of the of using GLIOLAN has been demonstrated. The use of GLIOLAN almost doubled the PFS at 6 months. This advantage was kept at 9 months. At longer FUPs time points the actual numbers analyzable were too low as expected for this disease.

Clinical safety

- Patient exposure

Demographic profile of patients treated with the test product (Safety-Analysis-Set)

Parameter	Study			
	ALS.20/BV N = 21	**ALS.8-I/GLI** N = 21	**ALS.28/GLI** N = 36	**ALS.3/GLI** N = 201
Age (years)				
Mean (SD)	29.4 (6.6)	59.0 (8.7)	56.8 (13.1)	57.6 (10.74)
Median (Range)	(20-45)	59.0 (37-70)	61.5 (21-72)	60.0 (19-73)
Gender n (%)				
Female	-	14 (67%)	17 (47.2%)	82 (40.8%)
Male	21 (100%)	7 (33%)	19 (52.8%)	119 (59.2%)
Body weight (kg)				
Mean (SD)	78.3 (8.9)	75.82 (13.58)	76.4 (11.7)	78.7 (14.75)
Median (Range)	(64-95)	72.50 (52 – 102)	75 (58-105)	79.0 (50-120)

- Adverse events

Adverse reactions observed after the use of this medicinal product for fluorescence-guided glioma resection are divided into the following two categories:

- immediate reactions occurring after oral administration of the medicinal product before induction of anaesthesia (= active substance-specific side effects)
- combined effects of 5-ALA, anaesthesia, and tumour resection (= procedure-specific side effects).

Substance-specific side effects:

Cardiac disorders	Uncommon: Hypotension
Gastrointestinal disorders	Uncommon: Nausea
Skin and subcutaneous tissue disorders	Uncommon: Photosensitivity reaction, photodermatosis

Abbreviations: Very common (≥ 1/10), common (≥ 1/100, < 1/10), uncommon (≥ 1/1,000, < 1/100), rare (≥ 1/10,000, < 1/1,000), very rare (≤ 1/10,000), not known (cannot be estimated from the available data). Within each frequency grouping, adverse reactions are presented in order of decreasing seriousness.

Procedure-related side effects

The extent and frequency of procedure-related neurological side effects depend on the localisation of the brain tumour and the degree of resection of tumour tissue lying in eloquent brain areas (see SPC section 4.4).

Blood and lymphatic system disorders	Very common: Anaemia, thrombocytopenia, leukocytosis
Nervous system disorders	Common: Neurological disorders (e.g. hemiparesis, aphasia, convulsions, hemianopsia) Very rare: Hypesthesia
Cardiac disorders	Uncommon: Hypotension
Vascular disorders	Common: Thromboembolism
Gastrointestinal disorders	Common : Vomiting, nausea Very rare: Diarrhoea
Hepatobiliary disorders	Very common: Blood bilirubin increased, Alanine aminotransferase increased,

	Aspartate aminotransferase increased, Gamma glutamyltransferase increased, Blood amylase increased

In a single-arm study including 21 healthy male volunteers, erythema of the skin could be provoked by direct exposure to UVA light up to 24 hours after oral application of 20 mg/kg body weight 5-ALA HCl. Possibly drug-related mild nausea was reported in 1out of 21 volunteers.

In another single-centre study, 21 patients with malignant glioma received 0.2, 2, or 20 mg/kg body weight 5-ALA HCl followed by fluorescence-guided tumour resection. The only adverse reaction reported in this trial was one case of mild sunburn occurring in a patient treated with the highest dose.

In a single-arm study including 36 patients with malignant glioma, drug-related adverse events were reported in 4 patients (one patient: mild diarrhoea, one patient: moderate hypesthesia, one patient: moderate chills, and one patient: arterial hypotension 30 minutes after application of 5-ALA HCl). All patients received the medicinal product in a dose of 20 mg/kg body weight and underwent fluorescence-guided resection. Follow-up time was 28 days.

