



Australian Government  
Department of Health  
Therapeutic Goods Administration

# Australian Public Assessment Report for Aminolevulinic acid HCl

Proprietary Product Name: Gliolan

Sponsor: Specialised Therapeutics Australia Pty  
Ltd

**March 2014**

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- The Therapeutic Goods Administration (TGA) is part of the Australian Government Department of Health, and is responsible for regulating medicines and medical devices.
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- The work of the TGA is based on applying scientific and clinical expertise to decision-making, to ensure that the benefits to consumers outweigh any risks associated with the use of medicines and medical devices.
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- AusPARs are prepared and published by the TGA.
- An AusPAR is prepared for submissions that relate to new chemical entities, generic medicines, major variations, and extensions of indications.
- An AusPAR is a static document, in that it will provide information that relates to a submission at a particular point in time.
- A new AusPAR will be developed to reflect changes to indications and/or major variations to a prescription medicine subject to evaluation by the TGA.

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## I. Introduction to product submission

### Submission details

<i>Type of Submission:</i>	New Chemical Entity
<i>Decision:</i>	Approved
<i>Date of Decision:</i>	31 October 2013
<i>Active ingredient:</i>	Aminolevulinic acid HCl
<i>Product Name:</i>	Gliolan
<i>Sponsor's Name and Address:</i>	Specialised Therapeutics Australia Pty Ltd Level I, 711 High Street East Kew Victoria 3102
<i>Dose form:</i>	Powder for Oral Solution
<i>Strength:</i>	30 mg/mL
<i>Container:</i>	Vial
<i>Pack size:</i>	Single
<i>Approved Therapeutic use:</i>	Gliolan is indicated in adult patients for visualisation of malignant tissue during surgery for malignant gliomas that are glioblastoma multiforme (GBM) on preoperative imaging, and who are intended for resection of the tumour.
<i>Route of administration:</i>	Oral
<i>Dosage (abbreviated):</i>	20 mg aminolevulinic acid hydrochloride per kilogram body weight. The solution should be administered orally three hours (range 2 to 4 hours) before anaesthesia.
<i>ARTG Number:</i>	202549

### Product background

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

Tumour tissue at the transition zone between tumour and normal tissue is visualized intra operatively with light from a xenon arc lamp [ $\lambda = 375$  to  $440$  nm]. Following excitation with blue light ( $\lambda=400$  to  $410$  nm), the PPIX, which has accumulated selectively in the malignant tissue, emits a strong red PpIX fluorescence (the peak is at  $635$  nm). PpIX is photolabile and fluorescence decreases over the course of light exposure. Gliolan is contraindicated in acute or chronic types of porphyria.

Specialised Therapeutics Australia Pty Ltd (STA) has applied to register Gliolan (aminolevulinic acid hydrochloride; 5-ALA) as a diagnostic agent for visualisation of malignant tissue during surgery of malignant glioma (brain tumours WHO grade III and IV) by means of photodynamic diagnosis. During surgery, tumour resection is improved as malignant tissue is differentiated more easily from normal brain tissue, due to a colour reaction to light. GBM is otherwise fatal following rapid deterioration of the patient. 5-ALA is an endogenous heme precursor. Gliolan was approved in the EU in 2007 and has been used under the TGA Special Access Scheme in Australia, by qualified and accredited neurosurgeons

This AusPAR describes the application by the sponsor to register Gliolan for the following indication:

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

During the evaluation process and prior to registration the proposed indication was altered to:

*'Gliolan is indicated in adult patients for visualisation of malignant tissue during surgery for malignant gliomas that are glioblastoma multiforme (GBM) on preoperative imaging, and who are intended for resection of the tumour.'*

## Regulatory status

The product received initial registration on the Australian Register of Therapeutic Goods (ARTG) on 7 November 2013

At the time TGA considered this application a similar application had been approved in the European Union (EU) on 7 September 2007 and in three additional countries. An application was under consideration in the United States of America (US) and two other countries.

## Product Information

The approved Product Information (PI) current at the time this AusPAR was prepared can be found as Attachment 1.

## II. Quality findings

### Introduction

Aminolevulinic acid hydrochloride (5-aminolevulinic acid hydrochloride, 5-ALA) is a new chemical entity.<sup>1</sup>

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<sup>1</sup> The methyl ester of aminolevulinic acid (methyl aminolevulinate) is registered as a 160 mg/g cream (Metvix) by Galderma Australia Pty Ltd for the treatment of various keratoses and skin cancers (ARTG Number: 3838).

The powder for oral solution is intended for single use but the dose administration depends on body weight. The recommended dose is 20 mg aminolevulinic acid hydrochloride per kilogram body weight. The oral solution is prepared by dissolving the amount of powder of one vial in 50 mL of drinking water and the reconstituted solution is a clear and colourless to slightly yellowish fluid. Any content remaining after first use must be discarded.

The solution should be administered orally three hours (range 2 to 4 hours) before anaesthesia. In the absence of compatibility studies, Gliolan must not be mixed with other medicinal products.

### **Drug substance (active ingredient)**

Aminolevulinic acid hydrochloride (5-aminolevulinic acid hydrochloride, 5-ALA) is a white to off white crystalline powder which is freely soluble in water and slightly soluble in ethanol/methanol. There is no United States Pharmacopoeia/European Pharmacopoeia monograph for aminolevulinic acid hydrochloride. The chemistry, manufacture and stability of the drug substance have already been evaluated in the separate report for aminolevulinic acid hydrochloride. The company has agreed to amend the acceptance limit for the impurity in the drug substance specification. The proposed acceptance limit has been cleared by the Medicines Toxicology Evaluation Section of TGA.

### **Drug product**

The drug product is to be manufactured by dissolving aminolevulinic acid hydrochloride in water for injection, followed by sterile filtering into 50 mL vials. The vials are placed into the sterilised freeze drier and the solution is freeze dried.

The powder for injection is well controlled with satisfactory expiry limits and release limits.

Stability data was provided to support the proposed shelf life of 36 months when stored below 25°C in original package for aminolevulinic acid hydrochloride powder for injection. The vials should be kept in the outer carton in order to protect from light.

The in use stability of the reconstituted drug product in water with a final concentration of 30 mg/mL (3%) is stable for oral administration for a period of 24 hours after reconstitution.

The carton and vial labels and the Provisional ARTG Records have been finalised.

### **Biopharmaceutics**

A bioavailability study was performed to compare the oral doses of 20 mg/kg bodyweight aminolevulinic acid hydrochloride with 2 mg/kg bodyweight intravenous administration in healthy male subjects. The results indicate that:

- Following oral administration of a single dose of 20.0 mg/kg body weight of 5-ALA HCl, the absolute bioavailability was 100%, calculated from plasma data [area under the plasma concentration time curve ( $AUC_{0-\infty}$ )] and 105%, calculated from urinary excretion data.

### **Advisory committee considerations**

Details of this submission were presented at the 151<sup>th</sup> meeting of the Pharmaceutical Subcommittee (PSC) of Advisory Committee on Prescription Medicines (ACPM) on 27 May 2013. The PSC endorsed all questions raised by PCS and had no objections to approval of this product provided all issues were addressed to the satisfaction of the TGA. The PSC reiterated the acceptance limits for each unknown impurity should be consistent with the recommended International Conference on Harmonization (ICH) identification threshold.

The PSC recommended several revisions to the quality aspects of the draft Product Information. Details of these revisions are beyond the scope of the AusPAR.

### **Quality summary and conclusions**

Approval of the company's application is recommended noting chemistry and quality control as the only outstanding issue regarding the acceptance limit for the impurity applied by both the drug substance and drug product manufacturers has been resolved with the company. The company agreed to amend the acceptance limit in the drug substance specification and the proposed acceptance limit has been cleared by the Medicines Toxicology Evaluation Section.

The chemistry and quality control aspects of the draft PI have been finalised to the satisfaction of the quality evaluator

The carton and vial labels and the Provisional ARTG Records have been finalised.

## **III. Nonclinical findings**

### **Introduction**

The nonclinical submission contained studies undertaken by the sponsor and numerous literature publications supporting the pharmacodynamics, safety pharmacology, pharmacokinetics and toxicity (with supportive toxicokinetic data), photogenotoxicity and phototoxicity. All submitted studies were reviewed by the evaluator. The nonclinical dossier was sufficient in scope according to the relevant EU guideline on photosafety toxicity testing<sup>2</sup>. Reproductive toxicity data were limited to published studies, justification was provided for the lack of carcinogenicity studies. Most toxicity studies used intravenous IV administration, not the clinical oral PO route.

### **Overall quality of the nonclinical dossier**

Most safety related studies performed by the sponsor were conducted under Good Laboratory Practice (GLP) conditions. However, the submission also relied in part on data from the published literature (including safety related studies) which were not GLP compliant. As mentioned, most studies used the IV route of administration, rather than the clinical PO route. Adequate justification was provided for the lack of carcinogenicity studies.

### **Pharmacology**

#### **Primary pharmacology**

##### ***Rationale and mechanism of action***

5-Aminolevulinic acid hydrochloride (5-ALA) is an endogenous precursor of heme that is metabolised in a series of enzymatic reactions to endogenously formed fluorescent photosensitiser porphyrins (mainly protoporphyrin IX [PpIX]). It is ferrochelatase that ultimately catalyses the incorporation of Fe<sup>2+</sup> into protoporphyrin IX to yield haemoglobin. Thus, all nucleated cells have at least a minimal capacity to synthesise haemoglobin since mammalian cells require haemoproteins [that is cytochromes] for aerobic energy metabolism/oxidative phosphorylation.

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<sup>2</sup> EMA. CPMP. Note for Guidance on Photosafety Testing (2002; CPMP/SWP/398/01).

Intracellular aminolevulinic acid synthesis by 5-ALA synthetase is regulated by an intracellular pool of free heme by a negative feedback loop. Administration of an excess of 5-ALA HCl bypasses this negative feedback mechanism with subsequent accumulation of PpIX in neoplastic cells, such as those of malignant gliomas. In the presence of visible violet-blue light ( $\lambda=400$  to  $410$  nm [although the absorbance peak is at  $635$  nm]), red-light fluorescence of PpIX occurs in target tissues (a photodynamic effect) which can be used for photodynamic diagnosis (PDD) and visualisation of malignant tissue during surgery of malignant gliomas (brain tumours WHO grade III and IV).

That 5-ALA is selectively taken up by tumour tissue and intracellularly metabolised to fluorescent porphyrins (predominantly PpIX) was generally explained in the submitted studies by:-

- higher 5-ALA uptake into tumours (explanations for higher 5-ALA uptake into tumour tissue include a disrupted blood-brain barrier, increased neo-vascularisation, and over expression of membrane transporters in glioma tissue) or
- an altered pattern of expression or activity of enzymes involved in haemoglobin biosynthesis in tumour cells (for example, ferrochelatase).

### ***Porphyrins in humans***

The porphyrias are a group of rare genetic or acquired rare human disorders of haem biosynthesis, in which accumulation of ALA and porphyrins occurs in tissues, manifest acutely as abdominal pain, vomiting, neuropathy, psychiatric symptoms, cardiac arrhythmias and tachycardia, and skin photosensitization. Particular attention was given to these symptoms in evaluation of the nonclinical data.

### ***In vitro studies***

5-Aminolevulinic acid (5-ALA) was tested *in vitro* with different tumour cell lines to investigate i) the mechanism of 5-ALA Photodynamic Therapy (PDT) and the associated potential photocytotoxic effects and ii) photobleaching (depletion of photosensitiser fluorescence intensity over time) following 5-ALA induced protoporphyrin IX (PpIX) levels.

#### ***Mechanism of 5-ALA Photodynamic Therapy (PDT) and photocytotoxic effects***

Neuroblastoma cells (MHH-NB-11) and human hepatoblastoma (HuH6) were incubated with 5-ALA ( $150$   $\mu\text{g}/\text{mL}$ ) for 4 hours followed by PDT; human fibroblastic cells served as controls. Selective fluorescence was observed for MHH-NB-11 and HuH6 and cells (compared to human fibroblastic cells) did not survive light doses over  $15$   $\text{J}/\text{cm}^2$  (while only about 20 % of the human fibroblastic cells were affected at  $20$   $\text{J}/\text{cm}^2$ ). The susceptibility to 5-ALA and PDT appears to be dependent on cell type as normal human fibroblasts were largely unaffected but human tumour cells (and normal human lymphocytes [see Genotoxicity below]) were very sensitive. The photocytotoxic effect of 5-ALA and PDT on two other glioma cell lines (U87 and GBM6840) was similar at the lethal dose, 50% ( $\text{LD}_{50}$ ) (at optimal uptake,  $1$   $\text{mM}$ ) at  $2$   $\text{J}/\text{cm}^2$ . The ability of both cell lines to migrate through a Matrigel artificial basement membrane (a measure of glioma cell invasiveness) was reduced following PDT. Furthermore, 5-ALA and PDT effectively inhibited both metalloproteinase MMP-2 and MMP-9 by down regulation, in a light dose dependent manner which may help reduce tumour progression.

The relationship between the intracellular location of 5-ALA induced PpIX formation and photodynamic efficacy was examined in human glioblastoma U373 MG cells, using ultra-sensitive fluorescence microscopy. PpIX located in mainly plasma and nuclear membranes, seemed to be the key factor for efficacy of 5-ALA induced PpIX formation together with PDT. In contrast, in T47D breast cancer cells in which the photodynamic efficacy was about half that of glioblastoma cells (per photosensitiser molecule), PpIX fluorescence was localized in small granules. Moreover, in 3 human glioma cell lines (U251MG, U87MG, U118MG)



following 5-ALA and PDT, there were increases in the activities of caspase-3 and -9, a decrease in mitochondrial membrane potential and a marked increase in cytochrome c in the cytosolic fraction. The findings suggest a dysfunctional mitochondrial membrane potential with mitochondrial cytochrome c release triggers apoptosis.

#### *Photobleaching*

Photobleaching (depletion in photosensitiser fluorescence intensity over time) of 5-ALA induced PpIX fluorescence was investigated in WiDr cells. Experiments using 5-ALA exposure times of up to 30 minutes revealed D10 values (exposure time to inactivate fluorescence in 90% of cells) of about 1 minute, 5 minutes and greater than 40 minutes at 1.0, 0.2 and 0.05 mM 5-ALA, respectively. Thus, PpIX was photolabile and 70–95 % of PpIX fluorescence in cells was degraded by light exposures (40–200 J/cm<sup>2</sup> at 630 nm). The rate of PpIX photobleaching was dependent on the initial concentration of the sensitiser. However, the excitation light proposed for 5-ALA for photodynamic diagnosis (PDD) in the clinical setting is different, that is, between 400 to 410 nm derived from a xenon arc lamp light source [ $\lambda = 375$  to 440 nm]. Due to this difference, it is possible that photobleaching may have a minor importance in the clinical setting, and this effect observed at  $\lambda = 630$  nm in this study, is of minor importance.

#### *In vivo studies*

No specific supporting nonclinical *in vivo* efficacy studies were submitted to define the optimal conditions for the use of 5-ALA. Several *in vitro* and *in vivo* studies on the pharmacodynamics of 5-ALA demonstrating efficacy in tumour models have already been published and these were submitted. These studies investigated the use of 5-ALA mainly as a sensitiser in photodynamic therapy (PDT) rather than its use in photodynamic diagnosis (PDD) of brain tumours.

**Note:** The sponsor proposes that the irradiation used for photodynamic therapy (PDT; red light [ $\lambda=630$  to 635 nm]) is more aggressive (greater irradiation intensity and deeper tissue penetration depth) than that used for PDD ( $\lambda=375$  to 440 nm; blue light]), so the potential damage to normal brain tissue following photodynamic diagnosis should in theory, be less frequent and less severe compared to damage to normal brain tissue following PDT (both are based on the selective induction of Pp-IX synthesis in tumours).

Five studies investigated:

- the ability of 5-ALA to detect the presence of brain tumours noninvasively
- induction of endogenous porphyrin synthesis
- photodynamic therapy (PDT) induced damage in brain tumour models and
- application of fluorescence guided resection (FGR) plus white light resection (WLR).

#### *Ability of 5-aminolevulinic acid (5-ALA) to detect the presence of brain tumours noninvasively*

The 9L rat gliosarcoma cell line transfected with GFP (9L-GFP; green fluorescent protein) and the human glioma cell line U251 were used for intracranial implantation into athymic nude mice which were used to investigate the ability to detect brain tumours noninvasively by spectroscopic measurements of PpIX fluorescence following 5-ALA dosing (100 mg/kg IP). High variability of fluorescence was observed indicating that sensitivity and specificity of such detection of tumours is influenced by PpIX production patterns of the model tumour tissue. However, PpIX fluorescence of the 9L-GFP and U251 groups were similar 2 hours after 5-ALA administration.

#### *5-ALA induced endogenous porphyrin synthesis*

Porphyrin synthesis in brain tumours (intracranial 9L or C6 tumours) was demonstrated *ex vivo* in tumour sections 2 to 8 hours following 5-ALA (200 mg/kg IV), with fluorescence in both tumours strongest after 6 hours). 9L tumours fluoresced homogeneously with sharp

demarcations towards normal brain tissue. Fluorescence was accentuated around blood vessels. C6 tumour fluorescence was patchy and necrotic sections (only seen in these tumours) showed no fluorescence with a poorly fluorescing edge. By 22 hours, porphyrin fluorescence had almost disappeared. Fluorescence was also detected in normal brain surrounding the tumour and in contralateral white matter, ventricle ependyma and pia mater (compared with the absence of fluorescence in rats without tumours) up to 6 hours after 5-ALA. It was likely this finding was probably due to either:-

- transport of porphyrins synthesised in tumours (as a result of oedema/plasma extravasation and transport by 'bulk flow') or
- due to circulating porphyrins or
- from 5-ALA leaking from tumour blood vessels. Additionally, in this study 9L and C6 tumour cell cultures were incubated with 5-ALA for 8 hours. Porphyrins were detected in vitro in tumour cells from 2 hours onwards.

