

Report on the Deliberation Results

March 15, 2013

Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau
Ministry of Health, Labour and Welfare

[Brand name] (a) Alabel Oral 1.5 g and (b) Alaglio Oral 1.5g
[Non-proprietary name] Aminolevulinic Acid Hydrochloride (JAN*)
[Applicant] (a) Nobelpharma Co., Ltd. and (b) SBI Pharmaceuticals Co., Ltd.
[Date of application] July 5, 2012

[Results of deliberation]

In the meeting held on March 13, 2013, the Second Committee on New Drugs concluded that the product may be approved and that this result should be presented to the Pharmaceutical Affairs Department of the Pharmaceutical Affairs and Food Sanitation Council.

The product is not classified as a biological product or a specified biological product, the re-examination period is 10 years, and neither the drug substance nor the drug product is classified as a poisonous drug or a powerful drug.

[Conditions for approval]

The applicant is required to conduct a post-marketing drug use-results survey over a certain period of time until the data about a certain number of patients are accumulated after market launch, covering all patients treated with the product, in order to understand background information about patients treated with the product because the product is an orphan drug and subjects for Japanese clinical studies are extremely few. At the same time, the data about the safety and efficacy of the product should be collected and necessary measures should be taken to ensure that the product is properly used.

**Japanese Accepted Name (modified INN)*

This English version of the Japanese review report is intended to be a reference material to provide convenience for users. In the event of inconsistency between the Japanese original and this English translation, the former shall prevail. The PMDA will not be responsible for any consequence resulting from the use of this English version.

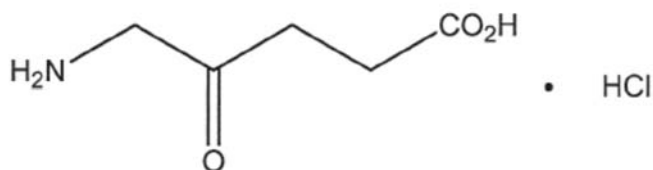
Review Report

March 5, 2013

Pharmaceuticals and Medical Devices Agency

The results of a regulatory review conducted by the Pharmaceuticals and Medical Devices Agency on the following pharmaceutical product submitted for registration are as follows.

[Brand name] (a) Alabel Oral 1.5 g and (b) Alaglio Oral 1.5 g
[Non-proprietary name] Aminolevulinic Acid Hydrochloride
[Name of applicant] (a) Nobelpharma Co., Ltd. and (b) SBI Pharmaceuticals Co., Ltd.
[Date of application] July 5, 2012
[Dosage form/Strength] A lyophilized preparation containing 1.5 g of Aminolevulinic Acid Hydrochloride per vial
[Application classification] Prescription drug (1) Drug with a new active ingredient
[Chemical structure]



Molecular formula: $C_5H_9NO_3 \cdot HCl$

Molecular weight: 167.59

Chemical name: 5-Amino-4-oxopentanoic acid monohydrochloride

[Items warranting special mention] Orphan drug (Notification No. 0914-1 from the Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare, dated September 14, 2010)

[Reviewing office] Office of New Drug II

This English version of the Japanese review report is intended to be a reference material to provide convenience for users. In the event of inconsistency between the Japanese original and this English translation, the former shall prevail. The PMDA will not be responsible for any consequence resulting from the use of this English version.

Review Results

March 5, 2013

[Brand name] (a) Alabel Oral 1.5 g and (b) Alaglio Oral 1.5 g
[Non-proprietary name] Aminolevulinic Acid Hydrochloride
[Name of applicant] (a) Nobelpharma Co., Ltd. and (b) SBI Pharmaceuticals Co., Ltd.
[Date of application] July 5, 2012

[Results of review]

Based on the submitted data, it is concluded that the efficacy of Alabel Oral 1.5 g and Alaglio Oral 1.5 g (hereinafter collectively referred to as “the product”) for visualization of malignant tissue during surgical resection of malignant glioma has been demonstrated and the safety is acceptable in view of its observed benefits. It is considered necessary to collect information concerning the presence or absence of tumor tissues of which resection is additionally decided based on the diagnosis with the product, the presence or absence of tumor cells in biopsies taken from the tumor margins under fluorescence guidance using the product, and the incidences of hepatic dysfunction and photosensitivity after administration of the product.

As a result of its regulatory review, the Pharmaceuticals and Medical Devices Agency has concluded that the product may be approved for the indication and dosage and administration as shown below, with the following conditions.

[Indication]

Visualization of malignant tissue during surgical resection of malignant glioma

[Dosage and administration]

The usual adult dosage is 20 mg/kg of Aminolevulinic Acid Hydrochloride for an oral solution prepared by dissolving in water. The solution should be administered orally 3 hours (range, 2-4 hours) prior to induction of anesthesia for surgery.

[Conditions for approval]

The applicant is required to conduct a post-marketing drug use-results survey over a certain period of time until the data about a certain number of patients are accumulated after market launch, covering all patients treated with the product, in order to understand background information about patients treated with the product because the product is an orphan drug and subjects for Japanese clinical studies are extremely few. At the same time, the data about the safety and efficacy of the product should be collected and necessary measures should be taken to ensure that the product is properly used.

Review Report (1)

January 25, 2013

I. Product Submitted for Registration

[Brand name]	(a) Alabel for Oral Administration 1.5 g and (b) Alaglio for Oral Administration 1.5 g (which are to be changed to (a) Alabel Oral 1.5 g and (b) Alaglio Oral 1.5 g, respectively)
[Non-proprietary name]	Aminolevulinic Acid Hydrochloride
[Name of applicant]	(a) Nobelpharma Co., Ltd. and (b) SBI Pharmaceuticals Co., Ltd.
[Date of application]	July 5, 2012
[Dosage form/Strength]	A lyophilized preparation containing 1.5 g of Aminolevulinic Acid Hydrochloride per vial
[Proposed indication]	Visualization of malignant tissue during surgical resection of malignant glioma
[Dosage and administration]	The dosage is 20 mg/kg given as an oral solution prepared by dissolving in water. The solution should be administered 3 hours (range, 2-4 hours) prior to induction of anesthesia for surgery.

II. Summary of the Submitted Data and Outline of the Review by Pharmaceuticals and Medical Devices Agency

A summary of the submitted data and an outline of the review by the Pharmaceuticals and Medical Devices Agency (PMDA) are as shown below.

1. Origin or history of discovery and usage conditions in foreign countries etc.

5-aminolevulinic acid (5-ALA) is a biological substance that is commonly found in various organisms and intracellularly converted to protoporphyrin IX (PPIX), which is a precursor in the heme synthesis. PPIX emits red fluorescence upon excitation with blue light. The activity of an enzyme involved in PPIX production is higher in malignant tumor cells than in normal cells while that of an enzyme catalyzing conversion of PPIX into heme is lower in malignant tumor cells than in normal cells, resulting in abundant accumulation of PPIX in tumor cells. German researchers designed a fluorescence diagnostic test of cancer using PPIX with such characteristics and published the first report on clinical studies of intraoperative 5-ALA-induced fluorescence diagnosis of malignant glioma in 1998 (*Neurosurgery*. 1998;42:518-26). Medac GmbH, Germany, developed 5-aminolevulinic acid hydrochloride (5-ALA HCl) intended for "Visualization of malignant tissue during surgery for malignant glioma (WHO grade III and IV) in adult patients." Consequently, 5-ALA HCl was approved in Europe in September 2007 and in Korea in January 2011.

In Japan, Nobelpharma Co., Ltd. started the development in January 2010. The third meeting of the Study Group on Unapproved and Off-label Drugs of High Medical Need (April 27, 2010) concluded that there is a high medical need for "Visualization of malignant tissue during surgery for malignant glioma (WHO grade III and IV)" for which 5-ALA HCl is indicated. Following the conclusion by the Study Group, the Ministry of Health, Labour and Welfare (MHLW) requested the applicant to develop 5-ALA HCl in May 2010. In September 2010, 5-ALA HCl was designated as an orphan drug for malignant glioma. Nobelpharma Co., Ltd. and SBI Pharmaceuticals Co., Ltd. have submitted the marketing application for Alabel for Oral Administration 1.5 g and Alaglio for Oral Administration 1.5 g (hereinafter collectively referred to as "the product"), respectively, based on the data including the results of Japanese phase III studies.

2. Data relating to quality

2.A Summary of the submitted data

2.A.(1) Drug substance

The drug substance, Aminolevulinic Acid Hydrochloride (5-ALA HCl), has been registered in the drug master file (DMF No. 224MF10095) by Heraeus Precious Metals GmbH & Co. KG (Germany).

2.A.(1.1) Characterization

The drug substance (5-ALA HCl) is a white to slightly grayish white crystalline powder. The general properties of the drug substance, including description, solubility, melting point, absorption maximum in ultraviolet (UV) light wavelength, dissociation constant (pKa), pH, optical activity, hygroscopicity, and crystalline polymorphism, have been determined.

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]. The chemical structure of the drug substance has been elucidated by elemental analysis, infrared spectrophotometry (IR), nuclear magnetic resonance spectroscopy (¹H-NMR, ¹³C-NMR), and mass spectrometry.

2.A.(1.2) Manufacturing process

The manufacturing process for 5-ALA HCl are described in Appendix.

2.A.(1.3) Control of drug substance

The proposed specifications for the drug substance include content, appearance (gross), identification (IR, thin-layer chromatography), purity (chloride [potentiometric titration], appearance of solution [colorimetry], heavy metals [heavy metal limit test], related substances [liquid chromatography (HPLC)],

residual solvents [gas chromatography (GC)], loss on drying, residue on ignition, microbial limit test, and assay (HPLC).

2.A.(1).4) Stability of drug substance

A stability study conducted for the drug substance is as shown in Table 1. The results of photostability testing indicated that the drug substance is photostable.

Table 1. Stability study of the drug substance

Study type	Primary batch	Temperature	Storage container	Storage period
Long term testing	3 production batches	-20°C	Polyethylene container	36 months

Based on the above, a retest period of 36 months has been proposed for the drug substance when stored in polypropylene containers at -20°C.

2.A.(2) Drug product

2.A.(2).1) Description and composition of the drug product

The drug product is a lyophilized preparation containing 1.5 g of the drug substance per vial.

2.A.(2).2) Manufacturing process

The drug product is produced through the manufacturing process comprising solution preparation, filling, lyophilization, capping and intermediates testing, carton packaging, packaging for transport and intermediates storage, quality testing, and final packaging and storage.

[REDACTED]

[REDACTED]

2.A.(2).3) Control of drug product

The proposed specifications for the drug product include content, appearance (gross), identification (IR, chlorides [qualitative test]), purity (appearance of solution [colorimetry], related substances [HPLC], residual solvents [GC]), water content, uniformity of dosage units (mass variation test), and assay (HPLC).

2.A.(2).4) Stability of drug product

Stability studies conducted for the drug product are as shown in Table 2. The results of photostability testing indicated that the drug product is photostable.

Table 2. Stability study of the drug product

Study type	Primary batch	Temperature	Humidity	Storage container	Storage period
Long term testing	7 production batches	25°C	60% RH	Brown vial: 5 batches Colorless vial: 2 batches	36 months
Accelerated testing	7 production batches	40°C	75% RH		6 months

2.B Outline of the review by PMDA

PMDA reviewed the submitted data and the applicant's responses to the inquiries from PMDA and concluded that the quality of the drug product is appropriately controlled.

3. Non-clinical data

3.(i) Summary of pharmacology studies

3.(i).A Summary of the submitted data

3.(i).A.(1) Primary pharmacodynamics

The mode of action of 5-ALA HCl has already been reported in many published articles, and therefore, the applicant conducted no primary pharmacodynamic studies and investigated the mode of action based on the existing information.

3.(i).A.(1).1 PPIX production and accumulation in various cells (*in vitro*) (4.2.1.1-1, Reference data)

Malignant tumor cell lines including rat bladder carcinoma cell line (NBT-II), murine squamous cell carcinoma (PAM), murine melanoma cell line (B16), human epidermoid carcinoma (A431), human transitional cell carcinoma (EJ) as well as normal cell lines including human fetal bladder cells (FHs738BL) and human skin fibroblast (HSF) cell line were incubated at 1.5×10^5 cells/well for 24 hours, and 1 mM of 5-ALA was added to the cells to synthesize PPIX. The level of PPIX accumulation was measured 1, 4 and 24 hours after addition of 5-ALA. The addition of 5-ALA led to increased formation of PPIX in all cells. Malignant tumor cells except A431 showed remarkable increases in accumulation of PPIX from 1 to 4 hours after the 5-ALA addition in comparison with normal cells. At 24 hours after the 5-ALA addition, intracellular PPIX levels in malignant tumor cell lines except A431 were higher than those in normal cells. In the above test system, 5 µg/mL desferal, which inhibits conversion of PPIX into heme, was added to the cells at the same time as 5-ALA. Consequently, intracellular PPIX levels 24 hours after the 5-ALA addition significantly increased in malignant tumor cells in comparison with those without desferal (mean increase of 38%-52%) while no significant increase was found in normal cells.