In a comparative, unblinded phase-III trial (MC-ALS.3/GLI), 201 patients with malignant gliomas received 5-ALA HCl in a dose of 20 mg/kg body weight and 176 of these patients underwent fluorescence-guided resection with subsequent radiotherapy. 173 patients received standard resection without administration of the medicinal product and subsequent radiotherapy. Follow-up time comprised at least 180 days after administration. At least possibly related adverse reactions were reported in 2/201 (1.0 %) patients: mild vomiting 48 hours after surgery, and mild photosensitivity 48 hours after study surgery. Another patient accidentally received an overdose of the medicinal product (3000 mg instead of 1580 mg). Respiratory insufficiency, which was reported in this patient, was managed by adaptation of ventilation and resolved completely. A more pronounced transient increase of liver enzymes without clinical symptoms was observed in the 5-ALA HCl- treated patients. Peak values occurred between 7 and 14 days after administration. Increased levels of amylase, total bilirubin, and leukocytes, but decreased levels of thrombocytes and erythrocytes were observed, however differences between treatment groups were not statistically significant.

Nervous system disorders

Most frequently observed adverse events in both treatment groups were of neurological origin followed by “sensory organs”. The overall incidence of non-serious events related to the nervous/sensory system was similar in the experimental and control arm of the pivotal trial with the exception of adverse events related to the sensory organs (FL vs WL: 17 vs 8.7%; P = 0.02). This might be an indication of a more aggressive surgery in the FL-arm (see table 2.7.4.2.1.1-4).

Nearly half of all reported AEs related to the neuro-/sensory system occurred during the first week after brain tumour surgery. Percentage of patients with deterioration of the NIH Sum Score were slightly higher in the experimental arm during the first three visits (significant at the 48 hours visit), however, this difference disappeared progressively during the further follow-up

Hepatobiliary disorders

A dose-dependent mild to moderate increase of the transaminases (AST/ALT) can be observed frequently with a higher incidence in the 5-ALA arm of study MCALS. 3/GLI than in the control group (see table 2.7.4.3-13). These laboratory changes peaked between 7 and 14 days after surgery and were not accompanied by clinical symptoms.

Skin and subcutaneous tissue disorders

According to special experiments (measurement of minimal erythema dose) performed in healthy subjects (study MC-ALS.20/BV), photosensitivity reactions can potentially be expected during the first 24 hours after administration of 5-ALA*HCl (table 2.7.2.2.-3). A photosensitivity reaction was reported in one of the glioma patients only. This low rate is probably due to the strict recommendation of reduced exposure to strong light sources up to 24 hours after surgery.

- Serious adverse event/deaths/other significant events

None of the 6 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 (see 2.7.4.2.1.2).
Only two drug-related SAEs have been reported. One patient (# 073; study ALS.3/GLI) suffered from a respiratory insufficiency after administration of an overdosage of 5-ALA which resolved after adaptation of ventilation. Another patient (#033; study ALS.28/GLI) experienced hypotension after administration of 5-ALA which resolved after adequate treatment.
More patients with convulsions [12 (6%) vs 5 (2.9%)], aphasia [7 (3.5%) vs 1 (0.6%)], and pulmonary embolism [13 (6.5%) vs 2 (1.2%) were reported in the 5-ALA-arm of the pivotal trial, however the differences were not statistically significant except for pulmonary embolism (P = 0.015; Fisher's exact test). The reason for the high number of pulmonary embolism in the 5- ALA arm is unclear, since 8 of these 13 events in the experimental arm occurred beyond 4 weeks after surgery and none of these events could be definitely related to the administration of 5-ALA.