#### *Photodynamic therapy (PDT) in brain tumours of rats and rabbits*

Significant prolongation of survival time was observed for treated BD-IX tumour bearing rats (BT<sub>4</sub>C glioma) following PDT at a low fluence rate (26 J, fluence rate of 90 minutes; 632 nm) administered 4 to 5 hours after 5-ALA injection (60 or 125 mg/kg, Intraperitoneal (IP), compared with controls. Fluorescence intensities were uneven and in various areas of adjacent brain, intensities of up to 20 to 25% that of tumour fluorescence was observed. Brain oedema was the main cause of morbidity and early mortality following PDT (despite steroid therapy). Microfluorometry of frozen tissue sections showed photosensitisation resulted in 200:1 tumour:normal tissue selectivity after 5-ALA dosing.

In tumour bearing rabbit brain (VX2 carcinoma), a photodynamic threshold model was used to quantify the intrinsic tissue sensitivity of normal white matter, cortex and VX2-tumour to PDT following 5-ALA administration (20 and 100 mg/kg, IV).<sup>3</sup> Photodynamic threshold values (number of photons absorbed by the photosensitizer per unit tissue to induce tissue necrosis) were found to be significantly lower in normal tissue than in the tumour. These findings showed there was very little white matter damage. Moreover, levels of the photosensitizer were significantly lower in white matter than in cortex and tumour. However, in the Lilge *et al.* (1996)<sup>2</sup> study (100 mg/kg 5-ALA, IV), neuronal damage was seen beyond the zone of frank necrosis following PDT. Tumour to normal brain (grey matter) ratios at 6 hours (20 mg/kg) were 22:1; the production of PpIX in white matter was lower with the tumour:white matter ratio at 6 hours (at 20 mg/kg) about 100:1. Normal brain structures lacking a blood-brain barrier showed high uptake of the sensitizer.

#### *Fluorescence guided resection*

Increased brain tumour resection using fluorescence image guidance in intracranial VX2 tumour bearing rabbits was demonstrated in comparison with white light visualization alone<sup>4</sup>. Fluorescence guided resection (FGR; excitation light of 405 nm, 6 mW/cm<sup>2</sup>) in addition to white light resection (WL) significantly increased the resection completeness, following endogenously induced PpIX fluorescence with 5-ALA administration (20 mg/kg, IV), 4 hours prior to surgery. FGR plus WLR significantly increased resection completeness 1.4 fold from 68 ± 38 % to 98 ± 3.5 % (p=0.010). The amount of residual tumour was reduced 16 fold from 32 ± 38 % to 2.0 ± 3.5 % of the initial tumour volume (p=0.010).

<sup>3</sup> Lilge L *et al.* (1996) The sensitivity of normal brain and intracranially implanted VX2 tumour to interstitial photodynamic therapy. *British Journal of Cancer* 73, 332-43; Lilge L and Wilson BC (1998) Photodynamic therapy of intracranial tissues: a preclinical comparative study of four different photosensitizers. *Journal of Clinical Laser Medicine & Surgery* 16, 81-91.

<sup>4</sup> Bogaards A *et al.* (2004) Increased brain tumour resection using fluorescence image guidance in a preclinical model. *Lasers Surg Med* 35, 181-90

## Overall findings

The two most clinically relevant findings are the use of 5-ALA for fluorescence for photodynamic diagnosis (PDD) of brain tumours entails the possibility of PpIX related photosensitization outside the tumour in surrounding normal tissue and basal porphyrin levels may not be observed until 24 hours after administration. Associated potential clinical risks with these findings are resection of healthy normal brain tissue and phototoxicity associated with light exposure and/or drug interactions with potentially phototoxic substances post surgery, respectively. For the former risk, it is reassuring that 5-ALA induced PpIX formation is significantly higher in malignant tissue than in normal brain. However, the risk for resection of normal brain in the clinical setting will still require assessment of the clinical studies by the clinical evaluator. For the risk of potential phototoxicity, appropriate precautions are provided in the draft Product Information and include avoiding strong light exposure 24 hours post surgery and avoiding co-administration with other potentially phototoxic substances (for example, tetracyclines, sulfonamides, fluoroquinolones, hypericin extracts).

No specific nonclinical efficacy studies were submitted to define the optimal use of 5-ALA for the proposed indication of photodynamic diagnosis of tumours during surgery in the clinical setting. This is an obvious shortcoming of the nonclinical section. However, the proposed time schedule for 5-ALA administration and subsequent fluorescence guided tumour resection in patients with malignant glioma is reasonable, based on the submitted nonclinical literature. Thus, in experimental glioma in animal studies, 5-ALA induced PpIX formation is significantly higher in malignant tissue than in normal brain, with maximal levels of 'tumour to normal brain fluorescence contrast ratios' observed at about 4 to 6 hours following 5-ALA administration. Levels of 5-ALA were also significantly lower in white matter than in tumour and cortex after IV doses of 20 and 100 mg/kg in rabbits; normal brain structures lacking a blood-brain barrier generally showed higher uptake of the sensitiser. Moreover, porphyrins were detected in tumour sections mainly from 2 to 8 hours (by 22 hours porphyrin fluorescence had almost disappeared). Therefore, administration of a 5-ALA solution 3 hours prior to anaesthesia/surgery is reasonable because absorption following oral dosing occurs rapidly and PO and IV dosing of 5-ALA show similar pharmacokinetic profiles. Overall, there is sufficient evidence from the available *in vivo* data that PpIX mediated fluorescence in brain tumours in rats and rabbits that occurs after about 3 hours and for at least 6 to 8 hours following 5-ALA dosing.

Moreover, in tissue distribution studies, brains of VX2 tumour bearing rabbits showed maximum fluorescence intensity in tumour tissue 4.5 hours after dosing (100 mg/kg 5-ALA, IV) which started to decline by about 6 hours. At 4.5 hours, tumour tissue had the highest PpIX fluorescence compared to normal contralateral cerebral cortex (with 8 fold higher PpIX levels). Additionally, 5-ALA was detected in the CSF within 2 hours post dose. The maximum selectivity of tumour to normal cerebral cortex fluorescence intensity, expressed as a ratio of 85 to 1, was observed at 24 hours. The increased selectivity of tumour to normal cerebral cortex fluorescence observed at 24 hours was also accompanied by very low absolute fluorescence levels.

Lastly, a paper (Bogaards *et al.*, 2004) describes findings from a study which performed the entire proposed clinical procedure for fluorescence guided resection (FGR) in patients with malignant glioma in an intracranial VX2 tumour model in rabbits. "The experiment was performed using a fixed 5-ALA dose and time interval prior to the planned clinical and preclinical glioma resection studies to optimize these two factors." IV dosing of 5-ALA (20 mg/kg) was 4 hours prior to surgery to induce endogenous PpIX formation (the proposed dose in the clinical setting is 20 mg/kg orally prior to surgery, 3 hours prior to anaesthesia (range 2.5 to 3.5 hours)). In this study, the surgical resection procedure of brain tumour was initially carried out in New Zealand White (NZW) rabbits using white light illumination only. When tumour resection under white light illumination was completed, the

fluorescence guided resection procedure followed by switching to the blue light (405 nm) recommended for the proposed indication (that is,  $\lambda=400$  to 410 nm). It was demonstrated that the entire procedure which started 4 hours after 5-ALA administration increased the completeness of tumour resection of the rabbit brain tumour (significantly increased resection completeness 1.4 fold and the amount of residual tumour was reduced 16 fold post resection).

On balance, it could be reasonably expected from the submitted nonclinical data that a lucid contrast for fluorescence guided glioma resection could be expected for at least 6 hours and possibly up to 6 to 8 hours following 5-ALA administration. This is in accord with the findings in humans.

### ***Adequacy of the data submitted***

The intracranial VX2 carcinoma cell model study in rabbits, which used similar methods to the clinical protocol, showed a significant increase in the completeness of resection and a significant decrease in residual tumour. However, no specific nonclinical *in vivo* (efficacy) studies were performed to define optimal conditions for use of 5-ALA for the proposed indication of photodynamic diagnosis of tumours during neurosurgery using the same parameters as those used in the clinical setting that is, with similar light wavelengths and exposure times of the filtered blue photoactivating light [ $\lambda=400$  to 410 nm] plus white light used in the surgery procedures. There were no studies of glioma resection in dogs, the main toxicity test species.

Outstanding issues raised from review of the nonclinical studies that could have addressed the optimal clinical utility of 5-ALA include:

- defining the optimal 5-ALA dose
- timing of 5-ALA administration prior to surgery including the time after administration of a drug when the maximum plasma concentration is reached ( $T_{max}$ ) for PpIX levels) and
- defining the characteristics/criteria for identifying the tumour/normal brain border during resection surgery.

Furthermore, there was no nonclinical discussion on the use of 5-ALA fluorescence guided resection of different tumour types/grades. These issues may have been addressed in the clinical setting. Nevertheless, additional nonclinical studies to define the optimal conditions for 5-ALA use may have provided useful information to optimise the efficacy of 5-ALA use as well as its safety.

### ***Secondary pharmacodynamics***

Limited studies described the secondary activity of 5-ALA including investigations on cellular haemoglobin synthesis, neuro biochemical mechanisms and whether photodynamic therapy (PDT) of experimental brain tumours with 5-ALA is possible without injuring normal or oedematous brain. No other studies were performed by the sponsor. However, there are some unclarified issues. Studies on the potential binding of 5-ALA to the typical broad panel of receptor types would have provided useful safety information. In addition, the interaction of 5-ALA with neurotransmitters only covered glutamate, gamma-aminobutyric acid (GABA) and aspartate (presumably because these transmitters may be involved in some neurological perturbations in human porphyrias), and a wider screening was desirable. It would be difficult to discern possible drug related effects on neurotransmitters in glioma patients.

#### ***Cellular haemoglobin synthesis***

It was shown in cultured hepatocytes from chick embryos that short term (3 hours) single exposure of cells to 5-ALA (up to 34  $\mu\text{M}$ ) did not alter cellular haemoglobin or cytochrome

P-450 levels in control cultures, or those treated with CYP450 inducers (whilst inducers of cytochrome 450 [CYP450] increased amounts of cytochrome c and haem by increasing the activity of ALA synthase and increasing conversion of protoporphyrin into haem).

In response to a question by the Committee for Medicinal Products for Human Use (EU CHMP) regarding the potential effect of disruption of 5-ALA homeostasis, the sponsor stated:

*'due to temporary (48 hour) high plasma levels of 5-ALA (and its metabolites) one could expect disturbances in heme metabolism, theoretically resulting in porphyria like symptoms or increased levels of haemoglobin. However, no such symptoms have been described in the literature, nor have they been observed in clinical studies performed by the applicant. All published clinical trials as well as those performed by the applicant clearly demonstrate that a single oral dose of 20 mg/kg 5-ALA is very well tolerated. Therefore it can be concluded that the temporarily observed high plasma levels of 5-ALA and PpIX do not have a clinical relevance.'*

In response, the Committee for Medicinal Products for Human Use (CHMP) has commented that:

*'the applicant bases their response on the fact that a single dose of 5-ALA is well tolerated. This is a nonresponse to the issue raised. Nevertheless, no adverse effects, especially those related to haemoglobin homeostasis, have been observed.'*

#### *Neuro biochemical mechanisms (neurotransmitter uptake and deoxyglucose uptake)*

Since GABA, glutamate and aspartate uptake by rat brain synaptosomes was inhibited by 5-ALA (0.05 to 2 mM), and uptake of 2-deoxy-glucose in cultured neurons from chick embryos was decreased following exposure to 5-ALA (10 µM to 1 mM), the findings indicate a possible effect of 5-ALA on GABA, glutamate and aspartate neurotransmission, as well as on neuronal glucose uptake. Thus, neurotransmitter disturbances and/or neuronal toxicity with 5-ALA use cannot be ruled out.

#### *Photodynamic therapy of brain tumours with 5-ALA*

5-ALA (100 mg/kg, IV) was shown to be effective with photodynamic therapy (PDT) in tumour models of malignant glioma (C6 tumours) in rats and in rabbit brain VX2 tumours. In rats, 5-ALA administration with subsequent irradiation resulted in only minor superficial cortical damage in normal or oedematous brain (following induction of cold injury, with findings of slightly increased depth of damage in oedematous compared with normal brain). However, there was selective damage to the glioma. In the rabbit study, normal tissue was found to have a lower photodynamic threshold (value) than tumour tissue. However, neuronal damage was seen beyond the zone of frank necrosis, suggesting normal brain tissue in rabbits is also sensitive to 5-ALA and PDT. Although this is a toxicologically relevant finding, PDT is typically more aggressive (with greater irradiation intensity and deeper tissue penetration depth) than photodynamic diagnosis (PDD) (as mentioned above). Thus, the finding in rabbit brain does not therefore highlight a particular toxicological concern and potential clinical risk.

#### **Safety pharmacology**

The majority of studies were conducted (according to the sponsor) in the dark, under 'light protection' conditions (that is, with a light source that did not emit UV light [less than 635 nm]), to exclude phototoxic reactions. Specialised safety pharmacology studies (not all conducted under GLP conditions) covered the Central Nervous System (CNS) (movement, sleeping time, neuromuscular function), cardiovascular, respiratory (using a pneumotachometer), gastrointestinal (isolated guinea pig ileum *in vitro*) and renal systems (diuresis and saluresis). These studies were supplemented by studies of the effect of 5-ALA on the CNS (neurotransmission) and phototoxicity.

Saluresis at 250 mg/kg was observed in rats with an increase in Na<sup>+</sup> excretion (24 hours; 34%) and K<sup>+</sup> excretion (0 to 1 hours) during urine collection (no treatment related effects were observed at 40 and 100 mg/kg). In a cardiovascular study in dogs, 5-ALA treatment at 45 mg/kg resulted in a slight decrease in peripheral arterial blood pressure (systolic: 9.5% and diastolic: 7.5%) and systolic LVP (15%), and a significant decrease in dp/dt max post dose (43%; p≤0.01). The sponsor proposes the effects are likely to be associated with the 5-ALA IV dosing route, as (pre dose) basal values were observed within 5 minutes of dosing. However, no influence was seen on these parameters after administration of the vehicle. The No Observable Effect Level (NOEL) was 15 mg/kg.

The draft Product Information appropriately advises: *'In patients with pre-existing cardiovascular disease, Gliolan should be used with caution since literature reports have shown decreased systolic and diastolic blood pressures, pulmonary artery systolic and diastolic pressure as well as pulmonary vascular resistance.'*

#### *Potential for QT prolongation<sup>5</sup>*

The ICH S7A guideline<sup>6</sup> recommends assessment of the cardiovascular system, and the ICH S7B guideline<sup>7</sup> recommends *in vitro* and *in vivo* assessment of the potential for QT prolongation. No *in vitro* studies on the potential for QT interval prolongation for example hERG channels, were submitted. In an S31 response the sponsor provided retrospective quantitative electrocardiogram (ECG) analyses from the 7 day (LPT 11652/98) and 14 day repeat dose toxicity studies (LP 11664/98) in dogs, which showed no differences in QT and QT<sub>c</sub> intervals before and 5 minutes after dosing. The measurement time was inadequate to detect potential changes due to PpIX, which had a T<sub>max</sub> of 2 to 4 hours. Relative systemic levels of PpIX were also lower in dogs than in humans. The sponsor stated that the heart would not be exposed to light during neurosurgery. A literature search by the sponsor did not reveal any publication describing arrhythmogenic effects/QT prolongation after administration of 5-ALA and/or PP-IX. The sponsor stated that no adverse effects concerning heart rate or arrhythmia were reported in the Gliolan clinical trials, nor in post marketing reports since EU registration in 2007. The Gliolan product information contains a caution for patients with pre-existing cardiovascular conditions.

#### *Effect of 5-ALA on glutamate, adenylate cyclase and serotonin*

Two literature reports investigated potential mechanisms involved in (endogenous) 5-ALA induced neurotoxicity in patients suffering porphyria (where up to 100 fold higher 5-ALA levels may occur). In a study in rat brain (to elucidate 5-ALA's *in vivo* excitatory properties), 5-ALA (0 to 10 mM) dose dependently inhibited glutamate uptake by astrocyte cultures with the inhibitory effect on glutamatergic neurotransmission shown to be due to oxidative damage (lipid peroxidation), of the GLT-1 (EAAT2) glutamate transporter. However, there was no effect of 5-ALA [up to 1 mM] on radioactively labelled [<sup>3</sup>H] glutamate binding to cortical membranes. Similarly, the effect of 5-ALA on production of cyclic adenosine monophosphate (cAMP) was investigated. 5-ALA dose dependently decreased cAMP levels in rat cerebella membranes (38 % at 1 mM) by inhibition of basal adenylate cyclase activity. The effect was mediated by oxidative damage (since antioxidants prevented the effect). However, in another study 5-ALA (15 mg/kg, IP) did not alter day or night time pineal serotonin or melatonin levels (involved in behaviour/mood regulation). It was suggested

<sup>5</sup> The QT interval is a measure of the time between the start of the Q wave and the end of the T wave in the heart's electrical cycle. A prolonged QT interval is a risk factor for ventricular tachyarrhythmias and sudden death. The QT interval is dependent on the heart rate (the faster the heart rate, the shorter the QT interval). To correct for changes in heart rate and thereby improve the detection of patients at increased risk of ventricular arrhythmia, a heart rate-corrected QT interval QT<sub>c</sub> is often calculated.

<sup>6</sup> ICH S7A. Safety pharmacology studies for human pharmaceuticals. 2000.

<sup>7</sup> ICH S7B. The non-clinical evaluation of the potential for delayed ventricular repolarization (QT interval prolongation) by human pharmaceuticals. 2005.

that 5-ALA may not trigger neurological disorders such as depression. It would be difficult to discern neurological effects related to ALA exposure in the proposed patient group.