3.(i).A.(1).2) Intracerebral distribution of photosensitizers after administration of 5-ALA HCl or porfimer sodium to tumor-bearing rabbits (4.2.1.1-2, Reference data)

VX2 tumor cells (virus-induced papilloma growing in the brain), which are malignant tumor cell lines, were implanted in the brain of male New Zealand White (NZW) rabbits (2-3.5 kg, 20 animals in total). At 13 days after implantation, 20 or 100 mg/kg of 5-ALA HCl or 2.5, 5 or 10 mg/kg of porfimer sodium was intravenously administered to the animals, and the concentrations of the photosensitizer (PPIX or porfimer sodium) in the white and gray matter, tumors and surrounding tumors were measured when excited at 514 nm at 6 and 24 hours after administration of 5-ALA HCl or 24 hours after administration of porfimer sodium. In the 5-ALA group, the concentration of PPIX was high in tumors, being ≥ 72 times that in the white matter, ≥ 21 times that in the gray matter, and ≥ 10 times that in tumor-surrounding regions 6 hours after administration of 5-ALA HCl, and ≥ 165 times that in the white matter, ≥ 24 times that in the gray matter, and ≥ 5 times that tumor-surrounding region 24 hours after administration of 5-ALA HCl. On the other hand, the porfimer sodium group showed small differences between tumors and other tissues; the concentration of porfimer sodium in tumors was 7 to 9 times that in the white matter, 6 to 11 times that in the gray matter, and 3 to 5 times that in tumor-surrounding regions.

3.(i).A.(1).3) Rate of complete resection of brain tumor after administration of 5-ALA HCl or porfimer sodium to tumor-bearing rabbits (4.2.1.1-3, Reference data)

VX2 tumor cells, malignant tumor cell lines, were implanted in the brain of male NZW rabbits (3.3-3.8 kg, n = 14). At 14 days after implantation, 20 mg/kg of 5-ALA HCl was intravenously administered, followed by the removal of brain tumor 4 hours after administration. After grayish tumor cells were resected under white light, residual reddish violet tumor cells were resected under 405-nm excitation light conditions, and finally the whole brain was removed. The calculation of volumes of removed brain tissues (normal and tumor tissues) revealed that $67.9\% \pm 38.4\%$ (mean \pm standard deviation [SD]) of the total tumor volume were removed under white light, and residual tumor cells ($30.1\% \pm 38.1\%$ of the total tumor volume) were removed under fluorescence guidance. As a result, $98.0\% \pm 3.5\%$ of the total tumor volume were removed by using white light resection followed by fluorescence-guided resection.

3.(i).A.(1).4) Effect on normal and edematous brain in photoirradiation to photosensitizers (4.2.1.1-4, Reference data)

Craniotomy was conducted in the right parietal region of male Wistar rats (240-260 g, n = 6), followed by (a) laser irradiation (200 J/cm^2 , 635 nm argon-pumped rhodamine dye laser); (b) laser irradiation 6 hours after intravenous administration of 100 mg/kg of 5-ALA HCl; (c) induction of brain edema by applying a copper stamp (1 mm in diameter) pre-cooled at -68°C to the cortex for 15 seconds; (d) induction of brain edema in the same way as (c) at 3 hours after intravenous administration of 100 mg/kg of 5-ALA HCl, and then laser irradiation another 3 hours later; or (e) laser irradiation 6 hours after intravenous administration of 5 mg/kg of porfimer sodium. Brain tissue samples removed 72 hours after treatment were stained with hematoxylin and eosin (HE), and the depth of superficial cortical damage was measured.

The depth of damage in the group (b) for laser irradiation after administration of 5-ALA HCl was similar to that of the group (a) for laser irradiation alone and the group (c) for induced brain edema. The depth was <0.5 mm in all groups. The depth of damage in the group (d) for laser irradiation after administration of 5-ALA HCl and induction of brain edema was 0.83 ± 0.31 mm. The depth of damage in the group (e) for laser irradiation after administration of porfimer sodium was 1.77 ± 0.22 mm and damages were found also in deeper tissues in this group than in other groups, i.e. (a) to (d).

3.(i).A.(1).5) Mechanism of PPIX accumulation in tumor cells

(a) Activities of ALA dehydratase, PBG deaminase and uroporphyrinogen decarboxylase in human normal and breast cancer cells (4.2.1.1-5, Reference data)

Malignant tumor and normal cells collected from tissues removed during breast cancer surgery were homogenized in sucrose solution within 4 hours after collection of the tissues and centrifuged to obtain the supernatant. The activities of PPIX synthases in the supernatant were determined.

ALA dehydratase activity was calculated by fluorometric measurement of porphobilinogen (PBG) that formed up to 1 hour after addition of 5-ALA HCl to the sample. PBG deaminase activity was calculated by fluorometric measurement of porphyrin that was produced by enzymes for different hours after addition of PBG to the sample. Uroporphyrinogen (URO) decarboxylase activity was calculated by measurement of the fluorescence intensity of porphyrin that formed under nitrogen environment up to 2 hours after addition of PBG to the sample.

Activities of ALA dehydratase, PBG deaminase and URO decarboxylase in tumor cells were 1.6 to 17.6 times, 2.5 to 67 times, and 2.2 to 32.5 times, respectively, higher than those in normal cells.

(b) PBG deaminase and ferrochelatase activities in rat normal hepatocyte- and hepatoma-derived cell lines (4.2.1.1-6, Reference data)

Using JAR-2 rat normal hepatocyte-derived cell lines (RL, RCL-10, RCL-24, M, Culb-TC) and rat hepatoma (Yoshida ascites hepatoma)-derived cell lines (JTC-1, JTC-2, JTC-15, JTC-16, JTC-27), activities of PBG deaminase, a PPIX synthase, and ferrochelatase, an enzyme catalyzing conversion of PPIX into heme, were measured.

PBG deaminase activity was calculated by fluorometric measurement of porphyrin that formed up to 1 hour after addition of PBG to the sample. Ferrochelatase activity was calculated as follows: the sample was added with mesoporphyrin and iron (II) sulfate followed by cooling, and iron chloride (^{59}Fe) was added to the solution to start enzymatic reaction. At 1 hour of the reaction, porphyrin was removed from the ethyl acetate layer of the solution, and radioactivity in the resultant ethyl acetate layer was measured to serve as the data for the calculation of enzyme activity.

PBG deaminase activity in hepatoma-derived cells except JTC-27 was higher than that in normal hepatocytes (RL). PBG deaminase activity in normal hepatocyte-derived cells that were transformed by drugs or spontaneously (RCL-10, RCL-24, M, Culb-TC) was lower than that in RL but the activity levels were almost similar. Ferrochelatase activity in all hepatoma-derived cells and in transformed cells derived from normal cells was lower than that in RL.

3.(i).A.(2) Secondary pharmacodynamics

No data were submitted.

3.(i).A.(3) Safety pharmacology

3.(i).A.(3).1 Effects on the central nervous system

(a) Effects on spontaneous motility in mice (4.2.1.3-1)

In a dark room protected from UV light (<635 nm), 40, 100, or 250 mg of 5-ALA HCl or saline was intravenously administered to female NMRI mice (20-22 g, n = 5). Consequently, 5-ALA HCl had no effect on slight static movements (e.g., grooming, but excluding moving around) or active moving (e.g., moving around) until 130 minutes after administration.

(b) Effects on hexobarbital-induced sleep (4.2.1.3-2)

In a dark room protected from UV light (<635 nm), 40, 100 or 250 mg of 5-ALA HCl or saline was intravenously administered to female NMRI mice (20-23 g, n = 5), and 45 mg/kg of hexobarbital was intravenously administered 5 minutes after. Consequently, 5-ALA HCl had no effect on hexobarbital-induced sleep time.

3.(i).A.(3).2 Effects on respiratory and cardiovascular systems (4.2.1.3-3)

In a dark room protected from UV light (<635 nm), 5-ALA HCl was intravenously administered at escalating doses of 0 (vehicle), 5, 15 and 45 mg/kg to chloralose/urethane-anesthetized female beagle dogs (3.5-4 years old, n = 5). Peripheral arterial pressure (both systolic and diastolic), pulmonary arterial pressure (both systolic and diastolic), heart rate, cardiac output, stroke volume, left ventricular pressure, maximum developed pressure (dp/dt max), central venous pressure, respiratory rate, respiratory volume, and blood gas (pH, pO₂, pCO₂) were measured 5, 15 and 30 minutes after administration of 5-ALA HCl. No changes were found in any of the parameters at ≤15 mg/kg, while the maximum developed pressure significantly decreased immediately after administration at 45 mg/kg in comparison with the pre-dose value but recovered within 5 minutes post-dose.

3.(i).A.(3).3 Effects of 5-ALA on urinary excretion and urine electrolytes (4.2.1.3-4)

In a dark room protected blocked from UV light (<635 nm), 40, 100 or 250 mg/kg of 5-ALA HCl or saline was intravenously administered to female Sprague-Dawley (SD) rats (160-184 g, n = 10), followed by an oral dose of 20 mL/kg of distilled water. Urine was collected up to 24 hours after administration. Urine volume and concentrations of urine electrolytes (chloride ion [Cl⁻], sodium ion

[Na⁺], potassium ion [K⁺]) were measured. Consequently, no effects on urine volume or urinary Cl⁻ excretion were found in any of the treatment groups. Urinary Na⁺ excretion up to 2 hours after administration in the 100 mg/kg group was significantly lower than that in the saline group, but no dose-dependency was observed. Urinary K⁺ excretion up to 1 hour after administration in the 250 mg/kg group was significantly higher than that in the saline group.

3.(i).A.(3).4 Effects on smooth muscle contraction (4.2.1.3-5)

Using the ileum isolated from female Dunkin-Hartley guinea pigs (250-280 g, n = 6), the effect of 5-ALA HCl (0.5, 5, 50, 500 and 5000 µg/mL) on the smooth muscle was evaluated in a dark room protected from UV-light (<635 nm). 5-ALA HCl was added to isolated ileums treated with a spasmogen (histamine, acetylcholine or barium chloride) to evaluate the effect of 5-ALA HCl on smooth muscle relaxation. Although 5-ALA HCl alone showed no action to contract and relax the smooth muscle, 500 and 5000 µg/mL of 5-ALA HCl inhibited histamine-stimulated contraction by 27.48% and 98.77%, respectively. Furthermore, 5000 µg/mL of 5-ALA HCl inhibited acetylcholine- and barium chloride-stimulated contraction by 59.60% and 89.67%, respectively.

3.(i).A.(4) Pharmacodynamic drug interactions

No data were submitted.

3.B Outline of the review by PMDA

PMDA asked the applicant to explain whether the effect of 5-ALA HCl on malignant glioma was appropriately estimated by the results of the primary pharmacodynamic studies (4.2.1.1-1 to 6) because cells used in these studies were not malignant glioma cells.

The applicant responded as follows:

Heme synthesis is enhanced in not only malignant glioma cells but also almost all malignant tumor cells, and 5-ALA-induced PPIX formation is higher in tumor cells than in normal cells. Therefore, the detection of PPIX as a fluorescent substance enables most malignant tumor cells to be identified.

The results of pharmacology studies submitted showed that the level of PPIX accumulation after administration of 5-ALA HCl was higher in various malignant cells (excluding brain tumor cells) than in normal cells. Although there are no reports in which various enzyme activities involved in the heme synthesis pathway are studied using normal brain cells and brain tumor cells, it has been reported that a significant decrease in mRNA expression of ferrochelatase, an enzyme catalyzing the conversion of PPIX into heme, was noted in human brain tumor (malignant glioma) tissues in comparison with normal brain tissues (*Brit J Cancer*. 2011;104:798-807).

Based on the above, the finding of the effect of 5-ALA HCl noted in cells other than brain tumor cells is presumed to be reproducible also in brain tumor cells. Therefore, the results of primary

pharmacodynamics studies submitted in the application would appropriately estimate the effect of 5-ALA HCl on malignant glioma.

PMDA considers as follows:

No results were shown from studies to directly examine activities of PPIX synthase or enzymes catalyzing conversion of PPIX into heme, or the accumulation of PPIX in malignant glioma and malignant glioma-derived cells. There is no proof demonstrating that PPIX accumulates in malignant glioma cells as with the case of tumor cells used in the primary pharmacodynamics studies submitted in the application. However, the studies using malignant tumor cells showed the following findings: (a) the activity of PPIX synthase was higher in malignant tumor cells used in the study than in normal cells, while the activity of enzymes catalyzing the conversion of PPIX into heme was lower in malignant tumor cells than those in normal cells, and thus the level of 5-ALA-induced PPIX accumulation was higher in malignant tumor cells than in normal cells; and (b) following intravenous administration of 5-ALA HCl to rabbits with malignant tumor VX2 cells implanted into the brain, the level of PPIX accumulation was higher in brain tumor tissues than in normal tissues. Furthermore, from the results of a nonclinical pharmacokinetic study submitted by the applicant, which was a study on intracranial PPIX distribution following intravenous administration of 5-ALA HCl to rats with C6 glioma cells (human malignant glioma model) implanted into the brain, [see “3.(ii).A.(2).1 Tissue distribution”], it was suggested that 5-ALA was distributed in the brain and that the level of PPIX accumulation in brain tumor tissues was higher than in normal tissues. Thus, PMDA has concluded that the submitted data support the finding that administration of 5-ALA HCl to a patient with malignant glioma leads to PPIX accumulation in the brain tumor cells, resulting in visualization of the tumor cells.