AEs/SAEs with respect to the neurosensory system

There were no statistically significant differences in the type and incidence of neurologic AEs (excluding SAEs) related to the nervous system between the experimental and the control arm (see table 2.7.4.2.1.5-1). In contrast, adverse events related to the sensory system was significantly more frequent in the experimental arm (17% vs 8.7%; P = 0.02).
This was mainly due to impaired vision (most frequently reported term: hemianopsia) which was twice as much observed in the 5-ALA group (14.8 vs 7.5%; P = 0.041). However, 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).

There were more patients with aphasia (3.5% vs 0.6%) and convulsions (6% vs 2.9%) reported as SAEs in the experimental arm, however, the number of reported events was low and the differences not statistically significant.
The above discussed small differences did not have a clinically meaningful negative impact on the overall (KPS) and/or neurological (NIH-SS) well-being of the patients.

Karnofsky Performance Score (KPS)

The more complete tumour surgery in the 5-ALA-group did not result in a worse KPS. Quite the contrary, there is a trend for less deterioration and more improvement of KPS six months after surgery in the experimental arm.

NIH-SS

Results of the three clinical studies in glioma patients have shown that after fluorescence-guided resection of malignant gliomas, observed neurological side effects are mostly mild to moderate, reaching at maximum 10 points on the 36 point scale. Indeed, the majority of patients had an NIH-sum score of 0 – 1 only and the change from baseline during follow-up were usually very small (mostly 1-3 points).
In study MC-ALS.3/GLI, percentage of patients with deterioration were slightly higher in the experimental arm during the first three visits (significant at the 48 hours visit), however, this difference disappeared progressively during the further follow-up.

- Overdosage

Within a clinical trial, a 63-year old patient with known cardiovascular disease was accidentally given an overdose of 5-ALA HCl (3000 mg instead of 1580 mg). During surgery he developed respiratory insufficiency, which was managed by adaptation of ventilation. After surgery the patient also displayed facial erythema. It was stated that the patient had been exposed to more light than permitted for the trial. Respiratory insufficiency and erythema completely resolved. In the event of overdose, supportive measures should be provided as necessary, including sufficient protection from strong light sources (e.g. direct sunlight).

Discussion on Clinical Safety

Special warnings and precautions for use have been reflected in the SPC (see section 4.4). 5-ALA-induced fluorescence of brain tissue does not provide information about the tissue's underlying neurological function. Therefore, resection of fluorescing tissue should be weighed up carefully against the neurological function of fluorescing tissue.

Special care must be taken in patients with a tumour in the immediate vicinity of an important neurological function and pre-existing focal deficits (e.g. aphasia, vision disturbances, paresis etc.) that do not improve on corticosteroid treatment. Fluorescence-guided resection in these patients has been found to impose a higher risk of critical neurological deficits. A safe distance to eloquent cortical areas and subcortical structures of at least one cm should be maintained independent of the degree of fluorescence.

In all patients with a tumour in the vicinity of an important neurological function, either pre- or intraoperative measures should be used to localise that function relative to the tumour in order to maintain safety distances.

After administration of this medicinal product, exposure of eyes and skin to strong light sources (e.g. operating illumination, direct sunlight or brightly focused indoor light) should be avoided for 24 hours.

Co-administration with other potentially phototoxic substances (e.g. tetracyclines, sulfonamides, fluoroquinolones, hypericin extracts) should be avoided (see also SPC section 5.3).

Within 24 hours after administration, other potentially hepatotoxic medicinal products should be avoided.

In patients with pre-existing cardiovascular disease, this medicinal product should be used with caution since literature reports have shown decreased systolic and diastolic blood pressures, pulmonary artery systolic and diastolic pressures as well as pulmonary vascular resistance.

One case of an increased phototoxic reaction (severe sunburn lasting for 5 days) has been reported in a patient after co-administration of 5-aminolevulinic acid and a hypericin extract (a known phototoxic agent).

There are no adequate data from the use of this medicinal product in pregnant woman. Some limited animal studies suggest an embryotoxic activity of 5-ALA plus light exposure (see SPC section 5.3). Therefore, this medicinal product should not be used during pregnancy.