#### *Phototoxicity*

##### *Dermal (phototoxic damage) with exposure to photoactivating light*

5-ALA induced PpIX was found to photosensitize the epidermis and epidermal appendages of whole body irradiated mice (white light from 100 Watt tungsten lamp for 6 hours, 21 mW/cm<sup>2</sup>) when 5-ALA was administered systemically (250 mg/kg, IP), but did not photosensitize vascular structures or connective tissue elements. Skin rapidly showed the characteristic red fluorescence of PpIX. The location and severity of damage correlated with the location and intensity of the red PpIX fluorescence. Light microscopy of skin was examined at 12, 24 and 36 hours as well as 2, 3, 4, 6, 10 and 55 days, and showed destruction of sebaceous glands with dilatation of hair follicles/parakeratosis, focal epidermal necrosis with a transient acute inflammation and diffuse reactive changes in keratinocytes. The dermis showed transient secondary oedema and inflammation. There was complete recovery of the light exposed skin, except for the number of hair follicles over 55 days.

##### *Effects on intraperitoneal organs from white light (used during surgical procedures)*

Following systemic 5-ALA administration (IV and IP, up to 100 and 200 mg/kg, respectively) and exposure of rats to a broad band light source (2 hours at a distance of 90 cm) with a standard white light operating room lamp, photosensitization of major internal abdominal organs (liver, small and large intestines, peritoneum, bladder and adjacent skin) was assessed. Damage of intraperitoneal organs was generally superficial (including liver reddening or blanching and oedema, and swelling of the small and large intestines with focalised haemorrhages on outer surface; microscopic findings of cell damage were limited to liver and intestines [mucosa]), and considered not likely to affect organ function. Maximum Tolerated Doses (MTDs) were 1, 10 and 100 mg/kg (for both IV and IP routes) after 2, 6 or 16 hours (following the initial period of 2 hours of illumination), respectively. The proposed patient avoidance of strong light for 24 hours post operatively is supported.

### **Pharmacodynamic interactions**

No drug interaction studies were performed with concomitant treatments that might interfere with 5-ALA's diagnostic action. However, findings of *in vitro* and *in vivo* pharmacodynamic drug interaction studies from submitted published literature reports demonstrated that (without light irradiation) melatonin or vitamin E (administered *in vivo*) protected against 5-ALA induced oxidative toxicity (lipid peroxidation) in rat brain. Similarly, with light irradiation (5 minutes [4.6 J/cm<sup>2</sup>, 630 nm]) free radical scavengers, amino acids and sulphur containing compounds including L-tryptophan, reduced glutathione, N-acetylcysteine, melatonin, L-methionine, L-cysteine, mannitol and glycine, are protective with regard to 5-ALA (0.2 mM) induced photodamage (cell survival) in an LM2 cell line derived from a mammary murine adenocarcinoma. These agents were seen to be useful in protecting from the light induced side effects of PDT before and during PDT, whereas protection after PDT did not improve cell survival. All these agents may be useful in reducing toxicity.

In an interaction study with human glioma (ACBT) spheroids, it was shown that motexafin gadolinium (MGd; a radiation sensitiser), potentiated the cytotoxic and migration (through a gel collagen matrix) inhibitory effects of 5-ALA and PDT (635 nm; total light fluence of 6, 12, or 18 J/cm<sup>2</sup> delivered at a fluence rate of 5 mW/cm<sup>2</sup>). MGd interacted with 5-ALA and PDT synergistically, with the degree of synergism increasing with increasing light fluence. At the highest light fluence (18 J/cm<sup>2</sup>), spheroids showing growth 4 weeks after an initial

exposure to (i) MGd (ii) 5-ALA and PDT or (iii) MGd plus 5-ALA/ PDT was 100%, 75%, 15%, respectively.

## Pharmacokinetics

Pharmacokinetic studies were performed in mice, rats, rabbits and dogs by a number of routes (PO, IV, IP). Studies conducted by the sponsor in the rat and dog, were supplemented with published information on the absorption, distribution, metabolism and excretion of 5-ALA with particular emphasis on tissue distribution after systemic administration of 5-ALA in tumour bearing animals, including glioma models in rats.

*Absorption.* The pharmacokinetic disposition of 5-ALA was similar in humans and dogs given the comparable (i) rapid oral absorption ( $T_{max}$  values of about 0.63 hours [Maximum Concentration ( $C_{max}$ ), 14.72  $\mu\text{g}/\text{mL}$ ; AUC, 22.3  $\mu\text{g}\cdot\text{h}/\text{mL}$ ] and (ii) elimination half life ( $T_{1/2}$ ) (0.62 hours) after an equivalent oral dose of 20 mg/kg (the proposed clinical dose). Plasma clearance was 0.940 L/kg/h and the volume of distribution was 0.826 L/kg. Following IV dosing, the kinetic profile ( $C_{max}$  40.9  $\mu\text{g}/\text{mL}$ ; AUC 25.9  $\mu\text{g}\cdot\text{h}/\text{mL}$ ) was similar to PO dosing of 20 mg/kg in dogs. However, PpIX levels at this PO dose in humans ( $C_{max}$  0.259  $\mu\text{g}/\text{mL}$ ) were much higher than in dogs ( $C_{max}$  PpIX levels of 0.033  $\mu\text{g}/\text{mL}$  [PO] and 0.035  $\mu\text{g}/\text{mL}$  [IV]; rats [PO]:  $C_{max}$  0.115  $\mu\text{g}/\text{mL}$  at 30 mg/kg). Bioavailability was 86% in dogs with PO dosing. 5-ALA was also rapidly absorbed by the oral route in rats, with  $T_{max}$  20 minutes after dosing at 30 mg/kg [ $C_{max}$ , 10.4  $\mu\text{g}/\text{mL}$ ; AUC, 25.4  $\mu\text{g}\cdot\text{h}/\text{mL}$ ] and  $T_{1/2}$  of 1.1 hour. Toxicokinetic data in rats (PO and IV) and dogs (IV) showed dose related increases in 5-ALA exposures. Dose related increases in PpIX exposures were observed in rats, while in dogs there were limited samples with measurable levels detected (except in males at 27 mg/kg, IV). Exposure to PpIX was several orders of magnitude greater in humans than in dogs. There was no accumulation of 5-ALA and PpIX with repeated daily dosing.

Uptake of 5-ALA into normal brain cells and into tumour cells was determined *in vitro*. In normal brain cells, 5-ALA was taken up by a non-saturable process and in relatively high concentrations (about 85% of porphyrins detected were found within cells). In a murine mammary adenocarcinoma cell line, 5-ALA was incorporated both by passive diffusion as well as by active transport by a BETA transporter (probably the high affinity GABA transporter, GAT-2).

*Distribution.* Plasma protein binding by 5-aminolevulinic acid (5-ALA) was low in humans (12%) in the range of 500 to 5000  $\mu\text{g}/\text{L}$  (but not reported for any nonclinical species). The volume of distribution measured in dogs was low (0.26 L/kg). 5-ALA distribution in normal or tumour bearing (thymus aplasia or adenocarcinoma) animals, mice, rats (including glioma models using 101.8, C6 and BT4C glioma cells; models for human malignant glioma), rabbits or dogs following systemic administration, resulted in widespread tissue porphyrin PpIX fluorescence with high levels in liver, kidney and gastrointestinal tract. Brightest fluorescence was seen in tumour tissue and mostly observed 3 to 6 hours post dose. Faint fluorescence was also seen in normal brain tissue, basal pia, choroid plexus and white matter tracts bordering the tumour. Maximum contrast ratio fluorescence intensities between tumour /normal brain surrounding the tumour ranged from 7:1 to 30:1; or in the case of glioma models in rats: 3.6:1, 5:1, 6:1 and 200:1 were observed 4 to 6 hours post dose. Levels in skin ranged from low (rats, dogs) to high (mice, rats). Levels in brain and other tissue 24 hours post dose were similar to basal levels. It was suggested that PpIX synthesis and accumulation in brain tumour tissue could be due to a degraded blood-brain barrier allowing 5-ALA to permeate into the tumour tissue and/or due to enhanced intrinsic metabolic capability to produce more porphyrins such as PpIX. Conversely, in normal brain, penetration of 5-ALA is very low due to the blood brain protection. A role of PEPT2 as efflux transporter in choroid plexus was shown, limiting exposure in CSF and brain. Except for the hypothalamus, all brain regions biosynthesised PpIX from 5-ALA.



*Metabolism.* Since 5-ALA is an endogenous heme precursor that plays a significant role in the biosynthesis of porphyrins, its biosynthesis and metabolism is well known. Although 5-ALA synthesis is regulated by intracellular free haemoglobin via a feedback mechanism, exogenous addition of high levels of 5-ALA bypasses this feedback mechanism and results in an increased production/accumulation of intracellular PpIX. Importantly, since mammalian cells require haemoproteins for aerobic energy metabolism, all nucleated cells have a minimal capacity to synthesise haemoglobin.

Published information was also provided on the metabolism of 5-ALA in tumour cells. In murine mammary adenocarcinoma cells, up to 40% of 5-ALA was metabolised to porphyrins. In a colon adenocarcinoma cell line, up to 91% of accumulated porphyrins was PpIX (copro-, hexa-, hepta- and uroporphyrins were also observed at levels of  $\leq 7\%$  of total porphyrins). In rat liver mitochondria, 5-ALA decarboxylation by the Krebs cycle was a minor process. In fibrosarcoma MethA cells, not only did relatively low levels of ferrochelatase but also increased uptake of 5-ALA contributed to the accumulation of PpIX. A literature report in dogs showed porphyrins detected in plasma following dosing of 5-ALA (100 mg/kg, IV) were coproporphyrin III and protoporphyrin IX; porphyrin levels in liver, pancreas, prostate, bladder, muscle and skin increased up to 6 to 10 hours (porphyrins in liver were mainly PpIX). There were no toxicological issues of concern that arose from the metabolism data submitted.

*Excretion.* In rats, PO dosing resulted in dose dependent excretion in the urine. In dogs, urinary 5-ALA excretion was highest 2–4 hours after dosing and the total urinary porphyrin excretion was maximal 4 to 8 hours after dosing. 5-ALA (and various porphyrin metabolites) were excreted in the urine by re-absorption/excretion mechanisms. A complete mass balance study was not performed. The nonclinical overview states: '*For comparison, in humans elevated plasma levels result in an increase of urinary excretion of 5-ALA, filtration in the glomeruli, tubular secretion and reabsorption. Porphyrins formed in the liver after systemic administration of 5-ALA are partly excreted with bile and urine and partly reabsorbed enterally.*' The draft Product Information states: '*ALA is eliminated quickly with a terminal half life of 1 to 3 hours. Approximately 30% of an orally administered dose of 20 mg/kg body weight is excreted unchanged in urine within 12 hours.*'

*Conclusion.* Comparisons of the pharmacokinetic profiles of 5-ALA in the laboratory animal species used in the repeat dose toxicity studies (mice, rats and dogs) indicate that sufficient similarities exist to allow them to serve as appropriate models for the assessment of 5-ALA toxicity in humans. However, PpIX levels in humans were much higher than in rats and dogs.

### **Pharmacokinetic drug interactions**

No interaction studies were performed with concomitant treatments that might interfere with 5-ALA use for the proposed indication, although a nonclinical interaction study with anticonvulsants would be worthwhile.

### **Toxicology**

The sponsor conducted GLP compliant single dose toxicity, repeat dose toxicity, genotoxicity, local tolerance, skin sensitisation and phototoxicity studies using 5-ALA HCl; reproductive and developmental toxicity studies were not submitted. Studies were performed "in the dark" (except the phototoxicity studies) under 'light protection' conditions (that is, with a light source that did not emit UV light [less than 635 nm]) or were conducted under subdued or dimmed light to exclude phototoxic reactions. Additional published studies were provided for toxicology evaluation.

## **Acute toxicity**

Acute toxicity studies in mice (IV, IP) and rats (IV, PO) were conducted in accordance with GLP, although animals were not exposed to normal lighting conditions or photoactivation fundamental to the proposed clinical use of 5-ALA in photodynamic diagnosis (PDD). 5-ALA was very well tolerated in mice following IP dosing and rats following PO dosing (the clinical route), up to the highest dose (in both cases, 2500 mg/kg) since no signs of adverse reactions were observed. The IV administration of high doses of 5-ALA to mice (greater than or equal to 1000 mg/kg) or to rats (greater than or equal to 500 mg/kg) lead to nonspecific findings of intolerance (including reduced motility, ataxia, dyspnoea, lateral position and muscular hypertonia, without any macroscopic abnormalities at necropsy or signs of delayed toxicity). The LD<sub>50</sub> by the IV route was about 1000 mg/kg in both mice and rats, in both sexes. Necropsies were conducted, but target organs were not identified. The maximum non lethal dose for 5-ALA by the oral route was 2500 mg/kg in rats (highest dose tested), showing a low order of acute toxicity by the clinical route.

### ***Acute toxicity - 5-ALA and photodynamic therapy (PDT)***

Three submitted published studies reported a side effect of significant oedema in experimental 5-ALA PDT in: i) tumour tissue (6.5 % compared with. controls) and in normal tissue (although considerably less [3.2%]) in normal and tumour (C6 glioma) bearing Wistar rats (PDT: 100 J/cm<sup>2</sup>, 635 nm); and iii) in normal brain of Fischer rats (125 mg/kg 5-ALA, IP; 26 J) and in iii) the dorsal skin fold chamber of the Syrian Golden hamster (500 mg/kg 5-ALA, IV; 100 J/cm<sup>2</sup>).

### ***Repeat dose toxicity***

Submitted GLP repeat dose toxicity studies conducted by the sponsor were up to 14 Days duration and performed in rats (PO [the clinical route], IV) and dogs (IV) and performed “in the dark” under ‘light protection’ (that is with a light source that did not emit UV light [less than 635 nm]) to avoid possible effects of photosensitisation due to porphyrin (PpIX) accumulation, and therefore excludes phototoxic reactions. Only the 14 Day study in rats involved oral administration. The duration of these studies, the species used (rats and dogs), group sizes and the use of both sexes were consistent with ICH guidelines. Published (non GLP) studies in mice were also submitted (that is, 5 to 8 week [PO] and 13 week [IP] repeat dose toxicity studies in mice). Both these studies were performed under normal lighting conditions. Short term repeat dose oral toxicity studies on 5-ALA in the rat (14 Days) and mouse (5 to 8 weeks) reflect the intended route for human use and are considered suitable to cover single dose administration in patients. However, the submitted 5 to 8 week unconventional mouse study (0, 2 mg/mL 5-ALA in drinking water) provided only limited data (assessment of liver porphyrins and of UROD (uroporphyrinogen decarboxylase) enzyme activity involved in porphyrin metabolism). No overt toxicity was reported in this study. While the non GLP 13 week repeat dose toxicity study in mice (0, 10, 50, 100 mg/kg; 3 injections per week) was co-located with a reproductive toxicity study and employed dosing by the IP route, substantial data toxicity was provided. Similarly, there were no reports of overt toxicity.

**Table 1 Overview of \*repeat dose toxicity studies**

Species & strain	Study	Duration	Route	Dose (mg/kg/day)	Date	GLP
Mouse (SWR, DBA/2) <i>Note: normal lighting</i>	Constantin <i>et al.</i> , 1996	5 and 8 Weeks	PO	2 mg/mL in drinking water	1996	No
Mouse (CD-1; random bred albino strain) <i>Note: normal lighting; Data in Reproductive Toxicity Section</i>	Kennedy <i>et al.</i> , 1976	13 weeks [3 injections per week]	IP	0, 10, 50, 100	1976	No
Rat (SD)	LPT 11651/98	14 Days	PO	0, 30, 100, 300	July 14, 2000	Yes
	LPT 10827/97	7 Days	IV	0, 100, 200, 400	January 29, 1998	Yes
	LPT 10828/97	14 Days	IV	0, 125, 250, 500	June 10, 1998	Yes
Dog (Beagle)	LPT 11652/98	7 Days	IV	25, 75	March 4, 1999	Yes
	LPT 11664/98	14 Days	IV	0, 3, 9, 27	July 5, 2000	Yes

(\*): GLP repeat-dose studies conducted by the sponsor performed "in the dark" under 'light protection' conditions (i.e., with a light source that did not emit UV light [ $< 635$  nm]) to exclude phototoxic reactions.

## Relative exposure

**Table 2 Relative exposure in repeat dose toxicity studies**

Species	Study duration Route of Administration	Dose mg/kg	Sex	AUC <sub>0-24 h</sub> $\mu\text{g}\cdot\text{h}/\text{mL}$ 5-ALA; [PpIX]	Exposure ratio# -ALA	Exposure ratio# PpIX
Rat (SD)	14 Days [PO]	30	M	34.2 [0.592]	1	0.3
			F	23.2 [0.307]	0.7	0.2
		300	M	233.2 [0.615]	6.8	0.3
			F	224.4 [0.191]	6.6	0.1
	14 Days [IV]	125	M	661.3 [0.587]	19.4	0.3
			F	686.1 [0.443]	20.1	0.2
		500	M	1359.6 [1.986]	39.8	1
			F	2051.3 [0.305]	60.1	0.2

Species	Study duration Route of Administration	Dose mg/kg	Sex	AUC <sub>0-24 h</sub> µg·h/mL 5-ALA; [PpIX]	Exposure ratio#5 -ALA	Exposure ratio# PpIX
Dog (Beagle)	14 Days [IV]	3	M	9.0 [-]	0.3	-
			F	10.2 [-]	0.3	-
		27	M	85.6 [0.129]	2.5	0.1
			F	87.7 [-]	2.6	-
Human (healthy males) MC- ALS.20/BV	Single Dose [PO]	^20 mg/kg	M	AUC <sub>0-∞</sub> 34.15 [1.906]	-	-

(#): Animal [AUC<sub>0-24 h</sub>] : Human plasma [AUC<sub>0-∞</sub>]; (^): Based on the recommended clinical dose of 20 mg/kg in patients (-): Not determined

Relative exposure based on systemic exposure (AUC) in the 14 day pivotal PO study in rats (clinical route) was moderate for 5-ALA (1 to 7 fold) and low for PpIx (0.1 to 0.3 fold) compared to that anticipated clinically. In the 14 day IV studies, relative exposure for 5 to ALA in rats (19 to 60 fold) and dogs (0.3 to 3 fold) and for PpIX in rats (0.2 to 1 fold) and dogs (0 to 0.1 fold), varied from low to high as shown in the Table above.