3.(ii) Summary of pharmacokinetic studies

3.(ii).A Summary of the submitted data

Seven studies to examine pharmacokinetics of exogenously administered 5-ALA (4.2.2.2-1 to 4.2.2.2-6, 4.2.2.4-4, 4.2.2.5-3) for nonclinical pharmacokinetic data were submitted as the evaluation data, while published literature was submitted as the reference data.

5-ALA is a precursor of porphyrin, a component of hemoglobin and cytochromes. The plasma concentrations of 5-ALA and porphyrin were determined in pharmacokinetic studies. Plasma 5-ALA concentrations in rats and dogs were assayed using validated high performance liquid chromatography with fluorescence detection (HPLC-FL). The lower limit of quantification differed depending on studies, ranging from 0.02971 to 0.01 µg/mL in rats and from 0.03105 to 0.01 µg/mL in dogs. Plasma PPIX concentrations were assayed using validated HPLC-FL and the lower limit of quantification was 0.00562 µg/mL for rats and 0.00434 µg/mL for dogs. Concentrations of plasma porphyrin-related compounds (uroporphyrin I, uroporphyrin III, pentacarboxylporphyrin, heptacarboxylporphyrin, coproporphyrin I, coproporphyrin III) were assayed using validated HPLC-FL, and the lower limit of quantification was 0.2 pmol/mL. Unless otherwise specified, pharmacokinetic parameters are presented as mean or mean

± SD.

3.(ii).A.(1) Absorption

3.(ii).A.(1).1 Single-dose studies (4.2.2.2-1 to 2, and 4.2.2.2-5)

Following single oral doses of 5-ALA HCl (30 and 300 mg/kg) to male and female rats ($n = 3/\text{sex}/\text{time point}$), plasma 5-ALA concentration was measured pre-dose and at 20 minutes, and 2, 4, and 24 hours post-dose. The pre-dose value was below the lower limit of quantification. The time to reach the maximum plasma concentration (t_{max}) was 20 minutes post-dose in all groups. The maximum plasma concentration (C_{max}) was 7.1 ± 6.5 and 13.7 ± 2.5 $\mu\text{g}/\text{mL}$ for males and females, respectively, in the 30 mg/kg group and 69.8 ± 8.2 and 68.6 ± 14.1 $\mu\text{g}/\text{mL}$, respectively, in the 300 mg/kg group. The elimination half-life ($t_{1/2}$) was 70.8 and 63.3 minutes, respectively, in the 30 mg/kg group and 108.5 and 143.4 minutes, respectively, in the 300 mg/kg group. Plasma 5-ALA decreased below the lower limit of quantification at 24 hours post-dose in all dose groups. Following single oral doses of 5-ALA phosphate (15, 60, 250 mg/kg) to male and female rats ($n = 3/\text{sex}/\text{time point}$), plasma 5-ALA concentration was measured at 0.5, 1, 2, 4, 8 and 24 hours post-dose. t_{max} was 0.5 hour post-dose in males and females of all groups, and C_{max} was 2.20 and 4.09 $\mu\text{g}/\text{mL}$ for males and females, respectively, in the 15 mg/kg groups, 9.61 and 14.4 $\mu\text{g}/\text{mL}$, respectively, in the 60 mg/kg groups, and 85.3 and 58.2 $\mu\text{g}/\text{mL}$, respectively, in the 250 mg/kg groups. The area under the plasma concentration-time curve to 24 hours after administration (AUC_{0-24}) was 3.16 and 3.80 $\mu\text{g}\cdot\text{h}/\text{mL}$ for males and females, respectively, in the 15 mg/kg groups, 12.0 and 19.2 $\mu\text{g}\cdot\text{h}/\text{mL}$, respectively, in the 60 mg/kg groups, and 142 and 91.5 $\mu\text{g}\cdot\text{h}/\text{mL}$, respectively, in the 250 mg/kg groups.

Following single oral and intravenous doses of 5-ALA HCl (20 mg/kg) to male and female dogs ($n = 3/\text{sex}$) using a cross-over design, t_{max} was 0.625 ± 0.262 and 0.058 ± 0.027 hours, respectively, and C_{max} was 14.715 ± 1.662 and 40.901 ± 5.540 $\mu\text{g}/\text{mL}$, respectively. The area under the plasma concentration-time curve to infinity ($\text{AUC}_{0-\infty}$) was 22.290 ± 5.016 and 25.896 ± 5.063 $\mu\text{g}\cdot\text{h}/\text{mL}$, respectively, and $t_{1/2}$ was 0.623 ± 0.129 and 0.652 ± 0.059 hours, respectively. The total body clearance (CL) and volume of distribution (V_d) following intravenous administration was 0.799 ± 0.163 $\text{L}/\text{kg}\cdot\text{h}$ and 0.751 ± 0.164 L/kg , respectively. The bioavailability (BA) of oral dose administration of 5-ALA HCl was 86%. Plasma PPIX concentrations following oral or intravenous administration of 5-ALA HCl were below the lower limit of quantification in almost all animals, and thus no pharmacokinetic parameters were determined.

3.(ii).A.(1).2 Repeat-dose studies (4.2.2.2-1, 4.2.2.2-3 to 4, 4.2.2.2-6)

Following repeated oral doses of 5-ALA HCl (30, 300 mg/kg) to male and female rats ($n = 3/\text{sex}/\text{time point}$) once a day for 14 days, plasma 5-ALA concentration was measured pre-dose and at 20 minutes, and 2, 4 and 24 hours post-dose on Day 14. Consequently, t_{max} was 20 minutes in males and females of all groups and $t_{1/2}$ was 43.1 to 88.1 minutes (mean range by sex and dose in this section). Following repeated intravenous doses of 5-ALA HCl (125, 500 mg/kg) to male and female rats ($n = 3/\text{sex}/\text{time point}$) once a day for 14 days, plasma 5-ALA concentration on Days 1 and 14 decreased immediately

after administration and $t_{1/2}$ was 35.1 to 45.6 minutes. The pharmacokinetics of 5-ALA was not affected significantly by repeated administration. No large difference in plasma PPIX concentrations was found between single and repeated administration or between males and females in both 125 and 500 mg/kg groups, and t_{max} was 2 to 8 hours. C_{max} was 0.055 to 0.100 in the 125 mg/kg group and 0.038 to 0.146 $\mu\text{g/mL}$ in the 500 mg/kg group, respectively. Plasma PPIX concentrations decreased to baseline levels 24 hours after administration.

Following repeated oral doses of 5-ALA HCl (1, 3, 10 mg/kg) to male and female dogs ($n = 3\text{-}4/\text{sex}/\text{time point}$) once a day for 28 days, t_{max} was 0.5 to 1.4 hours on Days 1 and 28, respectively. C_{max} or AUC_{0-24} after oral administration of 5-ALA HCl showed no large differences between males and females, and these values after repeated administration caused almost no change. C_{max} was 0.577 to 0.608 in the 1 mg/kg groups, 1.90 to 2.29 in the 3 mg/kg groups, and 4.72 to 9.36 $\mu\text{g/mL}$ in the 10 mg/kg groups and AUC_{0-24} was 1.05 to 1.18, 2.74 to 3.38, and 8.24 to 12.3 $\mu\text{g}\cdot\text{h/mL}$, respectively.

3.(ii).A.(2) Distribution

3.(ii).A.(2).1 Tissue distribution (4.2.2.3-1, 4.2.2.3-2, 4.2.2.3-6, and 4.2.2.3-7, Reference data)

Tissue distribution of 5-ALA and PPIX at 1, 2, 3, 4, 6, 12 and 24 hours post-dose was examined in male albino rats ($n = 3/\text{time point}$) following single oral or intravenous dose of 5-ALA HCl at 200 mg/kg. For both routes of administration, 5-ALA was rapidly distributed throughout tissues and its concentration was high in the kidney, bladder, spleen, duodenal aspirate, jejunum, colon, and liver. The concentration after oral administration was higher in the stomach, duodenal aspirate, jejunum, kidney, liver and plasma than that after intravenous administration. For both routes of administration, 5-ALA concentrations in all tissues reached the peak and decreased rapidly. After intravenous administration, 5-ALA concentrations in many tissues fell below the detection limit 3 hours post-dose, and 5-ALA was not detected in the duodenal aspirate, jejunum, spleen, kidney, or bladder by 6 hours post-dose. After oral administration, 5-ALA concentrations in the stomach, duodenal aspirate, jejunum, liver, kidney, and bladder became undetectable more slowly than that after intravenous administration. However, 5-ALA concentrations in these tissues were below the detection limit 24 hours post-dose for both routes of administration. Tissue PPIX concentrations reached the peak 2 to 4 hours after oral administration and 1 to 3 hours after intravenous administration and showed similarity in the maximum concentrations between the two routes. High PPIX concentrations were shown in tissues of the duodenal aspirate, jejunum, liver, kidney, colon, stomach, heart, lung, esophagus, spleen, bladder and nerves. PPIX concentrations were higher in these tissues than in plasma at all time points. The time course of PPIX concentrations in the skin was similar to that in plasma. Although PPIX concentrations in the kidney were increasing at 24 hours post-dose, which was approximately 2.5-fold background level, porphyrin concentrations in tissues except the kidney at 12 hours post-dose were at background level. After oral administration, 5-ALA and PPIX concentrations in brain reached the peak at 1 and 3 hours post-dose, respectively, and returned to the background level at 24 hours post-dose.

Following a single intravenous dose of 5-ALA HCl at 100 mg/kg to male dogs (n = 3-7), the total porphyrin concentration increased at 1 to 4 hours post-dose in the liver, spleen, prostate gland, bladder and muscles, while porphyrin was slightly distributed in the skin.

Glioma cells (C6 glioma), which are human malignant glioma model, were implanted in the brain of female rats (n = 4-5/time point). A single dose of ¹⁴C-labeled 5-ALA at 120 mg/kg was intravenously administered to the animals 14 days after implantation. Radioactivity was found in tumors at 5 minutes post-dose. Radioactivity concentration in tumor increased (59,634 dpm/g) until 15 minutes post-dose and decreased after that, however, a small amount of radioactivity (3653 dpm/g) was detected even at 8 hours post-dose. The maximum radioactivity concentration in tumors was approximately 3.4-fold that in normal brain. Radioactivity concentration in the liver reached the peak (150,281 dpm/g) at 30 minutes post-dose, while that in the skin reached the peak (128,245 dpm/g) at 15 minutes post-dose.

C6 glioma cell-implanted male rats (n = 6/time point) received intravenous administration of 100 mg/kg of 5-ALA HCl 9 days after implantation. Fluorescence intensity of PPIX in brain tumor tissues was significantly higher than that in normal brain tissues at 3, 6 and 9 hours post-dose and reached the peak at 6 hours post-dose.

3.(ii).A.(3) Metabolism

3.(ii).A.(3).1 Hemoglobin synthesis in cells (4.2.2.4-8, Reference data)

Heme synthesis pathway is as follows: 5-ALA formed in mitochondria is converted to PBG by ALA dehydratase, followed metabolism catalyzed by PBG deaminase and URO III synthase to yield URO III, and URO III is then metabolized by URO decarboxylase to coproporphyrinogen III, in the cytosol. Subsequently, in mitochondria, coproporphyrinogen oxidase catalyzes the conversion of coproporphyrinogen III into protoporphyrinogen IX which is then synthesized by protoporphyrinogen oxidase to PPIX. Ferrochelatase mediates the conversion of PPIX into heme by inserting ferrous iron.

3.(ii).A.(3).2 *in vivo* Metabolism (4.2.2.4-1 [Reference data], 4.2.2.4-4)

Repeated oral doses of 5-ALA phosphate (15, 60 and 250 mg/kg) were given to male and female rats (n = 3/sex/time point) once a day for 91 days. As a result, the quantitation of the following 6 compounds was possible: uroporphyrin I, uroporphyrin III, pentacarboxylporphyrin, heptacarboxylporphyrin, and coproporphyrin I, and coproporphyrin III. Their t_{max} was 0.5 to 4 hours and C_{max} and AUC_{0-24} increased almost dose-proportionally.

Following a single intravenous dose of 5-ALA HCl at 100 mg/kg to male dogs (n = 2-7), plasma porphyrin concentrations rapidly increased up to 1 hour post-dose, and gradually decreased. In the urine, 5-ALA, PBG, coproporphyrin III, which is a porphyrin compound, heptacarboxylporphyrin, hexacarboxylporphyrin and pentacarboxylporphyrin were detected.