It is unknown whether 5-ALA or its metabolite PPIX are excreted in human breast milk. The excretion of 5-ALA or PPIX in milk has not been studied in animals. Breast-feeding should be interrupted for 24 hours after treatment with this medicinal product.

This medicinal product has no influence on the ability to drive and use machines.
In the event of overdose, supportive measures should be provided as necessary, including sufficient protection from strong light sources (e.g. direct sunlight).

5 Pharmacovigilance

Detailed description of the Pharmacovigilance system

The CHMP considered that the Pharmacovigilance system as described by the applicant fulfils the legislative requirements.

Risk Management Plan
The MAA submitted a risk management plan, which included a risk minimisation plan.

Summary of the risk management plan

Safety concern	**Proposed pharmacovigilance activities**	**Proposed risk minimisation activities**
Substance-specific adverse events		
Hypotension	Routine pharmacovigilance	Warning in section 4.4 of the SPC for use in patients with pre-existing cardiovascular disease Listed as undesirable effect in section 4.8 of the SPC
Nausea	Routine pharmacovigilance	Listed as undesirable effect in section 4.8 of the SPC
Photosensitivity reaction; Photodermatosis	Routine pharmacovigilance	Warning in section 4.4 of the SPC to reduce exposure of eyes and skin to strong light sources and warning for co-administration with other potentially photoxic substances Listed as undesirable effect in section 4.8 of the SPC.
Procedure-related adverse events		
Anaemia; Thrombocytopenia; Leukocytosis	Routine pharmacovigilance	Listed as undesirable effects in section 4.8 of the SPC
Neurological disorders (e.g. hemiparesis, aphasia, convulsions, hemianopsia	Routine pharmacovigilance	Restriction in 4.2 of the SPC that Gliolan should only be used by neurosurgeons that have attended a training course in fluorescence-guided surgery. Warning in section 4.4 of the SPC for special care in patients with tumours in the vicinity of an important neurological function Listed as undesirable effects in section 4.8 of the SPC. Restricted prescription Training courses for neurosurgeons;
Hypoaesthesia	Routine pharmacovigilance	Listed as undesirable effect in section 4.8 of the SPC
Hypotension	Routine pharmacovigilance	Warning in section 4.4 of the SPC for use in patients with pre-existing cardiovascular disease Listed as undesirable effect in section 4.8 of the SPC
Thromboembolism	Routine pharmacovigilance	Listed as undesirable effect in section 4.8 of the SPC
Nausea, vomiting	Routine pharmacovigilance	Listed as undesirable effect in section 4.8 of the SPC
Diarrhoea	Routine pharmacovigilance	Listed as undesirable effect in section 4.8 of the SPC
Blood bilirubin increased; ALAT increased; ASAT increased; γ-GT increased	Routine pharmacovigilance	Warning in section 4.4 of the SPC to avoid co-administration of other potentially hepatotoxic medicinal products within 24 hours after administration of Gliolan Listed as undesirable effect in section 4.8 of the SPC
Blood amylase increased	Routine pharmacovigilance	Listed as undesirable effect in section 4.8 of the SPC

The CHMP, having considered the data submitted in this MA application is of the opinion that the following risk minimisation activities are necessary for the safe and effective use of the medicinal product: See as detailed in Section 2.3 of this CHMP Assessment Report. Risk minimisation in the use of Gliolan requires knowledge on the theory behind the Glioma-fluorescence-guided surgery technique and the accurate performance of fluorescence-guided resection, especially regarding neurological serious adverse events noted during that study. Risk minimisation also requires knowledge on the risk/benefit ratio of resection in malignant glioma surgery per se and the pitfalls involved in this type of surgery in general. Therefore, the use of Gliolan in glioma surgery requires adequate training (see SPC section 4.2).