### Major toxicities

The major targets for 5-ALA toxicity were the liver (increased weight in rats [PO dosing]; decreased liver weight in dogs [IV dosing] with black discolouration), as well as findings of bile duct changes in all the GLP 14 Day PO or IV dosing studies in rats and dogs. Bile duct changes were not reversible within the recovery periods (14 days) in the pivotal 14 day rat (PO, IV) or dog (IV) studies. Some notable clinical chemistry correlates were alterations in transaminases [alanine aminotransferase (ALT), aspartate transaminases (AST), alkaline phosphatase (ALP)], lactate dehydrogenase LDH, total cholesterol, creatinine, urea and total bilirubin. Vomiting was also seen (only in dogs). There were no sex differences. The treatment findings are likely due to the excessive increase in porphyrin levels, metabolism and excretion following repeated 5-ALA dosing.

### Rat pivotal PO and IV studies

Hepatic toxicity (increased liver weight) and irreversible bile duct toxicity were observed following 5-ALA treatment in the rat 14 day PO study. The relative liver weights (reported as g/kg) increased at greater than or equal to 100 mg/kg (about 7%; systemic exposure and exposure ratio were not determined). This was accompanied by increases in AST at greater than or equal to 30 mg/kg (6%; exposure ratio, approximately 1). Total bilirubin also increased at greater than or equal to 30 mg/kg. The histopathological correlate of findings of bile duct changes (including bile duct proliferation, enlargement of biliary epithelium,

peribiliar fibrosis and inflammatory infiltrates, intraductal bile plugs and peribiliar bile pigment accumulation) was observed at greater than or equal to 100 mg/kg and was not reversible within the 14 day recovery period. The NOEL was not established in this study and was less than 30 mg/kg.

#### ***Dog pivotal IV study***

In a 7 Day dose ranging IV study (1 dog/sex/dose), at the high dose (75 mg/kg; systemic exposure and exposure ratios were not determined), the female died 1 day after the study termination and the high dose male was euthanised 2 days after study termination due to poor health. At necropsy, about 20 reddish foci (1 to 2 mm) in the left ventricle of the heart were found in both dogs. There were also marked increases in blood urea at the low and high doses (25 and 75 mg/kg) of greater than or equal to 37% (at 27 mg/kg, the exposure ratio is approximately 3). Hepatic toxicity and bile duct toxicity were also observed in the pivotal 14 day IV study in dogs.

Decreased liver weights were observed at the high dose of 27 mg/kg (males: 19%, females: 8%; exposure ratio, approximately 3). Black discolouration of livers at necropsy was evident in 2/5 females at 27 mg/kg, and was accompanied by the histopathological hepatic correlate of bile duct toxicity. At the histopathology assessment, intrahepatic cholestasis (characterised by bile plugs in the extended bile canaliculi) was noted at all doses (greater than or equal to 3 mg/kg; exposure ratio, approximately 0.3). The finding was dose dependent in severity and present in all 5-ALA treated dogs. However, this finding was marginal (grade 1) in low dose dogs (3 mg/kg) and had completely subsided during following the 14 day recovery period. Laboratory investigations showed correlates of increased total bilirubin, ALT and AST at the low and mid dose levels of 9 mg/kg (systemic exposure and exposure ratios were not determined) and 27 mg/kg (exposure ratio, approximately 3) LDH also increased at 27 mg/kg. Body weight reduction, increased ALT activity and bile duct lesions were not completely reversible within the 14 day recovery period at 9 and 27 mg/kg. In this study, there were no left ventricle changes of the heart as seen in the dose ranging at 75 mg/kg. The NOEL was not determined and was less than 3 mg/kg.

#### ***CNS toxicity in rats and dogs***

Clinical signs indicative of CNS toxicity but with no histopathological changes were observed in rats and dogs. In the rat 14 day IV study, from day 5 onwards all rats showed ataxia, dyspnoea and a slow gait of 1 minute duration after injection at 500 mg/kg (relative exposure, 50). In the IV dose ranging study in dogs, from days 1 to 7 vomiting was observed at greater than or equal to 25 mg/kg (relative exposure, approximately 3 dogs vomited once and/or repeatedly from Days 1 to 7 [20 minutes to 2 hours post dose]); on Day 7, dogs also showed ataxia, reduced motor activity, tremor, reduced body temperature and dyspnoea at the high dose level of 75 mg/kg (systemic exposure and exposure ratio not determined). In the 14 day IV study in dogs, vomiting was also observed among animals once or repeatedly, between 20 minutes to 6 hours post dose at doses greater than or equal to 3 mg/kg (relative exposure, 0.3). Due to the high safety margin reported in rats and the finding of emesis limited only in dogs suggests these findings are not deemed to be of clinical relevance.

#### ***Mouse PO and IP studies (published literature reports)***

Oral administration of 5-ALA (2 mg/mL in the drinking water<sup>8</sup>) to mice for 5 to 8 weeks led to a significant increase in liver porphyrin levels, with no reports of overt toxicity. This study was performed under regular lighting conditions.

<sup>8</sup> Constantin D *et al.* (1996) Uroporphyrin induced by 5-aminolevulinic acid alone in AHrd SWR mice. *Biochemical Pharmacology* 52, 1407-13

A 13 week pilot subacute toxicity study in mice (also performed with normal lighting conditions,<sup>9</sup>) was carried out (as a pilot study) prior to a reproductive toxicity study with repeated administration of 5-ALA to both male and female mice at doses of 10 to 100 mg/kg (IP; tri weekly). Parameters examined included haematological profiles, clinical chemistry and gross and microscopic pathology. There were no findings of overt toxicity. In male mice, blood glucose levels were moderately increased (40 to 50%; about 83 mg/100 mL compared with controls, about 57 mg/100 mL;  $p < 0.05$ ) at 100 mg/kg (values were reported to be within the normal range). In female mice, white blood cell (WBC) counts were elevated at 100 mg/kg, although the increase was attributed to a high value in a single mouse. In both mouse studies, systemic exposure was not determined, so exposure ratios are not reported.

#### *Exposure margins*

Hepatic toxicity and irreversible bile duct changes were seen in the GLP pivotal 14 day studies in rats and dogs. Safety margins and No Observable Adverse Effect Level (NOAEL) values were not established since adverse effects were observed at the lowest doses tested. Therefore, the NOAEL would be below 30 mg/kg in the rat following PO dosing (exposure ratio, approximately 1), below 125 mg/kg in the rat following IV dosing (exposure ratio, approximately 20) and below 3 mg/kg in the dog following IV dosing (exposure ratio, 0.3). Based on these limited exposure comparisons, hepatic toxicity and irreversible bile duct changes are a potentially relevant clinical risk.

The safety margins for PpIX were also not established. However, in the rat, the low dose (LD) of 30 mg/kg PO (which produced only small increases in total bilirubin and AST, there were no other effects at this dose, as bile duct changes and liver weight changes were only observed at 100 and 300 mg/kg, respectively) produced comparable 5-ALA systemic exposure ( $AUC_{0-24h}$  34.2  $\mu\text{g}\cdot\text{h}/\text{mL}$ ) as that with the intended clinical dose in humans (20 mg/kg;  $AUC_{0-\infty}$  34.15  $\mu\text{g}\cdot\text{h}/\text{mL}$ ). The safety margin in rats for PpIX at an ALA dose of 30 mg/kg PO and based on the measured systemic PpIX levels results in only low exposure ratios of 0.3 and 0.2 for male and female rats, respectively, compared with the intended clinical exposure.

#### *Adequacy of the data submitted*

An obvious limitation in all the company sponsored toxicity studies was the absence of animal exposure to normal light and photoactivation, hence the animals were poorly exposed to the potential systemic effects of photoactive porphyrin cytotoxicity expected clinically. In the clinical setting, patients are typically exposed to white light during neurosurgery and therefore, at least acute toxicity studies mimicking the intended clinical use would have provided useful information. It is expected that this data would have been collected prior to first in human studies.

#### **Genotoxicity**

5-ALA was tested for genotoxic effects in a standard battery of tests conducted by the sponsor (*S. typhimurium* reverse mutation assay, gene mutation (HPRT) assay in mammalian cells [V79], a chromosome aberration assay in human peripheral lymphocytes and a mouse micronucleus test). These studies were in compliance with GLP and used appropriate doses/concentrations. 5-ALA and its metabolites generally showed no mutagenic potential in these studies performed in the dark (or under darkened conditions [with a light source that did not emit any UV light], that is, under so called 'light protection' conditions in order to exclude potential phototoxic reactions). However, several published studies reported genotoxicity since (i) in rat hepatocytes, chromosomal aberrations and micronuclei were induced in the dark; it was suggested either PpIX has genotoxic potential or the effect relates to the generation of reactive oxygen species by oxidation of 5-ALA; (ii)

<sup>9</sup> Kennedy GL Jr *et al.* (1976) Toxicity studies with delta-aminolaevulinic acid. *Food Cosmet Toxicol* Jan 14, 45-7.

5-ALA induced DNA damage in human lymphocytes, which were very sensitive, as 0.75 mM 5-ALA alone (without light) caused cytotoxicity (greater than 50 %) and DNA damage, approximately 15% cytotoxicity occurred at the lowest concentration of 0.05 mM, and (iii) 5-ALA increased levels of mutagenic markers of oxidative damage of DNA (in the dark) in various rat tissues. The nonclinical overview stated '*The DNA damaging potential of ALA in the dark on normal lymphocytes might be a serious side effect, which is concentration dependent.*'

Further published studies clearly showed phototoxicity and photogenotoxicity potential of 5-ALA with light exposure/irradiation since: (i) in a photo-micronucleus test in mammalian V79 cells; (ii) studies showed DNA damage (micronuclei, oxidative DNA damage) in AS52 (CHO), L1210 (mouse leukaemia) cells or lymphocytes; and (iii) studies also showed increased 8-oxo-7, 8-dihydro-2'-deoxyguanosine (8-oxodGuo; a mutagenic marker of oxidative damage) in DNA from murine leukaemia P388D1 cells.

Phototoxicity/photogenotoxicity was well correlated with increased formation of PpIX (measured in parallel) following 5-ALA administration. The Nonclinical Overview suggested that genotoxicity in some tests in the presence of visible light may not be relevant to diagnostic use in light of a limited range of wavelengths, and that light penetration of tissues would be greater in photodynamic therapy in comparison with photodiagnosis.

### **Carcinogenicity**

Carcinogenicity studies were not submitted, and are not required for a drug administered only once, unless there is cause for concern<sup>10</sup>. Unequivocal genotoxic compounds are presumed to be carcinogenic and do not require long term genotoxicity studies. 5-ALA was genotoxic in some, but not all genotoxicity studies, and the Nonclinical Overview suggested that genotoxicity in some tests in the presence of visible light may not be relevant to diagnostic use in light of a limited range of wavelengths. It is known that patients suffering from porphyrias with highly elevated 5-ALA levels have a potentially increased incidence of cancer, especially in the liver<sup>11</sup>, however there is no evidence of higher rates of skin cancer in patients with photosensitivity diseases due to the presence of increased PpIX levels in skin<sup>12</sup>. A published study in which hairless mice were exposed to solar ultraviolet and visible light and treated topically with 20% ALA every week showed a significant delay in the time to development of the first tumour<sup>13</sup>, however this model has some recognised limitations.<sup>14</sup> It is unclear whether a single exposure to high ALA and porphyrin levels might pose a significant human carcinogenic risk. In view of the proposed use in diagnosis and surgical resection of brain gliomas, the lack of carcinogenicity studies is acceptable. Monitoring for drug related carcinogenicity is probably not feasible given the median patient survival of less than 1 year.

### **Reproductive toxicity**

Conventional GLP reproductive toxicity studies were not submitted. However published *in vitro* and *in vivo* unconventional reproductive and developmental toxicity studies were submitted. These provided clear evidence that with concomitant light exposure, 5-ALA displays embryotoxic, fetotoxic and teratogenic potential.

<sup>10</sup> ICH S1A. Guideline on the need for carcinogenicity studies of pharmaceuticals. 1995

<sup>11</sup> Ladner DP *et al.* (2001) Photodynamic diagnosis of breast tumours after oral application of aminolevulinic acid. *Br J Cancer* 84: 33-7.

<sup>12</sup> Fuchs J *et al.* (2000) Genotoxic potential of porphyrin type photosensitizers with particular emphasis on 5-aminolevulinic acid: implications for clinical photodynamic therapy. *Free Radical Biology & Medicine* 28:537-4.

<sup>13</sup> Stender I.-M *et al.* (1997). Photodynamic therapy with topical  $\delta$ -aminolevulinic acid delays UV photocarcinogenesis in hairless mice. *Photochemistry and photobiology* 66: 493-496.

<sup>14</sup> EMA. CPMP. Note for guidance on photosafety testing (CPMP/SWP/398/01, 2002). Nonclinical Evaluation of Aminolevulinic acid HCl (GLIOLAN) Submission No. PM-2012-03095-3-2 24

**Table 3 Overview of reproductive toxicity studies**

Species & strain	Study type; [Route]	Dose (mg/kg); [Dosing Frequency]	Study	GLP
<b>Fertility and early embryonic development</b>				
Mouse (CD-1; random bred albino strain)	<i>Effects on preimplantation mouse embryos (in vitro)</i>	0, 0.1, 0.5, 1.0, 5.0 mM	Yang <i>et al.</i> , 1995	No
	<i>Effects on mating ability of male mice</i> [PO]	0, 250, 500, 1000 [Daily for one week]	Arnold <i>et al.</i> , 1975	No
	<i>Effects on fertility in male and female mice</i> [IP]	0, 10, 50, 100 [13 weeks, 3 injections/week]	Kennedy <i>et al.</i> , 1976	No
<b>Embryofetal development</b>				
Chicken (white leghorn)	<i>Effects on development of chick embryos in ovo.</i> [Intra-amniotic Injection]	0, 1.0, 3.0, 10.0, 30.0, 100.0, 300.0 µg / 3 µL [Single dose]	Peterka <i>et al.</i> , 2001	No
Rat (SD)	<i>Effects in early pregnancy</i> [IV]	0, 20, 200 [Single dose]	Yang <i>et al.</i> , 1994	No

Findings from an *in vitro* study<sup>15</sup> showed 5-ALA (0.1 to 5 mM) with light exposure had direct deleterious effects of photodynamic ablation on preimplanted mouse embryos. There were parallel findings in the same study showing preimplanted mouse embryos are capable of converting 5-ALA to PpIX. Following PO or IP treatment daily for 1 week (250, 500, 1000 mg/kg 5-ALA HCl; high dose (HD) group treated only once by IP route) male mice were mated with untreated nulliparous females immediately after the final dose (Arnold *et al.*, 1975). Females were changed over weekly for 6 consecutive weeks. Mating (fertility) incidences and litter values that is, for females (including the embryonic index) were not different compared with controls. However, the mating ability of males treated only once with 1000 mg/kg IP showed reduced fertility for the first 2 weeks following treatment (mating [fertility] index of 37.5% [Weeks 1 and 2] compared with 75% in controls [Weeks 1, 2]). This was likely due to toxicity rather than a genetic (mutagenic) effect since all other reproductive parameters were similar compared with control values.

In a study<sup>16</sup>, repeated administration to both male and female mice of 5-ALA at doses of 10, 50 or 100 mg/kg (IP) tri weekly for 13 weeks prior to and during the mating period (2 weeks) showed increases in the mean number of resorptions per pregnant female at 50 and 100 mg/kg IP (1.0 and 1.1, respectively versus saline control 0.4, untreated controls 0.5, 0.6,

<sup>15</sup> Yang JZ *et al.* (1995) Treatment with 5-aminolevulinic acid and photoactivating light causes destruction of preimplantation mouse embryos. *Fertility and Sterility* 63, 1088-93.

<sup>16</sup> Kennedy GL Jr *et al.* (1976) Toxicity studies with delta-aminolaevulinic acid. *Food Cosmet Toxicol* Jan 14(1), 45-7.



0.5 and 0.2), one of the 7 pregnant females in the 100 mg/kg IP group resorbed 7/9 implantations. There were other signs of toxicity.

5-ALA did not show any signs of embryotoxicity *in ovo*, in the dark at greater than or equal to 3 µg, up to the highest concentration (300 µg) in chick embryos. However, 5-ALA with concomitant light irradiation of embryos induced marked embryotoxic effects (3 to 300 µg) and multiple malformations (10 to 300 µg; including umbilical hernia and buphthalmia, cleft beak and defect of eyelid as well as polydactyly)<sup>17</sup>. PpIX however, exhibited embryotoxicity and produced multiple malformations in the dark (in the absence of irradiation) as well as with irradiation.

In an *in vivo* study<sup>18</sup> 3 hours following 5-ALA administration (IV, 20 or 200 mg/kg) on gestational day 10 (GD10), the abdominal cavity of rats was opened followed by exposure to one or both uterine horns to photoactivating light. Transmural exposure to light resulted in dose dependent detrimental effects on fetuses (resorption in early pregnancy) in rats. Rebreeding of female rats after a drug free period (8 to 12 weeks) for a second time, suggested that 5-ALA at 200 mg/kg plus light exposure did not irreversibly compromise endometrial function in the majority of rats (67%; based on foetal incidence per uterine horn and fecundity).