3.(ii).A.(4) Excretion

3.(ii).A.(4).1 Excretion in urine (4.2.2.5-1 [Reference data], 4.2.2.5-2 [Reference data], and 4.2.2.5-3)

Urinary 5-ALA concentrations at 1, 2, 3, 4, 6, 12 and 24 hours post-dose were measured in male rats (n = 3 /time point) following single oral or intravenous dose of 5-ALA at 200 mg/kg. For both routes of administration, 5-ALA was detected in urine 1 hour post-dose and urinary 5-ALA concentrations reached the peak 2 hours post-dose. Small amounts of 5-ALA were detected in urine 4 hours post-dose.

Following a single intravenous dose of 5-ALA HCl at 100 mg/kg to male dogs (n = 3-7), 5-ALA excretion in urine rapidly increased after administration and reached the peak from 2 to 4 hours post-dose. Urinary PBG gradually increased and reached the peak from 4 to 8 hours post-dose. Urinary total porphyrin increased after administration and reached the peak from 4 to 8 hours post-dose.

Repeated oral doses of 0.9% saline or 5-ALA HCl (3, 100, 300 mg/kg) were given to male and female rats (n = 10/sex/group) once a day for 14 days. As a result, urinary 5-ALA concentrations at 16 hours post-dose on Day 13 were 4.7 ± 2.0 , 6.6 ± 2.2 , 39.4 ± 35.3 and 340.4 ± 182.3 $\mu\text{g/mL}$, respectively, in male rats, 5.7 ± 1.4 , 6.9 ± 1.1 , 42.7 ± 16.9 and 251.4 ± 148.6 $\mu\text{g/mL}$, respectively, in female rats.

3.(ii).B Outline of the review by PMDA

Based on the results of nonclinical pharmacokinetic studies, 5-ALA was shown to be well-absorbed after oral administration of 5-ALA HCl and the exposure level of 5-ALA in animals in the toxicity studies has been confirmed. Furthermore, 5-ALA was rapidly distributed in tissues following oral and intravenous administration of 5-ALA HCl, and 5-ALA was rapidly eliminated from the tissues tested and PPIX (produced from 5-ALA) from the tissues tested except the kidney. Although pigmentation in the kidney was found in toxicity studies, nephrotoxicity was not shown. 5-ALA were rapidly excreted in urine even if it was not distributed in tissues but found in plasma. Therefore, the pharmacokinetic profile of 5-ALA indicated no safety concern for oral administration.

Information concerning brain distribution of PPIX indicates that 5-ALA administered exogenously is distributed to the brain as well and converted into PPIX in brain tissues. Therefore, the pharmacokinetic profile of 5-ALA HCl is shown to support the expected efficacy.

3.(iii) Summary of toxicology studies

3.(iii).A Summary of the submitted data

The following toxicity studies of 5-ALA HCl were conducted: single dose toxicity, repeat dose toxicity, genotoxicity, reproduction toxicity and phototoxicity. Single dose toxicity studies and genotoxicity studies (excluding chromosomal aberration assay using CHL cells) were conducted in the dark.

3.(iii).A.(1) Single-dose toxicity (4.2.3.1-1 to 4.2.3.1-3)

Single dose toxicity studies conducted include intravenous dose toxicity studies in mice and SD rats and oral dose toxicity studies in rats. Decreased activity, ataxia, and dyspnea were observed after administration.

Animals were housed in the dark for 72 hours post-dose.

3.(iii).A.(1.1) Single-dose oral toxicity study in mice (4.2.3.1-1)

Following a single intravenous dose of 5-ALA HCl (250, 500 1000 mg/kg) to male and female NMRI mice, 2 of 5 male mice and 3 of 5 female mice of the 1000 mg/kg group died. The median lethal dose (LD₅₀) was determined to be 1064 mg/kg in male mice and 949 mg/kg in female mice. Decreased activity, ataxia, and dyspnea were observed after administration. Dead animals were in a coma in the prone position.

3.(iii).A.(1.2) Single-dose oral toxicity study in rats (4.2.3.1-2)

Following a single oral dose of 5-ALA HCl (625, 1250 2500 mg/kg) to male and female SD rats, no animal died. The approximate lethal dose was determined to be >2500 mg/kg.

3.(iii).A.(1.3) Single-dose intravenous toxicity study in rats (4.2.3.1-3)

Following a single intravenous dose of 5-ALA HCl (125, 250, 500, 1000 mg/kg) to male and female SD rats, 3 of 5 male rats and 2 of 5 female rats of the 1000 mg/kg group died. The LD₅₀ was determined to be 949 mg/kg in male rats and 1,064 mg/kg in female rats. Decreased activity, ataxia, dyspnea, hypotonia and lateral decubitus posture were observed after administration.

3.(iii).A.(2) Repeat-dose toxicity

Repeat dose toxicity studies conducted include repeated oral dose toxicity studies in rats (4 and 13 weeks) and dogs (4 weeks). The major target organ was the liver (particularly bile duct), and major findings included increased aspartate aminotransferase (AST), alanine aminotransferase (ALT) and total bilirubin, and brown pigmentation in the liver. Furthermore, in rats, mild anemia, hepatic necrosis, bile duct hyperplasia, peribiliary infiltration, reddish brown urine, brown pigmentation in the kidney, and proximal tubule epithelial vacuolation were also found. 5-ALA is converted into PPIX in heme synthesis. Skin findings including ear and tail flushing, excoriation and hair loss were found in rats, which were considered to be induced by the phototoxicity of PPIX. Brown pigmentation in the liver and kidney and reddish brown urine is attributable to accumulation and excretion of porphyrin pigment and these findings were not considered to be toxicity findings.

The C_{max} and AUC of plasma 5-ALA at the no-observed-adverse-effect levels (NOAELs) as determined in the 4- and 13-week toxicity studies in rats and 4-week toxicity study in dogs (44 and 11 mg/kg for rats, respectively; 3 mg/kg for dogs) were compared with those of plasma 5-ALA at the human clinical

dose (20 mg/kg, 5.3.5.2-1) (AUC_{0-24} in the toxicity study and AUC_{∞} in the clinical study). As a result, the C_{max} and AUC values, relative to those in humans, were approximately 0.5- and 0.3-fold, respectively, in the 4-week study in rats; approximately 0.09- and 0.04-fold, respectively, in 13-week study in rats; and approximately 0.06- and 0.05-fold in 4-week study in dogs, respectively.

The results of studies are shown in the following sections.

3.(iii).A.(2).1) Four-week oral toxicity study in rats (4.2.3.2-2)

Following 4-week oral doses of 5-ALA phosphate (0 [water for injection, the same applies in the following studies, unless otherwise specified], 44, 183, 366, 731 mg/kg/day [in terms of 5-ALA hydrochloride]) to male and female Wistar rats ($n = 10/\text{sex}/\text{group}$), brown pigmentation was found in small bile ducts, hepatocytes, Kupffer cells and proximal tubule epithelial cells in males and females at ≥ 44 mg/kg/day.

In both males and females at ≥ 183 mg/kg/day, brown urine, decreases in hemoglobin concentration, mean corpuscular hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin concentration, and platelet count, increases in reticulocyte count and monocyte proportion, increases in AST, ALT, LDH, total cholesterol and phospholipid, decreased urine pH, dark-brown liver and kidney, increases in liver and kidney weights, focal hepatocellular necrosis or unicellular necrosis, bile duct hyperplasia, and proximal tubule epithelial vacuolation were noted. In the ≥ 366 mg/kg/day groups, ear or tail flushing and excoriation, which were probably due to the phototoxicity of PPIX, decreased hematocrit, increases in neutrophil proportion and white blood cell count, increases in ALP, blood urea nitrogen, total bilirubin and triglyceride, decreased creatinine, increased urine volume, and urine ketone body were noted in both males and females, and reduced food consumption was observed in males. In the 731 mg/kg/day group, reduced food consumption, dark-brown tail, and hair loss in both males and females, and lower body weight were found in males. Based on the above, the NOAEL was determined to be 44 mg/kg/day in males and females.

3.(iii).A.(2).2) Thirteen-week oral toxicity study in rats (4.2.3.2-3)

Oral doses of 5-ALA phosphate (0, 11, 44, 183 mg/kg/day [in terms of hydrochloride]) were given to male and female Wistar rats ($n = 10/\text{sex}/\text{group}$) for 13 weeks. In the ≥ 44 mg/kg/day groups, decreases in mean corpuscular hemoglobin and mean corpuscular volume, brown pigmentation in the liver and kidney, and bile duct hyperplasia were found in both males and females, and increases in reticulocyte count and total bilirubin, unicellular and focal hepatocellular necroses, and periductal lymphocytic infiltration were found in males. In the 183 mg/kg/day group, hair loss in the lumber region, reddish-brown urine, increases in total bilirubin and ALT, decreases in creatinine and A/G ratio, increases in liver and kidney weights, dark brown kidney, unicellular hepatocellular necroses, and periductal lymphocytic infiltration were found in both males and female, and lower body weight, decreases in hemoglobin concentration and mean corpuscular hemoglobin concentration, increases in AST and ALP,

decreases in total cholesterol, phospholipid and albumin, and increased spleen weight were found in males. Although anemia tended to be persistent after a 4-week washout period, other changes resolved or were resolving. Based on the above, the NOAEL was determined to be 11 mg/kg/day in males and females.

3.(iii).A.(2).3 Four-week oral toxicity study in dogs (4.2.3.2-5)

Oral doses of 5-ALA HCl (0, 1, 3, 10 mg/kg/day) were given to male and female beagle dogs (n = 3-4/sex/group) for 4 weeks. Vomiting or vomitus was found after dosing and the incidence tended to increase with the dose in both males and females at ≥ 1 mg/kg/day. Slight increases in AST and ALT, dark brown liver, and yellow pigmentation in bile canaliculi, Kupffer cells and hepatocytes were found in both males and females at 10 mg/kg/day. These changes tended to resolve after a 4-week washout period. Based on the above, the NOAEL was determined to be 3 mg/kg/day in males and females.

3.(iii).A.(3) Genotoxicity (4.2.3.3.1-1 to 4, 4.2.3.3.2-1)

Genotoxicity studies conducted include a bacterial reverse mutation assay of 5-ALA HCl performed in the dark, gene mutation assay with mammalian cultured cells (Chinese hamster-derived V79 cells and HPRT mutants), chromosomal aberration assay with mammalian cultured cells (human lymphoid cells), and micronucleus test in mice. Consequently, all studies indicated negative results. However, in a chromosomal aberration assay of 5-ALA phosphate using mammalian cultured cells (CHL cells) performed in the presence of light, an increasing tendency of cells with chromosomal aberration was noted in continuous treatment without S9. As a result, 5-ALA was determined to be false-positive.

It has been reported that DNA oxidative damage associated with porphyrin is found after irradiation of UV or visible light to cells treated with 5-ALA (*Carcinogenesis*. 2001;22:771-8, *Environ Mutagen Res*. 2001;23:97-102).

3.(iii).A.(4) Carcinogenicity

No data were submitted.

3.(iii).A.(5) Reproductive and developmental toxicity

Reproductive and developmental toxicity studies conducted include study of fertility and early embryonic development to implantation in rats, embryo-fetal development study in rats and rabbits, and study for effects on pre- and postnatal development, including maternal function in rats. Decreased mating in paternal rats, delayed development such as fetal low body weight and delayed ossification, and inhibited body weight gain and low survival rate in offspring were found in rats.

It has been reported that fetal survival rate decreases after intravenous administration of 5-ALA and direct light irradiation to the uterus of pregnant rats on gestation day 10 (*Fertil Steril*. 1994;62:1060-5). Similar studies in mice and chicken have also been published (*Fertil Steril*. 1995;63:1088-93, *Reprod*

Toxicol. 2001;15:111-6). Taking account of these reports, direct light irradiation after administration of 5-ALA HCl may induce fetal toxicity.

Based on the above, it was determined that 5-ALA HCl should be contraindicated in pregnant or potentially pregnant women.

3.(iii).A.(5).1) Study of fertility and early embryonic development to implantation in rats (4.2.3.5.1-1)

Oral doses of 5-ALA phosphate (0, 44, 132, 366 mg/kg/day [in terms of 5-ALA hydrochloride]) to male and female Wistar rats (n = 20/sex/group) were given from 2 weeks before mating to the day before necropsy in males, and from 2 weeks before mating to gestation day 7 in females. In the ≥ 132 mg/kg/day groups, reddish brown urine and dark brown kidney were found in both males and females, and reduced body weight gain in males. At 366 mg/kg/day, ear flushing and reduced food consumption were found in both males and females, reduced body weight gain in females, and dark brown liver and increased seminal vesiculitis weight in males. For the effect on reproductive function, decreased mating was found in males at 366 mg/kg/day. Based on the above, the NOAEL was determined as follows: 44 mg/kg/day for parental general toxicity, 132 mg/kg/day for paternal reproductive function, and 366 mg/kg/day for maternal reproductive function and fetal development in the early stage.