6 Overall conclusions, risk/benefit assessment and recommendation

Quality

The Quality of this product was considered to be acceptable when used in accordance with the conditions defined in the SPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way. There are no unresolved quality issues that could have an impact on the benefit/risk balance for this product.

Non-clinical pharmacology and toxicology

Standard safety pharmacology experiments were performed under light protection in the mouse, rat and dog. 5-ALA HCl administration does not influence the function of the gastro-intestinal and central nervous systems. A slight increase in saluresis cannot be excluded.

Single administration of high doses of 5-ALA HCl to mice or rats leads to unspecific findings of intolerance without macroscopic abnormalities or signs of delayed toxicity. Repeat-dose toxicity studies performed in rats and dogs demonstrate dose-dependent adverse reactions affecting changes in bile duct histology (non-reversible within a 14 day recovery period), transient increase in transaminases, LDH, total bilirubin, total cholesterin, creatinine, urea and vomiting (only in dogs). Signs of systemic toxicity (cardiovascular and respiratory parameters) occurred at higher doses in the anaesthetised dog: at 45 mg/kg body weight intravenously a slight decrease in peripheral arterial blood pressure and systolic left ventricular pressure was recorded. Five minutes after administration, the baseline values had been reached again. The cardiovascular effects seen are considered to be related to the intravenous route of administration.

Phototoxicity observed after 5-ALA HCl treatment *in vitro* and *in vivo* was closely related to dose- and time- dependent induction of fluorescent molecule protoporphyrin (PPIX) synthesis in the irradiated cells or tissues. Destruction of sebaceous cells, focal epidermal necrosis with a transient acute inflammation and diffuse reactive changes in the keratinocytes as well as transient secondary oedema and inflammation of dermis are observed. Light exposed skin recovered completely except for a persistent reduction in the number of hair follicles. Accordingly, general light protective measures of eyes and skin are recommended for at least 24 hours after administration of this medicinal product.

Although pivotal studies on the reproductive and developmental behaviour of 5-ALA have not been performed, based on pharmacological grounds there is a risk that 5-ALA induced porphyrin synthesis leads to embryotoxic activity in mouse, rat and chick embryos only under the condition of direct concomitant light exposure. This medicinal product should, therefore, not be administered to pregnant women. Excessive single dose treatment of rats with 5-ALA reversibly impaired male fertility for two weeks after dosing.

The majority of genotoxicity studies performed in the dark do not reveal a genotoxic potential of 5-ALA. The compound potentially induces photogenotoxicity after subsequent irradiation or light exposure which is related to the induction of porphyrin synthesis.

Long-term *in vivo* carcinogenicity studies have not been conducted. However, considering the therapeutic indication, a single oral treatment with 5-ALA HCl might not be related to any serious potential carcinogenic risk.

There were no issues concerning the non-clinical pharmacology or the toxicology of 5-ALA HCl that negatively affected the overall benefit-risk assessment.

Efficacy

In a phase-III trial with 349 patients with suspected malignant glioma amenable to complete resection of contrast-enhancing tumour were randomised to fluorescence-guided resection after administration of 20 mg/kg body weight 5-ALA HCl or conventional resection under white light. Contrast-enhancing tumour was resected in 64 % of patients in the experimental group compared to 38 % in the control-group ($p<0.001$). At the visit six months after tumour resection, 20.5 % of 5-ALA-treated-patients and 11 % of patients who underwent standard surgery were alive at the six-month visit without progression. The difference was statistically significant using the chi-square test ($p=0.015$). No significant increase in overall survival has been observed in this study, however, the trial was not powered to detect such a difference.

Safety

Adverse reactions observed after the use of this medicinal product for fluorescence-guided glioma resection are either active substance-specific side effects, characterised by an immediate reactions occurring after oral administration of the medicinal product before induction of anaesthesia, or procedure-specific side effects, which are combined effects of 5-ALA, anaesthesia, and tumour resection. The most common side effects observed with the use of 5-ALA HCl in the clinical trials were mild alterations of blood cell counts without clinical symptoms, slight increase of some enzymes (transaminases, γ-GT, amylase) or bilirubin in the blood.