The sponsor proposes an Australian Pregnancy Category C<sup>19</sup>, which is appropriate. The contraindication of 5-ALA in pregnancy is supported.

### **Local tolerance**

The local tolerance of aqueous solutions of 5-ALA was evaluated in rabbits under darkened housing conditions with a light source that did not emit any UV light [less than 635 nm]. Local tolerance was very good following IV or intra-arterial dosing (60mg/kg; no or minimal findings). Following paravenous (5 mg/kg), SC (10 mg/kg) and IM (5 mg/kg) administration, there were minor local irritant effects (possibly due to the low pH of the solution). Overall, there was no local toxicity observed after PO dosing, and only minor irritation by other routes in the specific local toxicity. No skin sensitisation was observed with 5-ALA HCl following the topical challenge of guinea pigs with a 25 or 50% 5-ALA aqueous suspension. Following the initial induction with intracutaneous dosing with a 10% aqueous suspension, slight skin irritation was observed.

### **Phototoxicity**

Whole body UV irradiation of mice carried out under darkened housing conditions with dimmed light (for 1 hour using a mercury lamp delivering UV doses of 30 J UV-A/cm<sup>2</sup> and 0.3 J UV-B/cm<sup>2</sup>) administered 4 or 24 hours following high IV doses of 5-ALA HCl (250 and 750 mg/kg) resulted in major phototoxic reactions, including death. Phototoxicity and mortality occurred i) at both the LD (250 mg/kg) and HD (750 mg/kg) when followed by UV irradiation 4 hours after dosing and ii) only in the HD group of mice when UV irradiation was administered 24 hours after dosing. There were no inflammatory or degenerative lesions of the eyeball in any 5-ALA treated mice. UV irradiation administered 4 hours post dose resulted in marked phototoxic reactions of oedema (Grade 2) in 3 out of 5 mice (not 3). All 5 HD animals died within 24 hours. Necropsy revealed inflammatory reactions of the skin (shoulder) and eyelids, consisting of granulocytic dermatitis, ulceration and epithelial necrosis. UV irradiation 24 hours post dose produced erythema (Grade 1 or 2) in all HD

<sup>17</sup> Peterka M and Klepáček I (2001) Light irradiation increases embryotoxicity of photodynamic therapy sensitizers (5-aminolevulinic acid and protoporphyrin IX) in chick embryos. *Reproductive Toxicology* 15, 111-6.

<sup>18</sup> Yang JZ *et al.* (1994) Photodynamic ablation of early pregnancy in the rat with 5-aminolevulinic acid: a potential new therapy for tubal ectopic pregnancy in the human. *Fertility and Sterility* 62, 1060-65.

<sup>19</sup> Pregnancy Category C: Drugs which, owing to their pharmacological effects, have caused or may be suspected of causing, harmful effects on the human fetus or neonate without causing malformations. These effects may be reversible. Accompanying texts should be consulted for further details.

mice; 72 hours post irradiation 2 out of 5 HD mice showed moderate to severe erythema (Grade 3).

Phototoxicity reactions observed following dosing of 5-ALA *in vivo* and *in vitro* are likely to be associated with time dependent intracellular increases of photosensitizing PpIX. This was demonstrated in a 'Phototoxicity' study using irradiated C6 glioma cells derived from a rat glial tumour, in which PpIX fluorescence emission kinetics and phototoxicity were examined. No 5-ALA toxicity was observed in the dark but light exposure (25 J/cm<sup>2</sup>; 15 minutes; 514 nm) following incubation with 5-ALA resulted in a reduction in cell survival. The 50% lethal cytotoxic concentration (6 hours incubation) was 45 µg/mL. Increasing the incubation time from 3 to 6 hours increased the photocytotoxicity (cell death). Similarly there was a concentration related increase in phototoxicity over 25 to 200 µg/mL.

Based on all the available nonclinical data for the proposed indication of photodynamic diagnosis of brain tumours, care must be taken during photoactivation of 5-ALA/PpIX to avoid potential phototoxic reactions in other organs/tissues.

### **Impurities**

No impurities required toxicological qualification.

### **Paediatric use**

No specific studies in young animals were submitted. The safety or efficacy of 5-ALA in children or adolescents (up to 18 years old) has not been determined.

## **Nonclinical summary and conclusions**

### **Summary**

- The nonclinical data consisted of published literature and sponsored reports, hence not all definitive studies were GLP compliant (for example 5 to 8 week mouse repeat dose toxicity, photogenotoxicity and reproductive toxicity studies). The majority of toxicity studies used the IV route, not the clinical PO route.
- No nonclinical efficacy studies were performed to define the optimal conditions for 5-ALA use for the proposed indication of photodynamic diagnosis of tumours during neurosurgery using the clinical protocol. However, published *in vitro* and *in vivo* studies on the primary pharmacodynamics of 5-ALA, mostly on the use of 5-ALA as a sensitizer in photodynamic therapy (PDT [ $\lambda=630$  to 635 nm]), rather than as a sensitizer for use in photodynamic diagnosis (PDD) of brain tumours, showed 5-ALA penetrates into brain tumours after systemic administration and efficacy in tumour models. It was also observed that adjacent healthy tissue may show PpIX fluorescence, depending on its ability to synthesise PpIX.
- One study<sup>20</sup> used a similar protocol to the entire clinical procedure for fluorescence guided resection (FGR) in an intracranial VX2 tumour model in rabbits. The entire procedure (starting 4 hours after 5-ALA administration) significantly increased the completeness of tumour resection by 1.4 fold and reduced residual tumour by 16 fold.
- Limited secondary pharmacology data suggested that short term single exposure (3 hours) to 5-ALA (up to 34 µM) did not alter cellular haemoglobin or cytochrome P-450 levels in cultured hepatocytes from chick embryos and that neurotransmitter disturbances and/or neuronal toxicity cannot be ruled out. Cardiovascular safety

<sup>20</sup> Bogaards A *et al.* (2004) Increased brain tumour resection using fluorescence image guidance in a preclinical model. *Lasers Surg Med* 35, 181-90.

studies in dogs showed 5-ALA (45 mg/kg, IV) resulted in a slight decrease in peripheral arterial blood pressure, systolic LVP and a decrease in dp/dt max post dose (43%). Five minutes after administration, the baseline values had been reached again. There were no *in vitro* studies of the potential for QT interval prolongation. Retrospective analyses of QTc intervals in dog repeat dose toxicity studies showed no effects at 5 minutes post dose, but the PpIX Tmax was 2 to 4 hours. Saluresis was observed in rats.

- Further safety pharmacology studies showed the potential of 5-ALA to induce toxicity in the CNS (inhibition of glutamate uptake and decreases in cAMP levels) and phototoxic damage to skin (destruction of sebaceous glands, hair follicles) of mice or the intraperitoneal organs (mild superficial damage) of rats with bright white (photoactivating) light.
- The kinetic profile of 5-ALA was not comprehensive but 5-ALA its biosynthesis and metabolism is well known. The 5-ALA kinetic profile in dogs was similar to humans due to rapid absorption ( $T_{max}$  0.63 hours,  $C_{max}$ , 14.72 µg/mL; AUC, 22.3 µg.h/mL) and  $t_{1/2}$  (0.62 hours) after an equivalent oral dose of 20 mg/kg (the proposed clinical dose). However, systemic PpIX levels in rats and dogs were substantially lower than in humans. In tumour bearing mice, all tissues examined showed porphyrin fluorescence, with highest levels in the tumour, normal brain tissues showed low levels of fluorescence. Renal excretion was a major route of 5-ALA and PpIX elimination in rats and dogs.
- All GLP compliant toxicity studies were performed in the dark (except phototoxicity studies) under 'light protection' to exclude phototoxicity.
- 5-ALA had a low acute oral toxicity in mice and rats.
- Repeat dose toxicity studies with mice (PO, IP; 8 to 13 Weeks, non GLP) and GLP compliant studies in rats (PO, IV) and dogs (IV) up to 14 days were adequate. Relative exposure in the 14 day pivotal PO study in rats (clinical route) was moderate for 5-ALA (1 to 7 fold) and lower for PpIX (0.1 to 0.3 fold) than that anticipated clinically. In the 14 day dog (IV) study, exposure for 5-ALA and PpIX was 0.3 to 3 fold and 0 to 0.1 fold, respectively).
- Hepatic toxicity (alterations in serum transaminases, LDH, total cholesterol, creatinine, urea and total bilirubin) and irreversible bile duct changes were observed in the pivotal 14 day toxicity studies in rats and dogs. Vomiting was observed only in dogs. There was no overt toxicity in the published mouse (PO, IP) toxicity studies. These toxicities can be attributed to the pharmacological effects of 5-ALA and are likely due to the increase in porphyrin levels. Animal to human exposure ratios of approximately 1 in rats and less than 1 in dogs suggest that hepatic toxicity and bile duct changes may be clinically relevant.
- Genotoxicity studies performed in the dark showed no mutagenicity in *S. typhimurium* or mammalian V79 cells, however ALA induced chromosomal aberrations and micronuclei in rat hepatocytes, and caused cytotoxicity and DNA damage in human lymphocytes. Light exposure increased DNA damage in CHO and mouse leukaemia cells. An *in vivo* mouse micronucleus test under subdued light was negative.
- Adequate justification for the lack of carcinogenicity studies was provided, taking into account the proposed indication.
- No conventional reproductive toxicity studies were submitted, but published papers showed 5-ALA may affect pregnancy and 5-ALA induced porphyrin synthesis leads to developmental toxicity (embryotoxic, fetotoxic and teratogenic effects).
- There was no local toxicity following PO dosing and only minor irritation by other routes; no skin sensitisation was observed in guinea pigs.

- UV irradiation exposure 4 or 24 hours post 5-ALA dosing produced time dependent phototoxic reactions. Overall, the nonclinical data showed that the skin, eyelids (and/or normal brain surrounding the malignant tumour) are potentially affected when directly exposed to photoirradiation (for at least 24 hours after dosing).

### Conclusions and recommendations

- The nonclinical data consisted of literature publications (all primary and secondary pharmacology, some safety pharmacology, some repeat dose toxicity and some genotoxicity studies, and all reproductive toxicity studies) and sponsored studies.
- Primary pharmacology literature publications focussed mainly on tumour treatment, rather than resection. 5-ALA induced fluorescence and phyto-cytotoxicity in human glioma cell lines *in vitro*, however levels of fluorescence varied between cell lines. Selective increases in PpIX levels and fluorescence in experimental brain tumours were demonstrated in treated mice, rats and rabbits, however some studies detected increased fluorescence in normal brain, contralateral white matter, ventricle ependyma and pia mater. Normal brain structures lacking a blood brain barrier showed high uptake of 5-ALA. Clinical evaluation of efficacy should take into consideration not only the amounts of tumour, but also normal tissue removed.
- The only *in vivo* study which used similar methods to the clinical protocol, a rabbit intracranial VX2 carcinoma cell model, showed a significant increase in resection completeness (mean 68% to 98%) and a significant decrease in residual tumour (mean 32% to 2%). Although nonclinical studies did not define the optimal conditions for 5-ALA use, data on maximum and relative tumour levels of 5-ALA, PpIX and fluorescence were generally consistent with administration of the clinical dose 3 hours (range 2 to 4 hours) prior to anaesthesia.
- Secondary/safety pharmacology data were based mainly on published literature, and data on potential cardiovascular and neurotransmitter effects were limited. There were no *in vitro* studies of the potential for QT prolongation. Retrospective analyses of QT<sub>c</sub> values in dog toxicity studies showed no effects, however measurements were only made 5 minutes post IV dose. In dogs given a 45 mg/kg IV dose, transient, slight decreases in peripheral arterial blood pressure, systolic ventricular pressure, and dp/dt max occurred within 5 minutes. 5-ALA inhibited glutamate uptake by rat astrocytes *in vitro*, it may be difficult to discern any drug related effects on neurotransmitters in glioma patients.
- Most toxicity studies were conducted by the IV route, not the clinical PO route, and were performed under light protection. The PO toxicity studies did not indicate gastrointestinal (GI) toxicity. Repeat dose IV toxicity studies in rats and dogs attained adequate systemic exposure ratios (AUC, C<sub>max</sub>) for 5-ALA, but exposure ratios for PpIX were ≤1. The liver was identified as a target organ in both species at 5-ALA exposure ratios of approximately 1 in rats and less than 1 in dogs, consistent with clinical findings. Bile duct changes in both species were not reversible over 14 days recovery. Phototoxicity studies showed toxicity in the skin of treated mice and in the superficial layers of exposed internal organs of treated rats. Patient avoidance of strong light for 24 hours post operatively is supported.
- Photogenotoxicity was reported in a range of test systems, with indications that it was dependent on PpIX formation. Genotoxicity tests in darkness or subdued light were mostly negative, for example *in vivo* mouse micronucleus test but there were notable exceptions, 5-ALA induced chromosomal aberrations and micronuclei in cultured rat hepatocytes at greater than or equal to 1 µg/mL and cytotoxicity and DNA damage in human lymphocytes at 0.75 mM. Positive genotoxicity findings are not a major concern for a product proposed for single use in glioma patients.

- Adequate justification was provided for the lack of carcinogenicity studies, taking into consideration the proposed indication.
- Reproductive toxicity data were limited to literature publications, which indicated malformations and lethality to chick embryos and lethality to mouse embryos directly exposed to 5-ALA and light, and resorption of fetuses of rats treated with 5-ALA followed by light exposure of the uterine horn. These effects were not evident under light protection. The proposed Australian Pregnancy Category C, and contraindication of Gliolan in pregnancy, are supported.
- In conclusion, clinical efficacy and safety data (potential cardiovascular effects including QT prolongation, effects on neurotransmitters, and the liver) will be required to cover some of the limitations of the nonclinical data.

## IV. Clinical findings

A summary of the clinical findings is presented in this section. Further details of these clinical findings can be found in Attachment 2.

### Introduction

#### Clinical rationale

##### *Clinical background information*

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

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

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

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

**Chemotherapy:** A meta analyses of data from 12 randomised trials and 3004 patients showed a significant prolongation of survival associated with chemotherapy, with a hazard ratio of 0.85 (95% CI 0.78 to 0.91, P = 0.00004) or 15% relative decrease in the risk of

death. A randomised study of radiation therapy versus radiation therapy plus temozolomide followed by 6 months of adjuvant temozolomide in 573 patients with newly diagnosed GBM demonstrated a statistically significant increase in median survival of 2.5 months in the combination treated group (12.1 versus 14.6 months). Today, adjuvant radiochemotherapy with temozolomide is recommended as standard treatment for all patients aged 18 to 70 with newly diagnosed glioblastoma multiforme.

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

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

Data from a more recent randomised Phase III trial and a meta analysis give substantially better survival rates than population based registries, showing a two years survival rate of 13 to 26.5% <sup>(4)</sup>. Data from clinical trials may be due in part to improvement in therapeutic options but may also reflect survival in selected patients with more favourable prognostic factors.

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

Malignant glioma tissue (WHO Grade III and IV, for example 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, for example medulloblastoma, ligodendroglioma) no fluorescence could be observed after application of the active substance. Brain metastases revealed inconsistent or no fluorescence.

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

increased neo-vascularisation and the over expression of membrane transporters in glioma tissue.

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

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

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

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

### Orphan drug designation

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

### Guidance

Compliance with TGA Guidelines: The relevant guideline is that of the European Medicines Agency (EMA): '*Guideline on clinical evaluation of diagnostic agents*'

The evaluator considered that the present application complies with these guidelines when the additional analyses of clinical outcome as requested by the EMA are included. The application did not include a determination of sensitivity and specificity as required in the guidelines but the evaluator calculated these from biopsy data that the evaluator regarded as Standard of Truth as defined in the Guidelines

### Contents of the clinical dossier

The clinical dossier documented a full clinical development program of pharmacology, efficacy and safety studies. No population pharmacokinetic analyses were provided.

The submission contained the following clinical information:

- One clinical pharmacology study (MC-ALS. 20/BV) in normal subjects provided absolute bioavailability data, pharmacokinetic data, and the extent and duration of skin photosensitisation after oral administration of ALA. Different doses were used in the latter study.
- The pivotal efficacy/safety study, MC-ALS.3/GLI, randomised 415 patients with glioma multiforme (GM), 207 of whom received a single dose of ALA prior to surgery.
- One dose finding study, MC-ALS. 8-I/GLI, enrolled 21 patients with GM, who received single doses of 0.2, 2 or 20 mg/kg to determine dose efficacy relationships.
- Two other studies (MC-ALS. 28/GLI and MC-ALS. 30/GLI) of safety and efficacy were performed in patients with GM. One study (MC-ALS. 32/GLI) assessed safety only.

- A PSUR from 8.3.2008 to 7.9.2008 was submitted, and an Australian Specific Annex to the EU Risk Management Plan.

### ***Paediatric data***

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

### ***Good clinical practice***

The pivotal study protocol was approved by an appropriately constituted Independent Ethics Committee (IECs) of the Ludwig-Maximilian University Munich before the start of the study. The study was conducted in accordance with ethical principles of the Declaration of Helsinki (Somerset West, South Africa 1996) and in compliance with all applicable local regulations and the International Conference on Harmonization (ICH), Good Clinical Practice (GCP) guidelines.

## **Pharmacokinetics**

### **Studies providing pharmacokinetics data**

Table 4 shows the studies relating to each pharmacokinetic topic.