3.(iii).A.(5).2) Embryo-fetal development study in rats (4.2.3.5.2-1)

Following oral doses of 5-ALA phosphate (0, 44, 132, 366 mg/kg/day [in terms of 5-ALA hydrochloride]) to pregnant Wistar rats (n = 17-20) from gestation days 7 to 17, reduced body weight gain, reduced food consumption, reddish brown urine, and dark brown kidney were found at 366 mg/kg/day. In fetuses, lower fetal body weight and delayed ossification in the sacral and caudal vertebrae. Based on the above, the NOAEL was determined as follows: 132 mg/kg/day for maternal general toxicity, 366 mg/kg/day for maternal reproductive function, and 132 mg/kg/day for embryo-fetal development.

3.(iii).A.(5).3) Embryo-fetal development study in rabbits (4.2.3.5.2-3)

Following oral doses of 5-ALA HCl (0, 15, 50, 150 mg/kg/day) to pregnant NZW rabbits (n = 15-20) from gestation days 6 to 18, abortion was noted in one animal each in the 50 and 150 mg/kg/day groups. Abortion in the 150 mg/kg/day group was considered to be caused by malnutrition while abortion in the 50 mg/kg/day group was not accompanied by reduced food consumption and the cause was unclear. However, since the incidence was within the range of background data in the testing facility, it was not considered to be related to administration of 5-ALA HCl. In the 150 mg/kg/day group, reduced food consumption and body weight loss were noted, however, no effect on embryo-fetal development was found. Based on the above, the NOAEL was determined as follows: 50 mg/kg/day for maternal general toxicity, and 150 mg/kg/day for maternal reproductive function and embryo-fetal development.

3.(iii).A.(5).4) Study for effects on pre- and postnatal development, including maternal function in rats (4.2.3.5.3-1)

Following oral doses of 5-ALA phosphate (0, 44, 132, 366 mg/kg/day [in terms of 5-ALA hydrochloride]) to pregnant Wistar rats (n = 22) from gestation day 7 to lactation day 20, reddish brown urine and dark brown kidney were found at ≥ 132 mg/kg/day, and ear flushing and reduced body weight gain and reduced food consumption at 366 mg/kg/day. In newborn pups, lower body weight and lower 4-day survival rate, and negative geotactic expression retardation were found at 366 mg/kg/day. In postweaning neonatal rats, lower body weight, reproductive growth retardation, and high mortality in postimplantation embryo were found at 366 mg/kg/day. Based on the above, the NOAEL was determined as follows: 44 mg/kg/day for maternal general toxicity, and 132 mg/kg/day for maternal reproductive function and neonatal development.

3.(iii).A.(6) Other toxicity study

3.(iii).A.(6).1) Intravenous phototoxicity study in mice (4.2.3.7-1)

Single intravenous dose of 5-ALA HCl (0 [saline], 250, 750 mg/kg) was administered to female NMRI mice (n = 5/group), and the animals were irradiated with ultraviolet (UV-A: 30 J UV-A/cm², UV-B: 0.3 J UV-B/cm²) for 1 hour at 4 or 24 hours after 5-ALA administration. Two animals of the 250 mg/kg group (one each within 48 and 72 hours after irradiation) and 5 animals of the 750 mg/kg group (all within 24 hours after irradiation) died after UV irradiation at 4 hours after 5-ALA HCl administration. The gross examination of the skin revealed mild edema in all animals of the 250 mg/kg group following UV irradiation at 4 hours after 5-ALA HCl administration, while no abnormality was found following UV irradiation at 24 hours after 5-ALA HCl administration. The gross examination could not be performed in animals following UV irradiation at 4 hours after administration of 5-ALA HCl at 750 mg/kg since all the animals died during the study. Very mild to mild erythema was observed in all animals following UV irradiation at 24 hours after 5-ALA HCl administration. Histopathological findings of the eyes and skin at the completion of study showed inflammatory reaction in the skin (shoulder), eyelid dermatitis, ulceration, and epithelial necrosis were found in several animals following UV irradiation at 4 hours after administration of 5-ALA HCl at 250 mg/kg, while no abnormality except bleeding was found in animals following UV irradiation at 24 hours after 5-ALA HCl administration. Dermatitis and epithelial necrosis were found only in 1 animal each following UV irradiation at 4 hours after administration of 5-ALA HCl at 750 mg/kg, while inflammatory reaction in the skin (shoulder), ulceration, epithelial necrosis, and bleeding were found in several animals following UV irradiation at 24 hours after administration of 5-ALA HCl at 750 mg/kg. The findings for the group which underwent UV irradiation at 4 hours after 5-ALA HCl administration were based on the samples from dead animals because they had all died during the study. For this reason, no comparison between different doses or irradiation timings was possible.

As described above, more severe phototoxic reactions (death, skin erythema, inflammatory reaction) occurred in animals following UV irradiation at 4 hours after intravenous administration of 5-ALA HCl

in comparison with those at 24 hours after 5-ALA HCl administration, showing that the phototoxicity was time-dependent. These reactions were determined to be associated with PPIX production kinetics.

3.(iii).B Outline of the review by PMDA

3.(iii).B.(1) Combination with anesthetics

PMDA asked the applicant to explain whether 5-ALA HCl may not be of toxicological concern in combination with anesthetics, considering that 5-ALA HCl is to be coadministered with anesthetics. PMDA also asked the applicant to explain whether there are any drugs requiring caution for the combination use, except anesthetics.

The applicant responded as follows:

Anesthesia-related drugs used in a Japanese phase III study (Study NPC-07-1) were remifentanyl for (intravenous infusion), sevoflurane (inhaled anesthetic agent), propofol (emulsion for injection), fentanyl (solution for injection), and droperidol (solution for injection). In terms of pharmacological characteristics, the above-mentioned drugs used for anesthesia in the study act on the central nervous system, respiratory system, cardiovascular system and muscles. Adverse drug reactions reported in the clinical study were generally events related to such sites of action. These drugs are pharmacokinetically eliminated relatively earlier and many of them have short anesthetic duration. Anesthetics used in the Japanese phase III study, unlike barbiturates, were not confirmed to induce 5-ALA synthase, thereby enhancing heme biosynthesis. Therefore, there is little concern about toxicity due to interaction between 5-ALA HCl and the anesthesia-related drugs used in the study. It has been reported that 5-ALA synthase induced by barbiturate including thiopental may exacerbate acute intermittent porphyria, resulting in crises (*the guideline on use of anesthetics and the analogous drugs. the third edition. Japanese Society of Anesthesiologists, 2009*). On the other hand, in the repeat dose toxicity studies, the major target organ was the liver and bile duct changes were particularly seen. This would be because 5-ALA is converted into PPIX during heme synthesis and then PPIX accumulates dominantly in the liver, causing disorder. Increases in γ -GTP, ALT and AST are also found in humans, which raises concern about the effect on the liver. 5-ALA HCl is contraindicated in patients with porphyria because the 5-ALA HCl treatment may cause liver disorder in patients. In contrast, barbiturate anesthetics are to be listed in the "Precautions for Concomitant Use" section in the package insert of 5-ALA HCl because careful use of the anesthetics is desirable.

Besides barbiturate general anesthetics, the package insert of lidocaine describes precautions that the drug may induce acute symptoms in patients with porphyria. Lidocaine was used in 28 of 45 subjects in the Japanese phase III study (Study NPC-07-1). Lidocaine is a local anesthetic and not used for general anesthesia. There is little concern that lidocaine may have effects such as induction of 5-ALA synthase. Therefore, it is unnecessary to advise caution against the concomitant use with lidocaine in the package insert (draft) of 5-ALA HCl. Furthermore, induction of 5-ALA synthase or precautions for patients with porphyria is not described in the package insert of drugs other than lidocaine. It is unlikely that other

drugs is combined with 5-ALA HCl, like anesthetics, during surgical operation. Therefore, it is unnecessary to advise caution against the concomitant use with other drugs in the package insert (draft) of 5-ALA HCl.

PMDA considers as follows:

It is appropriate that the applicant advise caution against the combination with barbiturates. PMDA accepted the above responses by the applicant because, at the moment, there is no concern about interaction between 5-ALA HCl in a single dose and anesthetics used in the Japanese phase III study (Study NPC-07-1).

3.(iii).B.(2) Phototoxicity

In the phototoxicity study, death occurred more frequently in animals of the low-dose 5-ALA HCl group with UV irradiation in comparison with single-dose toxicity studies in mice in the presence of light. Based on the results, PMDA asked the applicant to explain whether acute toxicity of 5-ALA HCl may be exacerbated by light irradiation.

The applicant responded as follows:

No animal of the 250 mg/kg group died in the single-dose toxicity studies in mice performed in the dark, while in the phototoxicity study, animals of the 250 mg/kg group died following UV irradiation at 4 hours after 5-ALA HCl administration. Furthermore, all of 5 animals of the 750 mg/kg group died following UV irradiation at 4 hours post-dose. No animal died following UV irradiation at 24 hours after 5-ALA HCl administration in the phototoxicity study and skin erythema and inflammatory reaction were weaker than those following UV irradiation at 4 hours after 5-ALA HCl administration. These findings are considered to be attributable to exacerbated acute toxicity (systemic toxicity) of 5-ALA because PPIX, a metabolite of 5-ALA, was excited by light.

PMDA considers as follows:

Since the LD₅₀ was 949 to 1064 mg/kg in the single intravenous dose study in mice in the dark while the approximate lethal dose was 250 mg/kg in the phototoxicity study, it is clear that 5-ALA is phototoxic. Therefore, it is important to provide appropriate information concerning necessary time to prevent exposure to light. The appropriateness of advising caution will be discussed based on the safety in clinical use as well [see “4.(iii).B.(5).2) Photosensitivity”].

Based on the facts that the exposure of 5-ALA at the NOAEL determined in repeat dose toxicity studies were lower than the exposure at the clinical dose and that the major target organ for repeat dose toxicity studies was the liver, the effect on the liver, which is also a concern in clinical use, should be discussed from a clinical perspective [see “4.(iii).B.(5) Safety”].

4. Clinical data

4.(i) Summary of biopharmaceutic studies and associated analytical methods

4.(i).A Summary of the submitted data

Plasma 5-ALA concentrations were determined using a validated high performance liquid chromatography with fluorescence detection (HPLC-FL) after conversion of 5-ALA into 2,6-diacetyl-1,5-dimethyl-7-(2-carboxyethyl)-3H-pyrrolizine. The lower limit of quantification was 30.00 ng/mL. Plasma PPIX concentrations were assayed by validated HPLC-FL and the lower limit of quantification was 5.00 ng/mL.

Unless otherwise specified, pharmacokinetic parameters are presented as mean \pm standard deviation (SD).

4.(i).A.(1) Absolute bioavailability (Study MC-ALS.20/BV, 5.3.1.1-1 [Reference data])

Twelve healthy foreign adult male subjects received a single oral dose of 5-ALA HCl at 20 mg/kg in the fasted state, followed by a 2-day washout period, and then received a single intravenous dose of 5-ALA HCl at 2.0 mg/kg for 3 minutes. The absolute bioavailability calculated from the area under the plasma concentration-time curve to infinity (AUC_{∞}) was 100.02%.

4.(ii) Summary of clinical pharmacology studies

4.(ii).A Summary of the submitted data

4.(ii).A.(1) Protein binding (4.2.2.3-10)

5-ALA (0.5 and 5 μ g/mL [final concentration]) was added to human plasma (n = 3) and determined by ultrafiltration. The plasma protein binding of 5-ALA was 12%.

4.(ii).A.(2) Pharmacokinetics in Japanese subjects

4.(ii).A.(2).1 Patients with malignant glioma (Study NPC-07-1, 5.3.5.2-1)

Following a single oral dose of 5-ALA HCl at 20 mg/kg to 6 Japanese patients with malignant glioma, plasma 5-ALA concentrations showed t_{\max} of 0.83 ± 0.26 hours, C_{\max} of 34.009 ± 12.737 mg/L, AUC_{∞} of 77.086 ± 40.724 mg·h/L, and $t_{1/2}$ of 2.27 ± 2.35 hours, while plasma PPIX concentrations showed t_{\max} of 6.17 ± 0.98 hours, C_{\max} of 0.350 ± 0.098 mg/L, AUC_{∞} of 4.187 ± 1.373 mg·h/L, and $t_{1/2}$ of 4.91 ± 1.90 hours.