From the safety database all the adverse reactions reported in clinical trials have been included in the Summary of Product Characteristics.

Having considered the safety concerns in the risk management plan, the CHMP considered that the proposed activities described in section 3.5 adequately addressed these.

- User consultation

The CHMP raised concerned regarding the quality and the readability of the package leaflet (PL). A former user consultation was not performed in view of the setting and indication whereby patients undergoing malignant glioma surgery will routinely undergo information session with the surgical health care professionals. The applicant was asked, during the procedure, to improve the readability of the package leaflet and provide a more understandable and patient-friendly text. The CHMP agreed to finalise the text of the PL via a short written procedure, after the June CHMP meeting. In addition, the applicant committed to conduct user testing and submit a revised package leaflet as a follow-up measure.

Risk-benefit assessment

There is general consensus that an optimal treatment of malignant glioma should involve the resection of as much tumour as possible, without causing neurological deficits.

Surgery reduces the number of cancer cells requiring treatment and often removes the hypoxic core of the tumour that is relatively resistant to radiation and inaccessible to chemotherapy.

The optimal dose was not determined, but the 20 mg/kg dose proved to be the best of the three doses tested, based on the efficacy and safety outcome observed in the pivotal trial.

The benefits observed with the use of fluorescence-guidance after oral administration of 5-ALA*HCl was observed in a large prospectively (before surgery) randomised phase-III trial, and was based on the increase of tumour visualisation and rate of radiological complete tumour resection. In this pivotal trial, it was demonstrated that that a more aggressive debulking of contrast-enhancing tumour rather than conventional resection under white light leads to a significant benefit in terms of PFS rate.

Compared to the control group, approximately twice as many patients in the experimental group were progression-free at 6 months. Since PFS was mostly based on an imaging criteria, the clinical relevance of the finding was questioned.

In order to explore the treatment effect on neurological progression-free survival, the time form randomization to radiological progression or neurological progression or death (whichever occured first) was analysed for the full analysis set. In analogy to the definition given by Macdonald *et al* (Macdonald DR, et al. Response criteria for phase II studies of supratentorial malignant glioma. *J Clin Oncol. 1990; 8(7):1277-80*) neurological progression was defined as deterioration in NIH sum score by at least 1 point in case of stable or increased corticosteroids.

A clear clinical benefit in patients receiving 5-ALA was observed (46% vs. 29.3%, p=0.0331) event-free at six months after surgery for first analysis; p=0.0316 in favour of the experimental arm for NIH deterioration (27.3% vs. 15.5%, p=0.0122) and in favour of Gliolan arm for steroids increase or radiologic progression or death. Analysis (Full-Analysis-Set) of cumulative incidence of re-operation after study surgery showed a statistically prolonged time to re-surgery for the Gliolan group compared to the conventional resection under white light group (at 6-months rate 0.10 vs. 0.15, and for 12 months 0.22 vs. 0.33; for 18 months 0.28 vs. 0.38 and for 24 months 0.30 vs. 0.39, respectively). These results analysed by test of Gray showed statistical significant difference p=0.0311.

The cumulative incidence of chemotherapy after study surgery (Full-Analysis-Set) for all administered chemotherapeutics regiments and for temozolomide only were also analused. There was no statistically significant differences between groups: accordingly p=0.0918 and p=0.635.

In terms of efficacy, postoperative adjuvant radiotherapy, chemotherapy and re-operation after tumour progression could have compensated the reduced tumour debulking in the control group and therefore biased a possible survival benefit. One of the main safety issue was the risk of increased brain function deficits associated with the more extensive surgery, as an indirect consequence of the use of 5-ALA.