**Table 4. Submitted pharmacokinetic studies**

<b>PK topic</b>	<b>Subtopic</b>	<b>Study ID</b>
PK in healthy adults	General PK    Single dose	MC-ALS. 20/BV
	Multi dose	
	Bioequivalence† Single dose	MC-ALS. 20/BV
	Multi dose	
	Food effect	
PK in special populations	Target population§ Single dose	MC-ALS. 8-I/GLI
	Multi dose	
	Hepatic impairment	
	Renal impairment	
	Neonates/infants/children/adolescents	
	Elderly	
Genetic/gender related PK	Males versus. females	
Population PK analyses	Healthy subjects	



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

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

### Evaluator's overall conclusions on pharmacokinetics

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

## Pharmacodynamics

### Studies providing pharmacodynamics data

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

**Table 5. Submitted Pharmacodynamic Studies**

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

PD Topic	Subtopic	Study ID
Population PD and PK-PD analyses	Not done	

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

### Evaluator's overall conclusions on pharmacodynamics

#### Photosensitization of the skin in normal subjects after ALA administration:

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

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

#### Demonstration of greater fluorescence of tumour compared to normal brain tissue:

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

### Dosage selection for the pivotal studies

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

### Efficacy

#### Studies providing efficacy data

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

### Evaluator's overall conclusions of efficacy

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

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

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

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

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

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

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

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

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

## **Safety**

### **Studies providing evaluable safety data**

The following studies provided evaluable safety data:

#### **Pivotal efficacy study MC-ALS.3/GLI**

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

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

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

*NIH Stroke Score:* One major disadvantage of a more complete brain tumour resection might be that the patient suffers from more neurological deficits postoperatively. No validated scales were available for assessing acute neurological deficits from surgery of brain tumours, so within the three trials in glioma patients, the NIH Stroke Scale (NIH-SS)<sub>6</sub> was

used for assessment of the neurologic outcome and degree of recovery from surgery. The NIH-SS was originally developed for characterising cerebral vascular strokes, which – in analogy to surgery – result in acute neurological deficits. A modified form of the NIH-SS was used. This score covered 16 single neurologically relevant items with a theoretically maximum (worst) score of 36 points. Absolute change in NIH-SS relative to baseline was tabulated and the number/percentages of patients with deterioration, no change or improvement presented.

The frequency of monitoring is shown in the following figure

**Table 6. Safety parameters monitored and frequency (pivotal trial)**

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

### Dose response and non pivotal efficacy studies

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

### Other studies evaluable for safety only

#### **Study MC-ALS.32/GLI:**

Trial MC-ALS.32/GLI was launched in September 2004 after recruitment to the Phase III trial MC-ALS.3/GLI had been terminated. The primary objective was to determine the incidence of adverse events after 5-ALA supported fluorescence guided resection of newly diagnosed patients with malignant gliomas.

### Summary of patient exposure

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

**Table 7. Exposure to ALA in clinical studies**

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

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

### Post marketing experience

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

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

No explanation was given for the absence of PSURs after December 2008. The sponsor was asked to comment on this in the formal questions.<sup>21</sup>

### Evaluator's overall conclusions on safety

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

**Clinical Safety of ALA:** When given as a single dose of 20 mg/kg, ALA was a safe drug. The reported drug related adverse events were of minimal clinical significance except for

<sup>21</sup> PSUR data were subsequently submitted by the sponsor and was taken into account by the Delegate.

pulmonary embolism, and easily managed or prevented with appropriate care. They included the following:

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

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

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

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

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

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

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

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

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

**Necessary surgical expertise:** The need for special training of neurosurgeons to perform the procedures is acknowledged in the Australian Annex for the Risk Management Plan submitted in the application. The claims in the study report of the pivotal trial that no

differences were found in the surgical outcomes of participating surgeons and trial centers is disputed so that implementation of the training required is an important safety issue.

### **First round benefit risk assessment**

#### ***First round assessment of benefits***

The benefits of the proposed usage of ALA are:

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

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

#### ***First round assessment of risks***

The risks of the proposed usage of ALA are:

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

neurosurgeons in this procedure in Europe has been replaced by training by manuals and video presentations in Australia. This risk was addressed in the RPM Australian Annex.

### ***First round assessment of benefit risk balance***

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

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

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

### ***First round recommendation regarding authorisation***

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

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

*"Gliolan is indicated in adult patients for visualisation of malignant tissue during surgery for malignant gliomas that are glioblastoma multiforme (GBM), and intended for gross macroscopic resection of all visible tumour."<sup>23</sup>*

## **Clinical questions**

### ***Pharmacokinetics***

Not applicable.

### ***Pharmacodynamics***

Not applicable.

### ***Efficacy***

Not applicable.

### ***Safety***

The sponsor should comment on the lack of recent PSURs. The latest of those submitted was 7 September 2008.<sup>23</sup>

## **Second round evaluation of clinical data submitted in response to questions**

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

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<sup>23</sup> PSUR data were subsequently submitted by the sponsor and was taken into account by the Delegate.



## V. Pharmacovigilance findings

### Risk management plan

The sponsor submitted a Risk Management Plan [EU-RMP Version 9.0 (dated 08 October/ 2010, DLP 30 June 2010) and Australian Specific Annex Version 1.0 (dated 26 September 2012)] which was reviewed by the TGA's Office of Product Review (OPR). A summary of the RMP appears in the following table.

**Table 8. Summary of risk management plan**

All figures and tables in this section that have been copied from the original dossier are considered by the evaluator to be an accurate representation of the reviewed data, unless qualified as such in the commentary of the report.

Safety concern	Proposed pharmacovigilance activities	Proposed risk minimization activities
Hypoaesthesia	Routine pharmacovigilance	Listed in the Adverse Effects section of the Product Information.
Hypotension	Routine pharmacovigilance	Warning in the Precautions section of the Product Information for use in patients with pre-existing cardiovascular disease. Listed in the Adverse Effects section of the Product Information.
Thromboembolism	Routine pharmacovigilance	Listed in the Adverse Effects section of the Product Information.
Nausea, vomiting	Routine pharmacovigilance	Listed in the Adverse Effects section of the Product Information.
Diarrhoea	Routine pharmacovigilance	Listed in the Adverse Effects section of the Product Information.
Blood amylase increased	Routine pharmacovigilance	Listed in the Adverse Effects section of the Product Information.
Blood bilirubin increased; ALAT increased; ASAT increased; $\gamma$ -GT increased	Routine pharmacovigilance	Warning in the Precautions section of the Product Information to avoid co-administration of other potentially hepatotoxic medicinal products within 24 hours after administration of Gliolan. Listed in the Adverse Effects section of the Product Information.
Photosensitivity reaction; Photodermatosis	Routine pharmacovigilance	Warning in the Precautions section of the Product Information to reduce exposure of eyes and skin to strong light sources and warning for co-administration with other potentially phototoxic substances.

Safety concern	Proposed pharmacovigilance activities	Proposed risk minimization activities
		Listed in the Adverse Effects section of the Product Information.
Anaemia; Thrombocytopenia; Leukocytosis	Routine pharmacovigilance	Listed in the Adverse Effects section of the Product Information.
Neurological disorders (for example hemiparesis, aphasia, convulsions, hemianopsia)	Routine pharmacovigilance	<p>Restriction in the Dosage and Administration section of the Product Information that Gliolan should only be used by neurosurgeons that have attended a training course in fluorescence guided surgery.</p> <p>Warning in the Precautions section of the Product Information for special care in patients with tumours in the vicinity of an important neurological function.</p> <p>Listed in the Adverse Effects section of the Product Information.</p> <p>Prescription Only Medicine.</p> <p>Training course for neurosurgeons.</p>
<b>Safety Concern</b>	<b>Prevention of neurological deficits</b>	
Routine Risk Minimisation	<p>Restriction in the Precautions and Dosage And Administration section of the Product Information that Gliolan should only be used by neurosurgeons that have attended a training course in fluorescence guided surgery.</p> <p>Warning in the Precautions section of the Product Information for special care in patients with tumours in the vicinity of an important neurological function.</p> <p>Listed as adverse reactions in the Adverse effects section of the Product Information.</p> <p>Legal status: Prescription Only Medicine</p>	
<b>Additional Risk Minimisation Activities</b>		
Action Proposed	Training of the neurosurgeons before using the method.	
Objective of proposed action	To minimise neurological adverse events associated with the Gliolan fluorescence guided surgery through adequate education.	
Rationale	Risk minimisation in the use of Gliolan requires intimate knowledge of the theory behind the technique and the accurate performance of fluorescence guided resection.	

Safety concern	Proposed pharmacovigilance activities	Proposed risk minimization activities
Criteria to be used to verify success of proposed risk minimisation activity	<p>Details of the number of neurosurgeons who have taken part in a training course, or have obtained and followed a specialised training kit, signed a declaration and attained a certificate, will be kept on file by the Sponsor.</p> <p>Details will be reported to the Therapeutic Goods Administration if requested.</p>	
Proposed review period	1 year	

### Summary of recommendations

The following table provides a summary of the OPR evaluation of the RMP issues raised with the sponsor, sponsors responses and OPR evaluation of these responses.

**Table 9. Reconciliation of issues outlined in the RMP report**

Recommendation in RMP evaluation report	Sponsor's response (or summary of the response)	OPR evaluator's comment
<p>Safety considerations may be raised by the nonclinical and clinical evaluators through the consolidated section 31 request and/or the Nonclinical and Clinical Evaluation Reports respectively. It is important to ensure that the information provided in response to these includes a consideration of the relevance for the Risk Management Plan, and any specific information needed to address this issue in the RMP. For any safety considerations so raised, please provide information that is relevant and necessary to address the issue in the RMP.</p>	<p><i>'This request is noted. An updated ASA to the RMP will be submitted after we have received the Delegate's Overview. This update will take into account all comments and actions raised in the Clinical and Non Clinical evaluation reports, the RMP Evaluation report and the future delegates report.'</i></p>	<p>This was considered acceptable.</p>
<p>Given the potential off label use and the lack of safety and efficacy data in persons aged 0 to 18 years (exclusion criteria for all the clinical trials), the Delegate may wish to consider limiting the proposed indication for Gliolan be limited to adults (persons aged 18 years and over).</p>	<p><i>'The Sponsor agrees that the indication should be restricted to adult patients only, since no data on Gliolan are available in patients under 18 years of age. The revised indication (see also responses to the Clinical Evaluation report) now accurately reflects the restriction to adult patients only. The revised indication is: "Gliolan is indicated in adult patients for visualisation of malignant tissue during surgery for malignant gliomas that are glioblastoma multiforme (GBM), and intended for gross macroscopic resection of all visible tumour".'</i></p>	<p>This was considered acceptable.</p>
<p>Given the benefit risk profile, that the clinical benefit of fluorescence guided resection of malignant gliomas with 5-ALA has not yet been established, it is recommended to the Delegate that close monitoring of neurological deficits, vision deficits and the risk of pulmonary embolism in the initial PSUR's be a requirement of registration.</p>	<p><i>'According to the RMP, physicians are trained on the adverse event profile of fluorescence guided glioma resection. Attention is especially focused on neurological/visual deficits and physicians are asked to report specifically such undesired effects. Pulmonary embolism is a serious adverse event and will also be ultimately reported to the company as an adverse event. All these events are closely monitored and will be reported in the PSURs.'</i></p>	<p>This was considered acceptable.</p>

Recommendation in RMP evaluation report	Sponsor's response (or summary of the response)	OPR evaluator's comment
<p>The incidence of adverse events were further explored in an ongoing clinical trial (MC-ALS.32/GLI) It is recommended that the sponsor confirms if the results of this study are available and that the results will be included in the PSUR and, if needed, the RMP updated based on the study results.</p>	<p><i>'The Final Study Report of trial MC-ALS.32/GLI is available and is provided in Annex 5 to the EU RMP (Version 9.0, updated 08 October 2010). These results have not changed the riskbenefit balance of Gliolan.'</i></p>	<p>This was considered acceptable.</p>
<p>In regard to the proposed routine risk minimisation activities, it was recommended to the Delegate that the draft product information document be revised as follows:</p> <p>To include under precautions reference to patients with pre-existing cardiovascular disease: the drug should be used with caution since literature reports have shown hypotension and decreased pulmonary vascular resistance in such patients.</p>	<p><i>'Upon advice from the RMP evaluator, revisions to the PI that were recommended by the OPR will not be made until the sponsor has received the Delegates Overview. The sponsor will address this issue in a future response, if required by the Delegate to do so.'</i></p>	<p>In their response, the sponsor has not committed to include the suggested changes. Hence, the recommendation to the Delegate remains:</p> <p>In the 'Precautions' section, the sponsor should include a statement that the drug should be used with caution in patients with pre-existing cardiovascular disease, since literature reports have shown hypotension and decreased pulmonary vascular resistance in such patients (or a statement to that effect).<sup>24</sup></p>
<p>It was noted to the Delegate that the proposed Australian CMI was not presented in the standard Australian format.</p>	<p><i>'The sponsor will update the CMI to the standard Australian format before approval.'</i></p>	<p>This was considered acceptable.</p>

<sup>24</sup> The current Product Information includes the statement *'In patients with pre-existing cardiovascular disease, Gliolan should be used with caution since literature reports have shown decreased systolic and diastolic blood pressures, pulmonary artery systolic and diastolic pressure as well as pulmonary vascular resistance.'*

## Summary and recommendation

There were no outstanding issues except for PI matters. Should the application for registration be approved the OPR recommended implementation of EU-RMP Version 9.0 (dated 08 October 2010, DLP 30 June 2010) and Australian Specific Annex Version 1.0 (dated 26 September 2012)

## VI. Overall conclusion and risk/benefit assessment

The submission was summarised in the following Delegate's overview and recommendations:

### Introduction

Aminolevulinic acid hydrochloride (5-aminolevulinic acid hydrochloride, 5-ALA, ALA) is an endogenous compound which plays a significant role in the biosynthesis of porphyrins (components of haemoglobin). ALA is prodrug that is metabolised intracellularly to form fluorescent molecules, predominantly protoporphyrin IX (PPIX).

The exogenous application of ALA leads to a highly selective accumulation of PPIX in tumour cells and epithelial tissues. Following excitation with blue light ( $\lambda = 400$  to  $410$  nm), the PPIX, which has accumulated selectively in the malignant tissue emits a red PPIX fluorescence (the peak is at  $\lambda = 635$  nm). PPIX is photolabile and fluorescence decreases over the course of light exposure.

The currently proposed indication is

*'Gliolan is indicated in adult patients for visualisation of malignant tissue during surgery for malignant gliomas that are glioblastoma multiforme (GBM) on preoperative imaging, and who are intended for resection of the tumour'*

The aim of the use of ALA in the presence of blue light is to visualise malignant tissue and improve differentiation from normal brain tissue due to the colour reaction. This visualisation of malignant tissue is claimed to facilitate surgery and to improve completeness of resection. The sponsor has included in the RMP plans for the training of neurosurgeons in Australia in fluorescence guided surgery.

The recommended dose is 20 mg aminolevulinic acid hydrochloride per kilogram body weight. The oral solution is prepared by dissolving the amount of powder of one vial in 50 mL of drinking water. The solution should be administered orally 3 hours (range 2 to 4 hours) before anaesthesia. In the absence of compatibility studies, Gliolan must not be mixed with other medicinal products.

Aminolevulinic acid hydrochloride was designated an orphan drug by TGA in 2012 for the indication *'Gliolan is indicated for photodynamic diagnosis of gliomas that are glioblastoma multiforme (GBM) on preoperative imaging, and intended for gross macroscopic resection of all visible tumour'*.

Aminolevulinic acid hydrochloride was designated an orphan drug in EU in 2002. A registration application has been approved in EU in 2007 for the indication *'Gliolan is indicated in adult patients for visualisation of malignant tissue during surgery for malignant glioma (WHO grade III and IV)'* supported by the same dossier. A Pre Investigational New Drug (IND) meeting appears to have been undertaken with US FDA in 2011.

## Quality

Aminolevulinic acid hydrochloride is a white to off white crystalline powder which is freely soluble in water and slightly soluble in ethanol/methanol.

The drug product is to be manufactured by dissolving aminolevulinic acid hydrochloride in water for injection, followed by sterile filtering into 50 mL vials. The vials are placed into the sterilised freeze drier and the solution is freeze dried. The powder is well controlled with satisfactory expiry limits and release limits. Even though the proposed acceptance limit for each unknown impurity in the finished product release specification is higher than the threshold specified in the ICH Q3B guidelines<sup>25</sup> for doses greater than 2.0 g aminolevulinic acid hydrochloride (for those patients whose weight is over 100 kg), an acceptable justification has been provided. Stability data support the proposed shelf life of 36 months when stored below 25°C. The reconstituted drug product with a final concentration of 30 mg/mL is stable for oral administration for 24 hours after reconstitution.

A bioavailability study which compared oral doses of 20 mg/kg bodyweight aminolevulinic acid hydrochloride with 2 mg/kg bodyweight IV administration in healthy male subjects reported that following oral administration of a single dose of 20.0 mg/kg body weight of ALA, the absolute bioavailability was 100%, calculated from plasma data ( $AUC_{0-\infty}$ ) and 105%, calculated from urinary excretion data ( $A_{e,ur}$ ).

The application has been considered by the Pharmaceutical Subcommittee of ACPM. All chemistry and quality control issues associated with the application have been subsequently resolved during subsequent evaluation.

## Nonclinical

The nonclinical data consisted of published literature and sponsored reports. Most safety related studies performed by the sponsor were conducted under GLP conditions. The published literature included studies which were not GLP compliant.

Some key nonclinical findings follow.