4.(ii).A.(3) Pharmacokinetics in foreign subjects

4.(ii).A.(3).1 Healthy foreign adults (Study MC-ALS.20/BV, 5.3.1.1-1 [Reference data])

Twelve healthy foreign adult subjects received a single oral dose of 5-ALA HCl at 20 mg/kg in the fasted state, followed by a 2-day washout period, and then received a single intravenous dose of 5-ALA HCl at 2.0 mg/kg for 3 minutes. Plasma 5-ALA concentrations after oral and intravenous administration showed median t_{\max} [min-max] of 0.76 [0.50-1.00] and 0.17 [0.15-0.17] hours, respectively, C_{\max} of 20.90 (1.25) and 6.12 (1.39) mg/L (geometric mean [geometric SD]), respectively, AUC_{∞} of 33.13 (1.26) and 3.31 (1.30) mg·h/L, respectively, $t_{1/2}$ of 0.92 (1.17) and 0.69 (1.36) hour, respectively, and the urinary

excretion rate of 5-ALA up to 12 hours post-dose (A_e) of $33.62 \pm 7.93\%$ and $35.40 \pm 12.84\%$, respectively. Following an oral dose of 5-ALA HCl at 20 mg/kg, plasma PPIX concentrations showed mean t_{max} [min-max] of 4.00 [2.50-8.00] hours, C_{max} of 0.279 (0.00136) mg/L, AUC_{∞} of 1.876 (0.00147) mg·h/L, and $t_{1/2}$ of 3.57 (1.82) hours.

4.(ii).A.(3).2 Patients with malignant glioma (Study MC-ALS.8-I/GLI, 5.3.5.1-1 [Reference data])

A single oral dose of 5-ALA HCl at 0.2, 2 or 20 mg/kg was administered to 21 foreign patients with malignant glioma (7 subjects/group). As a result, plasma 5-ALA concentrations showed median t_{max} [min-max] of 0.50 [0.23-1.00] hours in the 0.2 mg/kg group, 0.50 [0.25-1.47] hours in the 2 mg/kg group, and 1.00 [0.52-2.00] hours in the 20 mg/kg group; C_{max} of 0.257 [1.20] (geometric mean [geometric SD]), 2.104 [1.57] and 8.272 [1.11] mg/L, respectively; AUC_{∞} of 0.540 [1.98], 3.326 [1.60] and 26.915 [1.19] mg·h/L, respectively; and $t_{1/2}$ of 0.85 [1.71], 1.12 [2.00] and 3.05 [2.09] hours, respectively. In the 2 and 20 mg/kg groups, plasma PPIX concentrations showed mean t_{max} [min-max] of 4.92 [2.90-6.92] hours in the 2 mg/kg groups and 4.97 [2.97-6.95] hours in the 20 mg/kg groups; C_{max} of 0.0323 [0.00228] and 0.128 [0.00227] mg/L, respectively; AUC_{∞} of 0.256 [0.00246] and 0.780 [0.00273] mg·h/L, respectively; and $t_{1/2}$ of 2.90 [1.36] and 2.61 [1.63] hours, respectively. In the 20 mg/kg group, the parameters in 1 subject were below the lower limit of quantification at all time points, and the plasma concentration of another subject was polymodal with no data for $t_{1/2}$. Consequently, pharmacokinetic parameters of the 20 mg/kg group were calculated from the results of the remaining 5 subjects.

4.(ii).A.(4) Pharmacodynamic evaluation (Study MC-ALS.20/BV, 5.3.1.1-1 [Reference data])

Following a single oral dose of 5-ALA HCl at 20 mg/kg to 21 healthy foreign adult male subjects, plasma PPIX concentrations and minimum erythema dose (MED) at sites of UV irradiation were determined to examine skin photosensitization after administration of 5-ALA HCl. Following UV irradiation (irradiation: 5-56 J/cm², light intensity: approx. 60 mW/cm², wavelength: 330-450 nm) at 8 stages in the back and hip before and 12, 24 and 48 hours after administration of 5-ALA HCl, immediate and delayed reactions in the skin were observed 16 minutes and 24 hours, respectively, after UV irradiation. MED of immediate reaction before and 12, 24 and 48 hours after administration was 18.19 ± 4.38 , 7.38 ± 3.41 , 8.52 ± 3.39 and 17.33 ± 5.49 J/cm², respectively, and MED of delayed reaction was 23.81 ± 7.59 , 6.05 ± 2.22 , 21.71 ± 7.16 and 28.00 ± 12.87 J/cm², respectively. Plasma PPIX concentrations at 12 and 24 hours after administration of 5-ALA HCl was 104.44 and 10.12 µg/L, respectively, and plasma PPIX concentrations before and 48 hours after administration were below the lower limit of quantification.

4.(ii).B Outline of the review by PMDA

4.(ii).B.(1) Timing of 5-ALA HCl administration

The applicant explained the timing of 5-ALA HCl administration as follows:

Since plasma PPIX concentrations of 5-ALA in the Japanese phase III study (Study NPC-07-1) showed t_{max} of 6.17 ± 0.98 hours and $t_{1/2}$ of 4.91 ± 1.90 hours, plasma PPIX concentrations after administration

of 5-ALA HCl 3 hours prior to induction of anesthesia were expected to reach the peak approximately 3 hours after induction of anesthesia and sufficient fluorescence of PPIX was assumed to be obtained in surgical resection (5-10 hours after administration of 5-ALA HCl). In Addition, it has been confirmed that tumor tissues emit stable fluorescence for 3 to 12 hours after administration of 5-ALA HCl (*Japanese journal of clinical medicine*. 2005;63:380-3) and the proposed dosage regimen of 5-ALA HCl is also supported by clinical experiences.

PMDA considers as follows:

The results of nonclinical pharmacokinetic studies suggested that 5-ALA is rapidly distributed throughout the body including the brain after oral administration and it can be deduced therefore that changes in plasma PPIX concentrations after administration of 5-ALA HCl reflect PPIX distribution in the brain. Thus, t_{max} and $t_{1/2}$ of plasma PPIX support the appropriateness of the timing of 5-ALA HCl administration. Since it has been reported that PPIX accumulates in brain tumor tissues more frequently than in normal brain tissues after plasma 5-ALA reached t_{max} [see “3.(ii).A.(2) Distribution”] and that plasma 5-ALA reached t_{max} approximately 1 hour post-dose in Japanese patients with malignant glioma, administration of 5-ALA HCl 3 hours prior to induction of anesthesia can be supported. The appropriateness of the dosage and administration of 5-ALA HCl will be discussed in the latter part based on clinical study results [see “4.(iii).B.(7) Dosage and Administration”].

4.(ii).B.(2) Differences in pharmacokinetics between Japanese and foreign patients

Since the applicant explained the efficacy and safety of 5-ALA HCl in Japanese patients with malignant glioma using the results of foreign phase II and III studies submitted for this application, PMDA reviewed whether or not such submission is justified considering the differences in pharmacokinetics between Japanese and foreign patients.

The applicant explained the differences in pharmacokinetics as follows:

Pharmacokinetic parameters and changes in plasma concentrations of 5-ALA and PPIX were compared among 3 Japanese and foreign studies (Studies NPC-07-1, MC-ALS.20/BV, and MC-ALS.8-I/GLI) in which plasma pharmacokinetics after a single oral dose of 5-ALA HCl at 20 mg/kg was investigated. C_{max} and AUC_{∞} of 5-ALA and PPIX were highest in Study NPC-07-1 in Japanese patients, followed by those in Study MC-ALS.20/BV in foreign healthy adults and Study MC-ALS.8-I/GLI in foreign patients in order. This may be because a difference in the blood sample treatment may have affected the results. There was concern about how the samples were treated (i.e., in the presence or absence of light). Plasma samples were stored in light-shielded polypropylene/polyethylene tubes in the NPC-07-1 and MC-ALS.20/BV studies because PPIX is easily photodegraded, but plasma samples were not stored in light-shielded tubes and samples were not confirmed to be kept on ice in the dark in the MC-ALS.8-I/GLI study conducted in foreign patients with malignant glioma. When designing studies in Japan, the applicant planned to compare pharmacokinetic results between Japanese and foreign patients, however, taking account of the situation above, the applicant considered that it was appropriate to compare the

results of studies in Japanese patients with those of the study (Study MC-ALS.20/BV) in foreign healthy adults in which samples had been treated properly compared to other studies. Changes in plasma 5-ALA and PPIX concentrations in foreign healthy adults showed similar patterns to those in the Japanese phase III study (Study NPC-07-1). To be specific, 5-ALA was rapidly absorbed in foreign healthy adults similarly to that in Japanese patients, and then plasma 5-ALA concentrations increased. Subsequently, plasma PPIX concentrations also increased several hours later. Thus, the applicant considered that pharmacokinetics obtained in the Japanese clinical study were similar to those in the foreign clinical study conducted in healthy adults.

PMDA considers as follows:

Given the applicant's explanation that there had been problems in the treatment of samples requiring light protection in the foreign clinical study in patients with malignant glioma, resulting in lower plasma PPIX concentrations, it is difficult to evaluate the differences in pharmacokinetics between Japanese and foreign patients with malignant glioma by using the study results presented by the applicant. However, the changes in plasma PPIX concentrations in Japanese patients with malignant glioma were similar to those in foreign healthy adults and the results of nonclinical pharmacokinetic study suggested that 5-ALA was rapidly distributed throughout the body including the brain after oral administration. Thus, it can be deduced that changes in plasma PPIX concentrations after administration of 5-ALA HCl reflect the distribution of PPIX in the brain. Based on the findings above, the difference in plasma 5-ALA concentrations between Japanese and foreign subjects is not problematic from the standpoint of efficacy. On the other hand, from the standpoint of safety, it should be noted that the time course of plasma 5-ALA concentrations in Japanese patients remained higher than that in healthy foreign adults. However, based on the facts that 5-ALA HCl is to be administered in a single dose in clinical practice in principle and that the acute toxicity of 5-ALA suggests no problem in clinical use, PMDA has concluded that the differences in plasma 5-ALA concentrations between Japanese and foreign subjects are unlikely to have effects on safety of 5-ALA HCl.

Based on the above, PMDA considers that there is no problem, from a pharmacokinetic point of view, in using the foreign clinical study results to explain the efficacy of 5-ALA HCl in Japanese patients with malignant glioma.

4.(iii) Summary of clinical efficacy and safety

4.(iii).A *Summary of the submitted data*

As the evaluation data, the results from 1 Japanese phase III study were submitted for this application. The results from 6 foreign clinical studies were also submitted as the reference data. The results from the main clinical studies are as follows.

4.(iii).A.(1) Japanese studies

4.(iii).A.(1).1 Phase III study (5.3.5.2-1, Study NPC-07-1, from September 2010 to December 2011)

An open-label, uncontrolled study was conducted at 10 medical institutions in Japan to evaluate the efficacy and safety of 5-ALA HCl orally administered at a single dose of 20 mg/kg to patients with primary or recurrent malignant glioma (WHO grade III/IV) (target sample size of 36 [Step I, 8; Step II, 28]).

5-ALA HCl (1.5 g) was dissolved in water (50 mL) and administered at 3 hours (range, 2-4 hours) prior to induction of anesthesia for surgery. Investigators microscopically confirmed that tumor emitted fluorescence upon excitation with blue light ($\lambda = 400\text{-}410$ nm) prior to tumor resection, and the tumors were resected under white light in a similar manner to usual brain tumor resection. After that, the presence or absence of residual tumors was confirmed following excitation with blue light ($\lambda = 400\text{-}410$ nm) again, and tumors were resected to evaluate the diagnostic performance of 5-ALA HCl. The collection of biopsy tissue samples was performed as follows: Tissues were collected from a total of ≤ 6 sites per subject, i.e., ≤ 3 sites each at the strongly fluorescing areas (strong fluorescence) and at the weakly fluorescing areas (weak fluorescence), which were fluorescent under excitation light conditions after tumor resection. Furthermore, tissues were collected from 2 sites each per subject at the fluorescence-adjacent areas (non-fluorescence) and areas distant from a tumor (non-fluorescence), unless additional sample collection puts the subject at risk. To examine the presence or absence of malignant glioma cells in the tissues collected, sample analysis was performed by the pathology department of each study site under blinded conditions. For preoperative diagnostic imaging of brain tumors and postoperative detection of residual tumors, magnetic resonance imaging (MRI) was performed before administration of 5-ALA HCl and within 72 hours after surgical operation. After confirmation of the safety and pharmacokinetics in the small number of subjects as Step I, the study proceeded to Step II. The time to avoid strong light sources after administration of 5-ALA HCl was 24 hours.

The main inclusion criteria were patients aged 18-70 years who were estimated as primary or recurrent malignant glioma (WHO grade III or IV) by radiological diagnosis and for whom surgical resection was indicated. Since all of 45 subjects (Step I, 10; Step II, 35) enrolled in the study were given 5-ALA HCl, they were all included in the safety analysis set. Of those subjects in the safety analysis set, 4 subjects were excluded from the full analysis set (FAS) because they did not fall under the WHO grade III/IV as a result of rapid intraoperative pathological diagnosis and 3 subjects were also excluded due to no fluorescence of tumor core. The other 38 subjects from whom the efficacy data was obtained were included in the FAS, which was considered to be the primary analysis set for efficacy. From the safety analysis set, 2 subjects were excluded: one subject due to the study withdrawal requested by the subject and the other due to difficulties in continuing to participate in the clinical study. As a result, 43 subjects (Step I, 10; Step II, 33) completed the clinical study.