The CHMP considered that the issue of the net benefit of the use of Gliolan given the increased neurological deficit of patients having the larger resections with the use of Gliolan was debatable and requested to consult the CHMP Scientific Advisory Group (SAG) for Oncology on issues related to the efficacy and safety of Gliolan. Following the CHMP request, a Scientific Advisory Group meeting had been convened to provide advice on the list of questions raised by the CHMP.

Answers to the questions to the SAG

1. **What is the general view about more extensive surgery improving outcome in high grade gliomas based on any prospective or retrospective studies?**

   There are no prospective data available on the association between extent of surgery and treatment outcome in high grade gliomas. The available retrospective analyses consistently point to an association with outcome although for many studies the determination of extent of resection has been difficult, as it was based on the surgeon's judgment and not on MRI measurements. Whether the improved outcome observed with more extensive resection is due to the resection itself, or to fact that resectability is just the marker that characterises smaller and more superficially located tumours that are associated with a better outcome regardless of the extent of resection, remains to be established. Nevertheless, the current consensus among neurosurgeons and neuro-oncologists is that the aim of the surgery of primary high grade glioma is to achieve a resection that is safe and that is the most extensive as possible. Furthermore recent data also point to better outcome of patients with a more extensive resection with adjuvant therapy (concurrent chemo-irradiation with temozolomide).

2. **Are the benefits of Gliolan adequately demonstrated based on appropriate primary and secondary clinical benefit endpoints?**

   Progression-free survival is an appropriate clinical benefit endpoint per se, and is possibly also an informative surrogate endpoint for overall survival. The radiological endpoint based on MRI imaging is adequate. Overall, the benefits of Gliolan have been adequately demonstrated based on appropriate primary and secondary clinical benefit endpoints. Surprisingly, no benefits in terms of overall survival have been observed although it is acknowledged that the trial was not powered to

detect differences in overall survival. Also, this endpoint is disturbed by treatment given after progression. The extent of resection in the control arm was significantly superior so that the difference observed can be attributed to Gliolan.

However, use of Gliolan and operating using fluorescent light poses significant challenges in practice. Use of Gliolan should only be allowed after adequate training of operating with fluorescent light in a dark operating field.

3. **How does the observed increase in neurological deficit compare to the main clinical benefits for Gliolan as observed in terms of primary endpoints, and main secondary endpoints?**

   Differences in the incidence of hemianopsia, aphasia and epilepsy have been observed. The difference is associated with a small detriment in terms of performance status within approximately the first 3 months after surgery, but the difference disappears and actually reverses to an advantage in the Gliolan arm later on. Epilepsy is generally expected after surgery, it is generally manageable using antiepilectic drugs and does not constitute a concern. Concerning aphasia and emianopsia, the difference was small in absolute terms, and tended to be associated with SAEs in patients who already had deficits before surgery. A clear warning should be given to surgeons to keep stay within safe boundaries in case of pre-existing aphasia or other critical focal deficits that do not improve on corticosteroid treatment, since this may identify a group of patients that is at risk of deterioration after more extensive surgery.

The CHMP concluded that it is clearly demonstrated that the use of Gliolan leads to larger resections and to a benefit in PFS at 6 months. There is no direct evidence that this translates in a survival advantage but the trial was not designed to address this question. The data indicate that enhanced resections bear a slightly more increased risk of developing transient neurological deficits and a variety of different factors may be responsible for this. In this context it appears noteworthy that the KPS which was determined prior to and 6 weeks after surgery, did not differ between study arms and tended to be superior in the ALA arm at 6 months after surgery.

A risk management plan was submitted. The CHMP, having considered the data submitted, was of the opinion that the following additional risk minimisation activities were required: see as detailed in section 2.3.

Recommendation

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considered by consensus that the risk-benefit balance of Gliolan in adult patients for visualisation of malignant tissue during surgery for malignant glioma (WHO grade III and IV) was favourable and therefore recommended the granting of the marketing authorisation.