- One study<sup>26</sup> used a similar protocol to the entire clinical procedure for fluorescence guided resection (FGR) in an intracranial VX2 tumour model in rabbits. The entire procedure (starting 4 hours after ALA administration) significantly increased the completeness of tumour resection by 1.4 fold and reduced residual tumour by 16 fold. Other published *in vitro* and *in vivo* studies on the primary pharmacodynamics of ALA, mostly on the use of ALA as a sensitizer in photodynamic therapy (PDT [ $\lambda=630$  to 635 nm]), rather than as a sensitizer for use in photodynamic diagnosis (PDD) of brain tumours, showed (i) ALA penetrates into brain tumours after systemic administration and (ii) efficacy in tumour models. It was also observed that adjacent healthy tissue may show PPIX fluorescence, depending on its ability to synthesise PPIX.
- Safety pharmacology studies showed the potential of ALA to induce (i) toxicity in the CNS (inhibition of glutamate uptake and decreases in cAMP levels) and (ii) phototoxic damage to skin (destruction of sebaceous glands, hair follicles) of mice or the

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<sup>25</sup> International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), 'ICH Harmonised Tripartite Guideline, Impurities in New Drug Products Q3B(R2)', 2 June 2006, web accessed 30 January 2014, <[http://www.ich.org/fileadmin/Public\\_Web\\_Site/ICH\\_Products/Guidelines/Quality/Q3B\\_R2/Step4/Q3B\\_R2\\_Guideline.pdf](http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q3B_R2/Step4/Q3B_R2_Guideline.pdf)>

<sup>26</sup> Bogaards A *et al.* (2004) Increased brain tumour resection using fluorescence image guidance in a preclinical model. *Lasers Surg Med* 35, 181-90.

intraperitoneal organs (mild superficial damage) of rats with bright white (photoactivating) light.

- The kinetic profile of ALA was not comprehensive but ALA biosynthesis/metabolism is well known.
- All GLP compliant toxicity studies were performed in the dark (except phototoxicity studies). 5-ALA had a low acute oral toxicity in mice and rats. Repeat dose toxicity studies with mice (PO, IP; 8 to 13 Weeks, non GLP) and GLP compliant studies in rats (PO, IV) and dogs (IV) up to 14 days were adequate. Hepatic toxicity (alterations in serum transaminases, LDH, total cholesterol, creatinine, urea and total bilirubin) and irreversible bile duct changes were observed in the pivotal 14 day toxicity studies in rats and dogs. Animal to human exposure ratios suggest that hepatic toxicity and bile duct changes may be clinically relevant.
- Genotoxicity studies performed in the dark showed no mutagenicity in *S. typhimurium* or mammalian V79 cells. Adequate justification for the lack of carcinogenicity studies was provided, taking into account the proposed indication.
- No conventional reproductive toxicity studies were submitted but published papers showed ALA may affect pregnancy and ALA induced porphyrin synthesis leads to developmental toxicity (embryotoxic, fetotoxic and teratogenic effects).
- UV irradiation exposure 4 or 24 hours post ALA dosing produced time dependent phototoxic reactions. Overall, the nonclinical data showed that the skin, eyelids (and/or normal brain surrounding the malignant tumour) are potentially affected when directly exposed to photoirradiation (for at least 24 hours after dosing)

## Clinical

### Pharmacology

One clinical pharmacology study (MC-ALS. 20/BV) in normal subjects provided absolute bioavailability data and also determined some pharmacokinetic parameters. 12 initial subjects received a 20 mg/kg bodyweight oral solution of ALA, administered on an empty stomach. Three days later a 2 mg/kg bodyweight IV dose was administered. The lower IV dose was chosen because of limited experience with IV administration of ALA. As previously described, absolute BA was 100% as calculated from the plasma AUC<sub>0-</sub> comparison and 105% as calculated from renal excretion data.

Additional PK/PD investigations during the same study were based on a total of 21 healthy subjects: The PK parameters determined were: C<sub>max</sub>, T<sub>max</sub>, t<sub>1/2</sub>λ<sub>Z</sub>. The metabolite, PPIX was measured in this trial, because it is responsible for the fluorescence used to help define the tumour margins. The highest plasma concentrations of ALA and PPIX were reached after 0.76 and 4.04 hours, respectively

A secondary objective of the study was to evaluate the duration of photosensitisation of the skin by observing the Minimal Erythema Dose (MED) and corresponding PPIX plasma concentrations after oral treatment. At 12 and 24 hours after administration of ALA the MED measured shortly after end of irradiation (immediate reaction) was significantly reduced compared to baseline. 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 ALA. The study also showed that there was no correlation between PPIX plasma levels and immediate or late skin reaction to UVA light.



Study MC-ALS. 8-I/GLI is a Phase I/II clinical study which assessed PK and PD relationship of 5-ALA administered to patients for fluorescence guided resection of malignant gliomas. This is the only dose finding study. The study assessed 0.2 mg/kg, 2.0 mg/kg and 20 mg/kg oral doses of 5-ALA and related these doses to the fluorescence produced in the tumour core and normal brain. Pharmacokinetic parameters of ALA and its metabolite, PPIX (terminal half life, AUC, maximum plasma concentration and time of maximum plasma concentration), were also determined.

Study MC-ALS. 8-I/GLI was performed in 21 adult patients who had radiographic evidence (MRI at first diagnosis) of malignant glioma (WHO Grade III/IV) and for whom surgical treatment was indicated. During tumor 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. Dose efficacy relationship was assessed by determining the global fluorescence extent and fluorescence quality of the tumor core at the end of operation after the tumor had been resected, summarised by two surgeons as fluorescence extent and fluorescence quality. These subjective impressions of the surgeons were confirmed by quantitative tissue spectrophotometric measurements and histological investigations. Finally the principal investigator and the second surgeon rated whether the use of fluorescence simplified resection due to enhanced visual demarcation of the tumour.

All 21 randomised patients received the investigational drug, underwent tumour resection and completed 28 days of follow up. Treatment groups did not diverge notably with respect to demographic characteristics. 20/21 patients (95%) suffered from glioblastoma multiforme (WHO Grade IV), one patient had an anaplastic astrocytoma (WHO Grade III).

Fluorescence quality and extent in tumour core were highest (100%) at the highest ALA dose studied (20 mg/kg bodyweight) as seen in Table 10 below. Fluorescence assessed spectrophotometrically was also higher at the highest ALA dose as seen in Table 11. These differences between treatment groups were statistically different.

**Table 10. Global fluorescence extent and quality in tumour core assessed visually**

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

**Table 11. Fluorescence intensity of tumour of tumour core assessed spectrophotometrically**

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)	-

Similar results were obtained for the tumor margin. Normal tissue in the highest dose group had a fluorescence intensity of  $0.2114 \pm 0.1544$  a.u.

Biopsies were taken from the selected areas of tumor core and margin and inspected histologically for tumor cellularity by the reference pathologist who was also blinded with respect to the treatment group. Tumor cellularity was plotted against fluorescence

intensity. A significant correlation could only be shown for the highest dose level of 20 mg/kg bodyweight

The surgeons rank on a 4 point scale as to whether the administered ALA dose caused sufficient tumor fluorescence for demarcation of the tumor margin to simplify the tumor resection concludes that only the highest 5-ALA dose (20 mg/kg bodyweight) leads to pronounced simplification (100%).

The clinical evaluator's conclusions on pharmacology were that the PK studies showed rapid and complete absorption of ALA after an oral dose in normal subjects and rapid absorption in patients with GM. About 6% of ALA was metabolised to PPIX, the active metabolite, which reached a maximum plasma concentration around 4 hours and was not detectable at 48 hours. In normal subjects, renal excretion of ALA was slower at lower plasma concentration of ALA and slower urine flow rates.

Values for the PK parameters for ALA and PPIX differed between normal subjects and patients, the latter having slower absorption, lower plasma concentrations ( $C_{max}$  and AUC), and a longer half life for ALA. The half life of PPIX was similar in both groups. The plasma concentration of PPIX did not correlate with PD effects.

The clinical evaluator commented that PK studies had no important deficiencies but that it was difficult to draw clinical conclusions from them. Such conclusions are better based on PD assessment. In Study MC-ALS. 8-I/GLI the highest ALA dose (20 mg/kg bodyweight) leads to sufficient tumor visualisation both by subjective description as well as spectrophotometric/ histological investigations and simplification of tumor surgery.

**Efficacy** The application contained one pivotal study (MC-ALS.3/GLI) of 415 patients (205 in the ALA arm and 208 controls). Two supporting studies determined the positive predictive value of tissue fluorescence (MC-ALS.28/GLI and MC-ALS.30/GLI).

**Study MC-ALS.3/GLI** is a randomised, group sequential, partially blinded, parallel group, controlled multicentre Phase III study to compare standard surgical resection versus fluorescence guided resection after oral ALA administration in patients undergoing initial surgery for newly diagnosed malignant glioma. The study was conducted in 19 centres in Germany from 1999 to 2004.

Males or females aged 18 to 72 years with cranial magnetic resonance imaging (MRI) justifying diagnosis of unilocular malignant glioma (WHO Grades III to IV) for whom surgical treatment was indicated were included in this study. The location of contrast enhancing tumour was to be such that should allow complete resection. Patients with tumour that could not be fully resected, with more than one contrast agent accumulating lesion unrelated to the primary tumour or presenting with extracerebral metastases were excluded. Furthermore, patients with a Karnofsky Performance Score less than 70, known porphyria or hypersensitivity to porphyrins, renal or hepatic insufficiency or other malignancies were also excluded.

The initial primary efficacy outcome was percentage of patients with a histologically confirmed malignant glioma (WHO Grade III or IV) without definite residual contrast enhancing tumour in the early post operative control MRI (within 72 hours after surgery). A second primary endpoint was added after the study had commenced, which was progression free survival at the 6 month visit after primary surgical treatment of a histologically confirmed malignant glioma (WHO Grade III or IV).

Randomisation was performed centrally and stratified by a number of risk factors. It was impossible to blind the study treatment to the investigators at the trial sites but all study MRIs were blinded. Bias regarding adverse event reporting could not be excluded. The Full Analysis Set comprised all randomised patients who underwent surgery, had a histopathological diagnosis consistent with WHO Grade III/IV glioma (assessed centrally) and which had unilocular tumour with features characteristic for malignant glioma

(assessed by neuroradiologist blinded to treatment). The Per Protocol Set comprised all patients within the Full Analysis Set except those with major protocol deviations. Conventional Intent to Treat (ITT) analysis would have included patients without malignant glioma. Safety was assessed in one population consisting of all patients included in the Full Analysis Set, and in a population of randomised patients who received 5-ALA or did not receive 5-ALA but qualified for Full Analysis Set. The first statistical analysis was planned with 270 evaluable patients in the Full Analysis Set. If statistical significance was not attained, randomisation would continue until 350 patients were eligible in the Full Analysis Set. A nominal significance level of 0.05 was applied for the first primary endpoint, 0.022 and 0.043 were applied for second primary endpoint at interim and final analysis, respectively.

For the final analysis, 415 consecutive patients were randomised into the two treatment arms in a 1:1 ratio with 207 randomised to fluorescence Group (FL group) and 208 randomised to white light group (WL group). The Full Analysis Set consisted of 176 in the FL group and 173 in the WL group. The most common reasons for exclusion were ineligible histology (FL group: n=21; WL group: n=20), ineligible preoperative MRI findings (FL group: n=5; WL group: n=10), and patients withdrawing their consent before start of surgery (FL group: n=2; WL group: n=3). The Per Protocol Set excluded a total of 43 (20.8%) patients randomised to the FL group and a total of 46 (22.1%) patients randomised to the WL group. Additional reasons for exclusion included failure of fluorescence device, reoperation with one week after primary surgery and missing efficacy parameters (MRI) due to patient's early death.

Overall, the median age was 60 years (range 23 to 73). About two thirds of the patients (61.0%) were male and about one third (39.0%) were female. Body weights ranged from 43 to 120 kg without meaningful differences between treatment groups.

The first primary efficacy parameter was the percentage of patients without residual tumour on early postoperative MRI. For the Full Analysis Set, 63.6% of all patients in the FL group and 37.6% of all patients in the WL group did not show residual tumour on early postoperative MRI. This difference was highly statistically significant using the Chi square test (p less than 0.0001). The crude odds ratio was calculated as 2.91 (95% CI: 1.88 to 4.49). A very similar result was obtained for the Per Protocol Set. In the FL group, 63.8% of patients were operated on without residual tumour on the early MRI versus 39.2% of patients in the control arm. This result was statistically significant (p less than 0.0001) with a crude odds ratio of 2.73 (95% CI: 1.75 to 4.28). Thus, results were homogenous in both patient sets analysed.

The second primary efficacy parameter was progression free survival at the 6 month visit after primary surgical treatment. CER Table 8 (see Attachment 2; table copied ?delete? elow) shows results overall and in subgroup analysis.

**Table 12. Progression free survival rate [%] at 6 month visit**

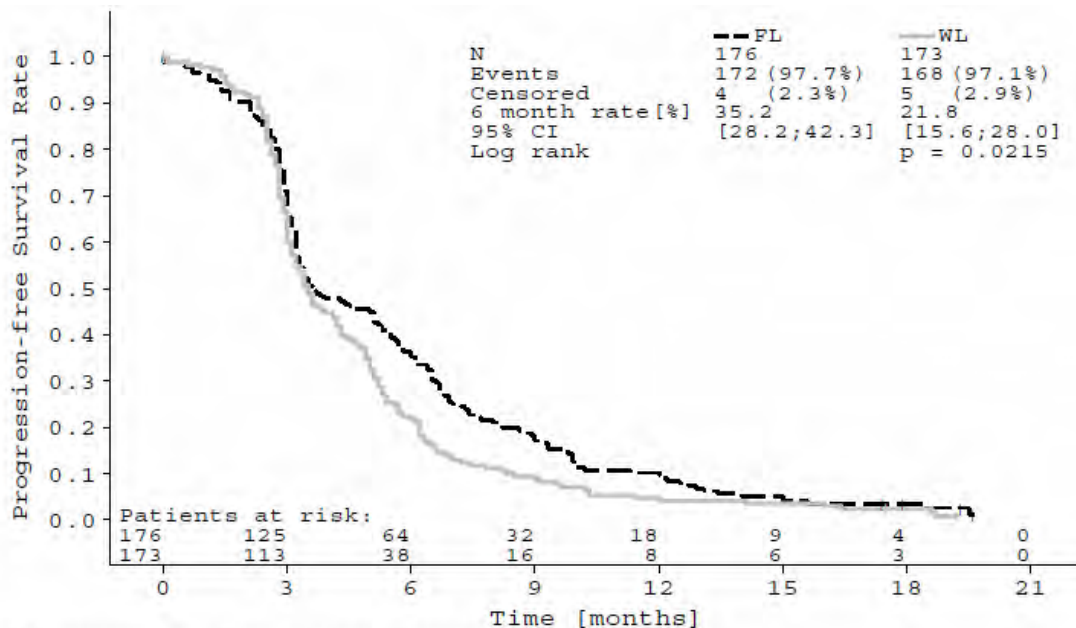
Parameter	5-ALA group	Control group	P value*	Odds ratio (95% CI)
All patients	36 (20.5%)	19 (11.0%)	P = 0.0152	2.08 (1.12-3.88)
Age ≤ 55 years	14 (25.5%)	7 (13.5%)	P = 0.1185	2.20 (0.78-6.17)
Age > 55 years	22 (18.2%)	12 (9.9%)	P = 0.0643	2.02 (0.93-4.40)
KPS ≤ 80%	6 (16.2%)	5 (12.2%)	P = 0.6104	1.39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

\* Chi-square test

7 patients in the FL- and 5 in the WL-group with missing 6-month MRI were considered as having progression

A Cox proportional hazards model showed a hazard ratio of 0.792 (95% CI: 0.638 to 0.983) for the FL group indicating a 21% reduction in the risk of radiologic progression at the 6 month visit (p = 0.0341).

Figure 1 shows the Kaplan-Meier estimates for progression free survival in the Full Analysis Set.

**Figure 1. Progression free survival, Kaplan Meier estimates**

The median residual tumour volume in the early postoperative MRI was smaller in the experimental arm than in the control group (0.0 cm<sup>3</sup> [range: 0 to 45.1 cm<sup>3</sup>] versus 0.5 cm<sup>3</sup> [range 0 to 32.6 cm<sup>3</sup>]).

PFS rates at 9, 12, 15 and 18 months favoured the experimental arm with odds ratios around 2, there were decreasing numbers of patients with PFS and differences did not reach the level of statistical significance.

Analysis of overall survival in this study was of explorative nature because second line treatment after tumour progression was not standardised. Median overall survival was

comparable in both treatment arms (FL versus WL: 14.3 versus 13.7 months;  $p = 0.9170$ , log rank test) and the crude hazards ratio was 0.99 (95% CI: 0.78 to 1.24).

Exploratory analyses related to median overall survival in patients with and without residual disease and effects of post surgical therapies were discussed in the CER

The EMA required supplementary efficacy analyses of tumour progression defined by radiologically determined increase in tumour size, radiological appearance of new lesions or steroid increase or death. In the FL group, 27.3% of patients were event free six months after surgery compared to 15.5% in the control group ( $p = 0.0122$ ). “Neurologically worse” was defined as a deterioration in the NIH stroke score by at least 1 point relative to the preceding visit. In the FL group, 46% of patients were event free six months after surgery compared to 29.3% in the control group ( $p = 0.0331$ ). If “neurologically worse” was defined as a deterioration in the NIH stroke score by at least 2 points relative to the preceding visit, the benefit was again statistically significant ( $p = 0.0316$ ).

EMA required supplementary analyses of effects of post surgical therapies. The study report claims that the time to re operation after surgery was significantly shorter in the FL group compared to the time in the WL group. The supplementary analysis used more correct and sophisticated cumulative incidence methods which showed a significant benefit for the FL group with 46% versus 29.3% event free at 6 months ( $p=0.033$ ), and a lower incidence of re operation in the FL group at time points 6, 12, 18 and 24 months. Neither cumulative incidence of chemotherapy after study surgery (Full Analysis Set) nor cumulative incidence of chemotherapy with temozolomide after study surgery (Full Analysis Set) were significantly different in the FL and WL groups ( $p=0.092$  and  $0.064$  respectively).

Studies MC-ALS. 28/GLI and MC-ALS.30/GLI are other non pivotal efficacy studies. Both studies had a primary objective to determine 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.