For the efficacy, the positive predictive rate of tissue fluorescence in fluorescent sites per subject, the primary endpoint for efficacy, is shown in Table 3. The lower limit of 95% confidence interval (CI) of

the positive predictive rate of tissue fluorescence was 48.6%, which did not exceed 53%, the pre-defined threshold limit value.

Table 3. Positive predictive rate of tissue fluorescence in fluorescent sites per subject (FAS)

Classification	Number of subjects ^a	Number of subjects showing positive tumor cell identification in all biopsies taken from areas of any fluorescence ^b	Positive predictive rate (%)	95% CI
Strong fluorescence	36	34	94.4	[81.3, 99.3]
Weak fluorescence	38	25	65.8	[48.6, 80.4]
Any fluorescence	38	25	65.8	[48.6, 80.4]

a: The subjects from whom ≥ 1 sample was collected were included in the analysis.

b: The number of subjects showing positive tumor cell identification in all biopsies (≤ 6 sites in total) taken from areas of weak fluorescence (≤ 3 sites) and of strong fluorescence (≤ 3 sites)

The secondary endpoints, positive predictive rate of tissue fluorescence per biopsy sample taken from fluorescent sites, distribution of complete resection rate, rate of tumor positive identification in biopsies taken from non-fluorescent areas, and positive predictive rate of tissue fluorescence in fluorescent sites per patient with primary or recurrent malignant glioma are shown in the table below.

Table 4. Positive predictive rate of tissue fluorescence per biopsy sample taken from fluorescent sites (FAS)

Classification	Number of tissue samples	Number of tissue samples identified as positive	Positive predictive rate (%)	95% CI
Strong fluorescence	108	102	94.4	[88.3, 97.9]
Weak fluorescence	114	88	77.2	[68.4, 84.5]
Any fluorescence	222	190	85.6	[80.3, 89.9]

Table 5. Distribution of complete resection rate (FAS)

Complete resection rate	Number of subjects (%)
100%	15 (39.5)
95%	12 (31.6)
90%	3 (7.9)
75%	6 (15.8)
50%	2 (5.3)
<50%	0 (0)

Number of subjects: 38

Table 6. Rate of tumor positive identification in biopsies taken from non-fluorescent areas (FAS)

Region	Number of tissue samples	Number of tissue samples identified as positive	Positive rate (%)	95% CI
Fluorescence-adjacent area	72	44	61.1	[48.9, 72.4]
Fluorescence-distant area	61	29	47.5	[34.6, 60.7]

Number of subjects: 38

Table 7. Positive predictive rate of tissue fluorescence in fluorescent sites per subject with primary or recurrent malignant glioma (FAS)

	Classification	Number of subjects ^a	Number of subjects showing positive tumor cell identification in all biopsies taken from areas of any fluorescence ^b	Positive predictive rate (%)	95% CI
Primary	Strong fluorescence	22	22	100	[84.6, 100]
	Weak fluorescence	22	14	63.6	[40.7, 82.8]
	Any fluorescence	22	14	63.6	[40.7, 82.8]
Recurrent	Strong fluorescence	14	12	85.7	[57.2, 98.2]
	Weak fluorescence	16	11	68.8	[41.3, 89.0]
	Any fluorescence	16	11	68.8	[41.3, 89.0]

a: The subjects from whom ≥ 1 sample was collected were included in the analysis.

b: The number of subjects showing positive tumor cell identification in all biopsies (≤ 6 sites in total) taken from areas of weak fluorescence (≤ 3 sites) and of strong fluorescence (≤ 3 sites)

For the safety, the incidence of adverse events were 93.3% (42 of 45 subjects) and adverse events occurring in ≥ 3 subjects during the observation period are shown in Table 8. One subject with septic shock and hepatic dysfunction resulted in death. Septic shock was not considered to be related to 5-ALA HCl while hepatic dysfunction was considered to be related to 5-ALA HCl. Other 8 serious adverse events (drug eruption, postoperative wound infection and hepatic dysfunction, bacterial meningitis and hydrocephalus, decreased platelet count, fever, and tumor hemorrhage) were reported in 6 subjects. Decreased platelet count (PLT: 34,000/ μ L), which was considered to be related to 5-ALA HCl, occurred 32 days after administration of 5-ALA HCl. The event improved by treatment 3 days after the onset of event, and resolved 7 days after the onset of event. Fever occurred 22 days after administration of 5-ALA HCl and was also considered to be related to 5-ALA HCl. The event resolved by treatment 14 days after the onset of event.

Table 8. Adverse events occurring in ≥ 3 subjects during the observation period (safety analysis set)

Adverse Events	Number of subjects with the event (incidence [%])
Nausea	14 (31.1)
Vomiting	14 (31.1)
Headache	12 (26.7)
Fever	9 (20.0)
Constipation	6 (13.3)
Hepatic dysfunction	5 (11.1)
Wound complication	5 (11.1)
Increased γ -glutamyltransferase	5 (11.1)
Eyelid edema	4 (8.9)
Diarrhea	4 (8.9)
Seizure	4 (8.9)
Anemia	3 (6.7)
Increased blood amylase	3 (6.7)
Increased C-reactive protein	3 (6.7)
Decreased lymphocyte count	3 (6.7)
Decreased neutrophil count	3 (6.7)
Decreased leukocyte count	3 (6.7)
Hypophagia	3 (6.7)
Back pain	3 (6.7)
Cerebral infarction	3 (6.7)
Hemiparesis	3 (6.7)
Insomnia	3 (6.7)
Restlessness	3 (6.7)
Pruritus	3 (6.7)

4.(iii).A.(2) Foreign studies

4.(iii).A.(2).1) Phase I/II studies (5.3.5.1-1, Study No. MC-ALS.8-I/GLI, 20 to 20, Reference data)

An randomized, double-blind, comparative study (target sample size of 7 subjects per group) was conducted at 1 foreign medical institution to evaluate the dose-response relationship of 5-ALA HCl orally administered at a single dose of 0.2, 2 or 20 mg/kg to patients with primary malignant glioma (WHO grade III/IV).

5-ALA HCl (1.5 g) was dissolved in water (50 mL) and administered 3 hours (range, 2.5-3.5 hours) prior to induction of anesthesia for surgery. Tumors were resected under white light and blue light excitation conditions ($\lambda = 380-440$ nm). After tumor resection that was as complete as possible, the investigators identified the tumor core of the resected tumor under white light. The extent of fluorescence in the tumor core was assessed at 4 levels (0/3, 1/3, 2/3, and 3/3); and the fluorescence quality was assessed in 3 stages, strong (strong fluorescence), weak (weak fluorescence), and none.

The main inclusion criteria were patients aged 18-75 years who were estimated as primary malignant glioma (WHO grade III or IV) by radiological diagnosis and for whom surgical tumor resection was indicated. A total of 21 subjects enrolled were randomized, all of them were given 5-ALA HCl and completed the clinical study. Consequently, the 21 subjects were included in the safety analysis set and were evaluable for efficacy.

For the efficacy, the primary endpoint for efficacy, the fluorescence extent and quality of the tumor core by dose are shown in Figure 1 and Figure 2. The extent of fluorescence increased and the fluorescence quality improved with dose ($p < 0.0001$, Jonckheere-Terpstra test, one-sided significance level 5%).

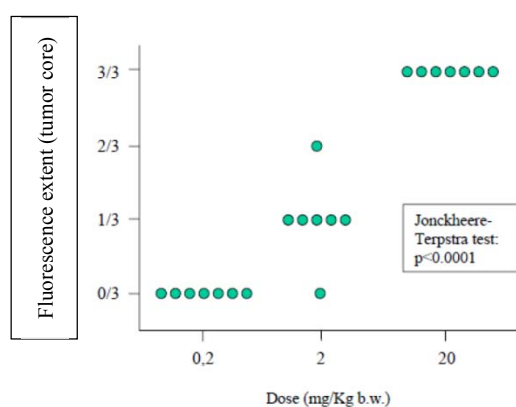


Figure 1. Fluorescence extent of the tumor core per dose (FAS)

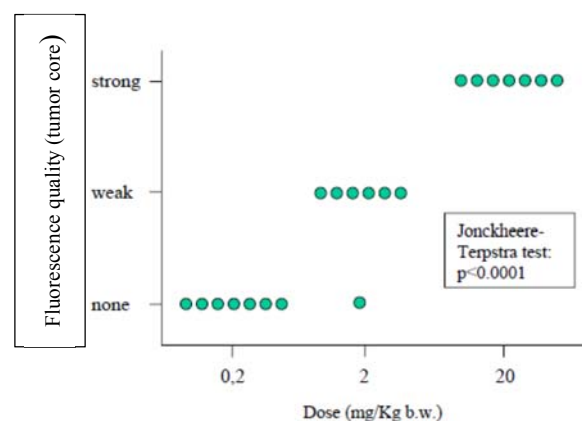


Figure 2. Fluorescence quality in the tumor core per dose (FAS)

For the safety, the incidence of adverse events were 57.1% (4 of 7 subjects) in the 0.2 mg/kg group, 57.1% (4 of 7 subjects) in the 2 mg/kg group, and 71.4% (5 of 7 subjects) in the 20 mg/kg group. Adverse

events occurring in ≥ 2 subjects of any group during the observation period are shown in Table 9. No death was observed. As serious adverse events, 2 events were reported (subgaleal hemorrhage and pneumonia) in 2 subjects of the 0.2 mg/kg group, 1 event (bleeding) in 1 subject of the 2 mg/kg group, and 2 events (meningitis and fever) in 1 subject.

Table 9. Adverse events occurring in ≥ 2 subjects in any group during the observation period (safety analysis set)

Adverse Events	0.2 mg/kg group	2 mg/kg group	20 mg/kg group
Fever/infection/cold symptom	2 (28.6)	4 (57.1)	3 (42.9)
Hypertension	1 (14.3)	2 (28.6)	3 (42.9)
Nausea	1 (14.3)	2 (28.6)	0 (0)
Hypotension	2 (28.6)	1 (14.3)	0 (0)
Headache	0 (0)	2 (28.6)	1 (14.3)
Language disorder	2 (28.6)	0 (0)	0 (0)
Performance status	2 (28.6)	0 (0)	1 (14.3)

The figure presents the number of subjects (%).

4.(iii).A.(2).2) Phase II study (5.3.5.2-2, Study No. MC-ALS.28/GLI, 20 to 20, Reference data)

An open-label, uncontrolled study was conducted at 4 foreign medical institutions to evaluate the efficacy and safety of 5-ALA HCl orally administered at a single dose of 20 mg/kg to patients with primary malignant glioma (WHO grade III/IV) (target sample size of 33 subjects).

5-ALA HCl (1.5 g) was dissolved in water (50 mL) and administered 3 hours (range, 2.5-3.5 hours) prior to induction of anesthesia for surgery. After tumor resection under white light, the following procedures were performed under blue light excitation conditions ($\lambda = 380-440$ nm): Investigators selected 3 sites each at the areas of strong fluorescence and of weak fluorescence, from which tissue samples were collected. The time to avoid exposure to strong light sources after administration of 5-ALA HCl was 48 hours.

The main inclusion criteria were patients aged 18-75 years who were estimated as primary malignant glioma (WHO grade III or IV) by radiological diagnosis; for whom surgical tumorresection was indicated; and who did not undergo pretreatment of tumor. Of 39 enrolled subjects, 36 subjects were included in the safety analysis set, and 3 subjects were excluded because they were not given the investigational product. Of 36 subjects included in the safety analysis set, 2 subjects were excluded from the FAS because their histological findings did not meet the inclusion criteria and 1 subject was also excluded from the FAS because surgical operation was not performed due to inability to intubate. The other 33 subjects were included in the FAS for efficacy because the efficacy (primary endpoint) data were obtained.

For the efficacy, the positive predictive rate of tissue fluorescence in fluorescent sites per subject, the primary endpoint for efficacy, is shown in Table 10.

Table 10. Positive predictive rate of tissue fluorescence in fluorescent sites per subject (FAS)

Fluorescence quality	Number of subjects	Number of positive biopsy samples ^a	Positive predictive rate (%)	90% CI
Strong fluorescence	32	32	100	[91.1, 100]
Weak fluorescence	30	25	83.3	[68.1, 93.2]
Any fluorescence	33	28	84.8	[70.7, 93.8]

a: The number of patients showing positive tumor cell identification in all biopsies

For the safety, the incidence of adverse events were 69.4% (25 of 36 subjects). Adverse events occurring in ≥ 3 subjects during the observation period are shown in Table 11. One subject who developed bilateral medullary infarction resulted in death, however, bilateral medullary infarction reported was unlikely to be related to 5-ALA HCl. Other 11 serious adverse events (convulsion [3]; aphasia and cerebral infarction [2 each]; hemiparesis, hypotension, postoperative infection, and aspiration pneumonia [1 each]) were reported in 8 subjects. Hypotension, which was considered to be related to 5-ALA HCl, occurred 1 to 2 hours after administration of 5-ALA HCl, and resolved by treatment.