Study 28/GLI, 39 patients (males or females aged 18 to 75 years with cranial magnetic resonance imaging (MRI) justifying diagnosis of malignant glioma [WHO Grades III to IV] for whom first surgical treatment was indicated) were assigned to undergo fluorescence guided resection which yielded 33 patients qualifying for the Full Analysis Set. In Study 30/GLI, 40 patients (males or females aged 18 to 75 years with diagnosis of recurrent malignant glioma for whom repeat surgery was indicated) were assigned to undergo fluorescence guided resection which yielded 36 patients qualifying for the Full Analysis Set (not the proposed indication).

Patients in 28/GLI had an average of 6 biopsies each, taken from different areas of fluorescence. The primary endpoint, the positive predictive value showing tumour cells on biopsy, was 100% (90% CI: 91.1 to 100.0%) for patients whose areas of strong fluorescence had been biopsied, and for areas of weak fluorescence, was 83.3% (90% CI: 68.1% to 93.2%). The number of tumour positive biopsies among all biopsies taken from areas of any fluorescence (weak and strong fluorescence), was 96.2% (90% CI: 93.0% to 98.2%). In study 30/GLI, the positive predictive value of strong tissue fluorescence was 91.7%; 95% CI: 77.5% to 98.2%) and for areas of weak fluorescence was 83.3%; 95% CI: 67.2% to 93.6%).

The clinical evaluator used data from Study 28/GLI to calculate sensitivity and specificity data.

## Safety

A total of 548 patients received a 20 mg/kg oral dose of ALA. 201 patients received ALA in the pivotal efficacy study MC-ALS.3/GLI, compared to 173 control patients, and 243

subjects received 5-ALA in a compassionate use study (MC-ALA.32/GLI) conducted in Germany after MC-ALS.3/GLI completed enrolment.

Most frequently observed adverse events in both treatment groups are shown in Table 13) with neurological and sensory organ events most frequent.

**Table 13 Adverse events summarised by CTC category and treatment group (excluding SAEs) – sorted by incidence**

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

The incidence of specific AEs (greater than 3%) in patients in each trial group up to 7 days after surgery is shown in CER Table 21 (shown below as Table 14). Early after brain tumor surgery (up to Day 7), most reported AEs were related to the neuro-sensory system. The clinical evaluator noted that a more complete brain tumor resection may have a disadvantage of more neurological deficits in patients post operatively.

**Table 14. Frequently (greater than 3%) reported AEs (excl SAEs) up to 7 days after surgery**

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

In the pivotal study, two patients experienced drug related AEs; one, mild vomiting 48 hours after surgery and the second mild photosensitivity 48 hours after surgery. In other studies 9 of 541 patients (1.7%) treated with 20 mg/kg ALA had possibly drug related AEs.

In the pivotal study within 30 days of follow up post surgery, 5 patients of 158 (2.5%) in the FL group and 3 of 131 (1.7%) in the WL group died. In the FL group: three patients died of suspected pulmonary embolism, one patient died of transtentorial herniation, another patient's death was of cardiac cause (ventricular fibrillation). For patients in WL group: one patient died due to pulmonary embolism, one patient died due to sepsis and circulatory failure and for another patient sudden cardiac death was diagnosed after suspected pulmonary embolism. With follow up to 180 days post surgery, 17 of 201 (8.5%) patients in the FL group and 11 of 173 (6.4%) patients in the WL group died as an outcome of a SAE. Pulmonary embolism was more frequent in the FL group (4%) compared to WL group (0.6%) although the difference was not statistically significant. In other studies none of the 10 deaths observed within 30 days could be related to study drug and were considered consequences of brain surgery or pre-existing cardiac disease.

In the pivotal study the overall incidence of patients with any serious AE (SAE) reported up to 180 days after surgery in the Safety Analysis Set was similar for both treatment groups, with at least one SAE reported in 60/201 (29.9%) of patients in the FL group and 40/173 (23.1%) of patients in the WL group. For both treatment groups, the most frequently reported SAEs were those assigned to the WHO system organ class "Central and Peripheral nervous system disorders" (FL group: 12.4% versus WL group: 11.6%), followed by "Platelet, Bleeding and Clotting disorders" (FL group: 9.5% versus WL group: 4.0%) and "Respiratory System disorders" (FL group: 3.5% versus WL group: 4.0%). The difference in the "Platelet, Bleeding and Clotting disorders" was mainly due to different numbers with pulmonary embolism. The median time to onset of pulmonary embolism was 35 days. There were few other observable differences between treatment groups, except for convulsions, hemiparesis and aphasia, as can be seen in Table 15 which follows.

**Table 15. Frequency distribution of all serious adverse events experienced by more than one patient up to 180 days after surgery in either treatment group (Safety Analysis Set)**

System Organ Class / WHO preferred term	FL	WL
Number of patients	201 (100.0%)	173 (100.0%)
Body As A Whole - General Disorders Condition Aggravated	3 ( 1.5)	2 ( 1.2)
Cardiovascular Disorders, General Cardiac Failure	1 ( 0.5)	2 ( 1.2)
Centr & Periph Nerv Syst Disorders		
Aphasia	7 ( 3.5)	1 ( 0.6)
Convulsions	12 ( 6.0)	5 ( 2.9)
Convulsions Grand Mal	7 ( 3.5)	5 ( 2.9)
Hemiparesis	8 ( 4.0)	4 ( 2.3)
Hypertension Intracranial	0 ( 0.0)	4 ( 2.3)
Stupor	1 ( 0.5)	3 ( 1.7)
Heart Rate And Rhythm Disorders Arrhythmia	3 ( 1.5)	1 ( 0.6)
Platelet,bleeding & Clotting Disorders		
Embolism Pulmonary	13 ( 6.5)	2 ( 1.2)
Haematoma	2 ( 1.0)	1 ( 0.6)
Thrombosis	3 ( 1.5)	3 ( 1.7)
Psychiatric Disorders Psychosis	3 ( 1.5)	1 ( 0.6)
Resistance Mechanism Disorders Abscess	2 ( 1.0)	2 ( 1.2)
Respiratory System Disorders Pneumonia	4 ( 2.0)	5 ( 2.9)
Secondary Terms - Events Cyst Nos	2 ( 1.0)	1 ( 0.6)
Skin And Appendages Disorders Hygroma Cystic	2 ( 1.0)	0 ( 0.0)

For the pivotal study and other studies, a dose dependent mild to moderate increase of the transaminases (AST/ALT) was frequently observed with a higher incidence in the 5-ALA arm of study MC-ALS.3/GLI than in the control group. These laboratory changes were not accompanied by clinical symptoms and recovery occurred in a short time. High concentrations of serum amylase (Grade 3 or 4) occurred more frequently in the ALA arm of the pivotal trial, peaking 24 hours after surgery (13.9% of patients affected compared with 10.4%), returning to normal in 7 days.

In Study MC-ALS.3/GLI, 87% in the FL group and 80% in the WL group had significant decrease in erythrocyte count after surgery. Leukocytosis was present in a majority of patients, with no post surgery differences between treatment groups. Coagulation studies showed no major differences between treatment groups or changes during the 6 week follow up.



Two clinical neurological assessments after surgery were carried out at the request of EMA. The Karnofsky Performance Score (KPS) as well as the NIH Stroke Score were assessed before and during Study MC-ALS.3/GLI. The more complete tumour surgery in the 5-ALA group did not result in a worse Karnofsky Performance Score. There was a trend for less deterioration (35.7 versus 49.1%) and more improvement (25.7 versus 17.5%) of KPS 6 months after surgery in the FL group. For NIH Stroke Score the percentage of patients with deterioration were slightly higher in the FL group during the first three visits (up to 6 weeks post surgery and significant at the 48 hours visit). This difference disappeared progressively during the further 3 monthly follow up. Periodic Safety Update Reports, now provided with a reporting period to March 2012, have not identified new safety concerns.

### **Clinical evaluator's benefit risk assessment.**

The clinical evaluator considered the single Phase III trial supporting efficacy and safety was acceptable only in the context of a fatal disease for which no curative treatment is available, a disease which affects relatively small numbers of patients and the difficulty of conduct of comparative studies when complex surgery is involved. ALA has shown a statistically significant increase in progression free survival at 6 months, which by Kaplan Meier analysis was 35.2% and 21.8% ( $p=0.02$ ) in favour of the treatment arm. Consistent results were seen in subgroup analyses. No overall survival benefit was shown. There were reservations about accepting the PFS rate as a clinical benefit. Based on clinical neurological assessment by NIH Stroke Score reduced "neurological deterioration" at 6 months after surgery was reported in 46% in treatment group compared with 29.3% in control group. A benefit was also demonstrated in the ALA group for time to further surgery following progressive disease. The risks of the proposed usage of ALA are: those associated with the drug itself, namely pulmonary embolism, photosensitivity, hypotension and increased liver enzyme and serum amylase concentrations; and those associated with the drug and surgical procedure. The greater excision of brain tissue during surgery when ALA was used was a procedural risk factor, inseparable from the requested use of the drug. Impaired vision occurred at a higher rate in the FL group. The study lacked the power to demonstrate a significant difference between the treatment arms with respect to post surgical convulsions, hemiparesis and aphasia. The KPS was not worse in the FL group from 6 weeks after surgery. The NIH Stroke Score showed higher percentage of patients with deterioration at 48 hours. There was trend to higher percentage with deterioration up to 6 weeks post surgery but with the difference progressively decreasing. When assessed by deterioration in NIH stroke score by at least 1 point relative to preceding visit patients in the FL group has clinical benefit (46% event free at 6 months after surgery) compared to the WL group (29.3%). An additional risk was the possibility that the operating surgeon may be inadequately trained or inexperienced in this method of excision, possibly leading to excessive removal of normal tissue in sensitive areas of the brain

### **Risk management plan**

There are no outstanding issues in relation to the proposed Risk Management Plan. The advice of the Advisory Committee on the Safety of Medicines (ACSOM) was not sought on this submission. There is one risk minimisation measure over routine which is a specific educational training program for neurosurgeons, which is aimed at reducing neurological adverse effects

## Risk benefit analysis

### Delegate considerations

A single dose finding study was conducted involving 0.2 mg/kg, 2.0 mg/kg and 20 mg/kg oral doses of 5 ALA. It is accepted that 20 mg/kg dose showed strong fluorescence and was adequate for tumour visualisation. The difference between the 2.0 mg/kg and the 20.0 mg/kg is 10 fold so that intermediate doses may have been as effective. The sponsor's Clinical Overview justified the 20 mg/kg dose level based on acceptable drug related side effects profile.

A single pivotal study supports efficacy. In Study MC-ALS.3/GLI the final efficacy analysis was based on 415 patients, with 349 patients in the full analysis set. In this study the second primary efficacy outcome, PFS assessed by MRI at 6 months, was added to the protocol in 2002 whereas the study had begun in 1999. The 6 month time point is the time point of maximum difference between FL group and WL group in PFS. Secondary efficacy outcomes PFS at 9, 12, 15 and 18 months after primary surgical treatment and overall survival were also added to the protocol in 2002. The EMA required supplementary analyses of efficacy and safety. The clinical evaluator considered that the clinical benefits in the pivotal study are marginal.

The pivotal study was conducted in Germany between 1999 and 2004. The pivotal study included gliomas intended for complete resection. The Indications initially proposed included the phrase '*intended for gross macroscopic of all visible tumour*' whereas the Indication' now proposed states "*who are intended for resection of the tumour*". The currently proposed Indications might be argued to include repeat surgery for recurrent malignant glioma for which support is provided from only one small study with limited follow up. Post surgical care was not standardised. There is some current use of 5-ALA under the TGA's Special Access Scheme.

In a supportive efficacy study (28/GLI), the primary endpoint was the positive predictive value showing tumour cells on biopsy, was 100% (90% CI: 91.1 to 100.0%) for patients whose areas of strong fluorescence had been biopsied, and for areas of weak fluorescence, was 83.3% (90% CI: 68.1% to 93.2%). In Study 28/GLI, the clinical evaluator has calculated sensitivity and specificity data. The sponsor, in a response to the clinical evaluation report, noted that study design asked for tissue sampling based on fluorescence quality. This leads to verification bias with under representation of fluorescence negative results so that observed sensitivity is increased and observed specificity is decreased.

Safety data base is modest with a total of 548 patients who received a 20 mg/kg oral dose of 5-ALA. Investigators are unblinded when reporting adverse effects. The single pivotal study has excluded patients outside the age range 18 to 72 years and patients with hepatic or renal insufficiency.

### Proposed action

The evaluator had no reason not to recommend that Gliolan should be approved for registration for the specified indication.

### Request for ACPM advice

The evaluator thanked the ACPM for discussing and providing advice on the following issues:

- Whether the data from the studies submitted are sufficient to adequately characterise the efficacy and safety profile of aminolevulinic acid hydrochloride in relation to use in adult patients for visualisation of malignant tissue during surgery for malignant

gliomas that are glioblastoma multiforme on preoperative imaging, and who are intended for resection of the tumour?

- Has the dose been adequately defined?
- In the pivotal efficacy study is the secondary primary endpoint valid?
- Does the ACPM consider results from the pivotal study can be generalised to current clinical practice in Australia?
- Are currently proposed indications appropriate?
- Does verification bias make the reporting of sensitivity and specificity results inappropriate in Study 28/GLI?

The Committee was also requested to provide advice on any other issues that it thinks may be relevant to a decision on whether or not to approve this application.

### **Response from sponsor**

#### ***Review of the Product Information***

Details of the Product Information are beyond the scope of the AusPAR.

#### ***Clinical Evaluation Report (CER)***

The sponsor finds the conclusions in the CER acceptable and reasonable.

#### ***Revised Australian Specific Annex (ASA) of the Risk Management Plan***

The sponsor provided an updated ASA for the ACPM members.

### **Advisory committee considerations**

The Advisory Committee on Prescription Medicines (ACPM), having considered the evaluations and the Delegate's overview, as well as the sponsor's response to these documents, advised the following:

The submission seeks to register a new chemical entity.

The ACPM, taking into account the submitted evidence of efficacy, safety and quality, agreed with the Delegate and considered Gliolan powder for oral solution containing 1.5g of Aminolevulinic acid hydrochloride to have an overall positive benefit-risk profile for the indication as proposed.

The committee was requested to provide advice on the following specific issues:

- Whether the data from the studies submitted are sufficient to adequately characterise the efficacy and safety profile of aminolevulinic acid hydrochloride in relation to use in adult patients for visualisation of malignant tissue during surgery for malignant gliomas that are glioblastoma multiforme on preoperative imaging, and who are intended for resection of the tumour?
  - As much as is ever likely to be possible, the data from the studies submitted do characterise the efficacy and safety of aminolevulinic acid hydrochloride used in adult patients for visualisation of malignant tissue during surgery for malignant gliomas that are glioblastoma multiforme on preoperative imaging, and who are intended for resection of the tumour. Aminolevulinic acid hydrochloride guided surgery facilitates a more extensive resection which could result in greater attendant neurological morbidity for these patients.
- Has the dose been adequately defined?
  - The appropriate dose has been defined as adequately as possible.

- In the pivotal efficacy study is the secondary primary endpoint valid?
  - The secondary primary endpoint in the pivotal study was not part of the study as initially described, which renders it invalid in the formal design of the study. Median survival in both arms (14.3, 13.7 months) was less than the 15 months reported with the current standard of care (radiotherapy + temozolomide). However, it may be useful to prescribers. Progression free survival at 6 months favoured the experimental group.
- Does the ACPM consider results from MC-ALS.3/GLI are generalisable to current clinical practice in Australia?
  - The results from MC-ALS.3/GLI are likely to be generalisable to current clinical practice in Australia.
- Are currently proposed indications appropriate
  - The pivotal trial includes Grade III (anaplastic) astrocytoma as well as Grade IV (glioblastoma multiforme GBM) tumours, thus it could be argued that Grade III tumours should be included in the indication. The committee considered that definitive histological grading would often occur post operatively and therefore the proposed indication would not unduly exclude patients who may benefit from extensive resection. It would not be expected that this affects results meaningfully.
- Does verification bias make the reporting of sensitivity and specificity results inappropriate in Study 28/GLI?
  - Verification bias almost certainly compromises the estimation of sensitivity and specificity results in Study 28/GLI but it is difficult to see how this could have been avoided. The low sensitivity, with tumour cells often found in biopsies from non fluorescent areas, merely confirms the conventional wisdom that tumour relapse is due to incomplete resection, even when the resection is extensive. The precise sensitivity and specificity is not clinically relevant given that there is no published randomised trial confirming the efficacy of surgery in GBM.

#### **Proposed conditions of registration:**

The ACPM agreed with the Delegate on the proposed conditions of registration.

#### **Proposed Product Information (PI)/Consumer Medicine Information (CMI) amendments:**

The ACPM agreed with the Delegate to the proposed amendments to the Product Information (PI) and Consumer Medicine Information (CMI).

The ACPM advised that the implementation by the sponsor of the recommendations outlined above to the satisfaction of the TGA, in addition to the evidence of efficacy and safety provided would support the safe and effective use of this product.

#### **Outcome**

Based on a review of quality, safety and efficacy, TGA approved the registration of Gliolan powder for oral solution containing aminolevulinic acid hydrochloride 30 mg/mL, vial, indicated for:

*Gliolan is indicated in adult patients for visualisation of malignant tissue during surgery for malignant gliomas that are glioblastoma multiforme (GBM) on preoperative imaging, and who are intended for resection of the tumour.*

### **Specific conditions of registration applying to these therapeutic goods**

The Gliolan (aminolevulinic acid hydrochloride) powder for oral solution Risk Management Plan (EU-RMP) Version 9.0 (dated 08/10/2010, DLP 30/06/2010) and Australian Specific Annex Version 1.0 (dated 26/09/2012), and any future updates, as agreed with the TGA will be implemented in Australia

### **Attachment 1. Product Information**

The Product Information approved at the time this AusPAR was published is at Attachment 1. For the most recent Product Information please refer to the TGA website at <<http://www.tga.gov.au/hp/information-medicines-pi.htm>>.

### **Attachment 2. Extract from the Clinical Evaluation Report**

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