Table 11. Adverse events occurring in ≥ 3 subjects during the observation period (safety analysis set)

Adverse Events	Number of subjects with the event (incidence [%])
Nerve disorder–motor	7 (19.4)
Language disorder	7 (19.4)
Visual disturbance	7 (19.4)
Fever	6 (16.7)
Vomiting	4 (11.1)
Nerve disorder–sensory	4 (11.1)
Nerve disorder–headache	4 (11.1)
Nausea	3 (8.3)
Nerve disorder–cerebrocortical	3 (8.3)

4.(iii).A.(2).3) Phase III study (5.3.5.1-2, Study No. MC-ALS.3/GLI, October 1999 to July 2004, Reference data)

A randomized, rater-blinded, comparative study was conducted at 19 foreign medical institutions to compare the efficacy and the safety of fluorescence-guided resection for malignant glioma after a single oral dose of 5-ALA HCl at 20 mg/kg with that of conventional white light resection in patients with primary malignant glioma (WHO grade III/IV) (target sample size of 270).

Subjects underwent MRI during the period of 1 to 14 days before operation. 5-ALA HCl (1.5 g) was dissolved in water (50 mL) and administered to the subjects of the 5-ALA HCl group 3 hours (range, 2-4 hours) prior to induction of anesthesia for surgery. Tumors were resected under white light or blue light excitation conditions ($\lambda = 380-440$ nm). Subjects of the both groups underwent MRI scans within 72 hours after operation. MRI images were evaluated by a central reference radiologist who was blinded to the randomization of the subjects to one of the two treatment groups. The time to avoid exposure to strong light sources after administration of 5-ALA HCl was 24 hours.

The main inclusion criteria were patients aged 18-72 years who were estimated as primary malignant glioma (WHO grade III or IV) with a single lesion by radiological diagnosis; for whom surgical tumor

resection was indicated and complete resection was considered to be possible; and who did not undergo pretreatment of tumor.

In this study, interim analysis was to be performed when 270 subjects were included in the FAS. In the interim analysis, if the percentage of subjects without residual tumor on postoperative MRI, the first primary endpoint, was significantly different between the groups, then the 6-month progression-free survival, the second primary endpoint, was compared between the groups. If the significant difference (two-sided significance level, 0.022) between the groups was found for the second primary endpoint, 5-ALA HCl was determined to be effective and the study was discontinued. On the other hand, if the significant difference was not confirmed for the second primary endpoint, the enrollment of subjects were continued until 350 subjects were included in the FAS. The 6-month progression-free survival was compared between the groups (final analysis, two-sided significance level, 0.043).¹ The significance level was adjusted after the interim analysis using Wang-Tsiatis boundary ($\Delta = 0$, *Biometrics*. 1987;43:193-9).

At the time of the interim analysis, 322 subjects (161 subjects in the 5-ALA group and 161 subjects in the control group) were enrolled, and of them, 34 subjects (16 and 18 subjects, respectively) who did not meet the pathological diagnostic criteria, 13 subjects (5 and 8 subjects, respectively) who did not meet the radiological diagnostic criteria, 4 subjects (1 and 3 subjects, respectively) with consent withdrawal prior to surgery, and 1 other subject (control group) were excluded from the FAS. The other 270 subjects (139 in the 5-ALA group and 131 in the control group) were included in the FAS for efficacy. The percentage of subjects without residual tumor on postoperative MRI, the first primary endpoint, was 64.7% (90 of 139 subjects) in the 5-ALA group and 35.9% (47 of 131 subjects) in the control group, showing the significant difference between the groups (χ^2 test, $p < 0.0001$). The 6-month progression-free survival, the second primary endpoint, was 23.0% (32 of 139 subjects) in the 5-ALA group and 11.5% (15 of 131 subjects) in the control group, showing the significant difference between the groups (χ^2 test, $p = 0.0122$). As a result, the study was discontinued in accordance with the above-mentioned rules. Although the efficacy of 5-ALA HCl was confirmed in the interim analysis, the enrollment was continued during the period of data management for interim analysis and analysis, resulting in a total of 415 subjects enrolled. Final study results are shown below.

Of the 415 subjects enrolled (207 subjects in the 5-ALA group and 208 subjects in the control group), 41 subjects (21 and 20 subjects, respectively) who did not meet the pathological diagnostic criteria, 15 subjects (5 and 10 subjects, respectively) who did not meet the radiological diagnostic criteria, 5 subjects (2 and 3 subjects, respectively) with consent withdrawal prior to surgery, 3 subjects (2 and 1 subjects, respectively) who did not undergo tumor resection, and 2 other subjects (1 subject each) were excluded from the FAS, while 349 subjects (176 and 173 subjects, respectively) were included in the FAS for

¹ In the interim analysis, if the first primary endpoint was not significantly different between the groups, the hypothesis tested was determined not to be verified and the study was discontinued.

efficacy. Of the enrolled subjects, 374 subjects (201 subjects in the 5-ALA group and 173 subjects in the control group) were included in the safety analysis set, while 6 subjects who were not given the investigational product in the 5-ALA group, and 35 subjects who were not evaluable for efficacy in the control group, were excluded.

For the efficacy, the percentage of subjects without residual tumor on postoperative MRI, the first primary endpoint, was 63.6% (112 of 176 subjects) in the 5-ALA group and 37.6% (65 of 173 subjects) in the control group, showing the significant difference between the groups (χ^2 test, $p < 0.0001$). The 6-month progression-free survival, the second primary endpoint, was 20.5% (36 of 176 subjects) in the 5-ALA group and 11.0% (19 of 173 subjects) in the control group, showing the significant difference between the groups (χ^2 test, $p = 0.0152$; two-sided significance level, 0.022).

The progression-free survival was estimated using the Kaplan-Meier method and showed significant difference between the groups (log-rank test, $p = 0.0215$).

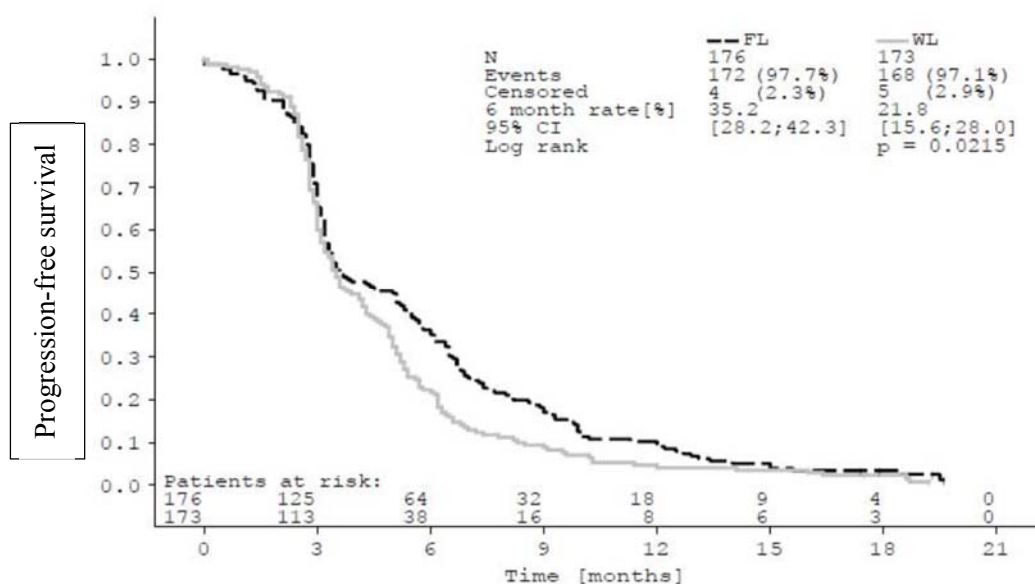


Figure 3. Progression-free survival
(Kaplan-Meier method, FAS; FL, 5-ALA group; WL, control group)

The median residual tumor volume on postoperative MRI (range), the secondary endpoint, was 0.0 cm³ (0.0-45.1 cm³) in the 5-ALA group and 0.5 cm³ (0.0-32.6 cm³) in the control group.

For the safety, the incidence of adverse events were 58.7% (118 of 201 subjects) in the 5-ALA group and 57.8% (100 of 173 subjects) in the control group. Adverse events occurring in $\geq 5\%$ of subjects in any group during the observation period are shown in Table 12. Death within 30 days after surgery was reported in 5 subjects of the 5-ALA group and 3 subjects of the control group. Of the 5 subjects of the 5-ALA group, 3 subjects were suspected to have pulmonary embolism, 1 subject had transtentorial

herniation due to cerebral edema following bilateral posterior cerebral artery infarction, and 1 other subject resulted in cardiac death (ventricular fibrillation). All of the 5 deaths were not considered to be related to the investigational product. For the 3 subjects of the control group, pulmonary embolism, sepsis and circulatory failure, and sudden cardiac death (pulmonary embolism suspected after death) occurred in 1 subject each. Serious adverse events were reported in 60 subjects of the 5-ALA group and 40 subjects of the control group. Serious adverse events occurring in ≥ 5 subjects in any group within 180 days after surgery included pulmonary embolism (in 13 subjects in the 5-ALA group and 2 subjects in the control group), convulsion (in 12 and 5 subjects, respectively), hemiparesis (in 8 and 4 subjects, respectively), aphasia (in 7 and 1 subjects, respectively), grand mal convulsion (in 7 and 5 subjects, respectively), and pneumonia (in 4 and 5 subjects, respectively).

Table 12. Adverse events occurring in $\geq 5\%$ of subjects in any group during the observation period (safety analysis set)

Adverse Events	5-ALA group (n = 201)	Control group (n = 173)
Language disorder	27 (13.4)	23 (13.3)
Sensory disturbance	27 (13.4)	13 (7.5)
Nerve disorder–motor	25 (12.4)	20 (11.6)
Personality change	17 (8.5)	9 (5.2)
Nerve disorder–headache	15 (7.5)	13 (7.5)
Ataxia	13 (6.5)	6 (3.5)
Nerve disorder–cerebrocortical	11 (5.5)	7 (4.0)
Seizure	11 (5.5)	10 (5.8)

The figure presents the number of subjects (%).

4.(iii).A.(2).4 Phase II study (5.3.5.2-3, Study No. MC-ALS.30/GLI, 20 to 20, Reference data)

An open-label, uncontrolled study was conducted at 4 foreign medical institutions to evaluate the efficacy and safety of 5-ALA HCl orally administered at a single dose of 20 mg/kg to patients with recurrent malignant glioma (WHO grade III or IV) (target sample size of 36 subjects).

5-ALA HCl (1.5 g) was dissolved in water (50 mL) and administered 3 hours (range, 2.5-3.5 hours) prior to induction of anesthesia for surgery. Tumors were resected under white light. Subsequently, under blue light excitation conditions ($\lambda = 380-440$ nm), 3 areas with pathological change noted under white light and 3 areas determined to be tumor margins were chosen. Tissues of strong and of weak fluorescence were collected from 1 site each at the areas. The time to avoid exposure to strong light sources after administration of 5-ALA HCl was 48 hours.

The main inclusion criteria were patients aged 18-75 years who were diagnosed with malignant glioma and underwent craniotomy, who are estimated as recurrent malignant glioma (WHO grade III or IV) by radiological diagnosis, and for whom surgical tumor resection was indicated. All of 40 subjects enrolled in the study were given 5-ALA HCl and were included in the safety analysis set. Of the 40 subjects, 4 subjects were excluded from the FAS for the following reasons: 2 subjects whose histological findings did not meet the inclusion criteria, 1 subject who did not undergo surgery prior to enrollment, and 1

subject due to microscope malfunction. Since the other 36 subjects underwent surgery and the efficacy (primary endpoint) data were obtained, all of them were included in the FAS for efficacy.

For the efficacy, the positive predictive rate of tissue fluorescence in fluorescent sites per subject and the tumor cell density per fluorescence quality (mean surface rate occupied with tumor cells *in situ*), both of which were the primary endpoints for efficacy, are shown in Tables 13 and 14.

Table 13. Positive predictive rate of tissue fluorescence in fluorescent sites per subject (FAS)

	Fluorescence quality	Number of subjects	Number of subjects determined to be positive ^a	Positive predictive rate (%)	95% CI
Tissues pathologically changed	Strong fluorescence	36	35	97.2	[85.5, 99.9]
	Weak fluorescence	34	34	100	[89.7, 100]
	Any fluorescence	36	35	97.2	[85.5, 99.9]
Tumor margin	Strong fluorescence	24	22	91.7	[73.0, 99.0]
	Weak fluorescence	34	28	82.4	[65.5, 93.2]
	Any fluorescence	34	27	79.4	[62.1, 91.3]
All tissues	Strong fluorescence	36	33	91.7	[77.5, 98.2]
	Weak fluorescence	36	30	83.3	[67.2, 93.6]
	Any fluorescence	36	28	77.8	[60.8, 89.9]

a: The number of patients showing positive tumor cell identification in all biopsies

Table 14. Tumor cell density by tumor sites and fluorescence quality (FAS)

Tumor site ^a (under white light)	Fluorescence quality	Histological findings ^b	Number of subjects	Tumor cell density (%) (Mean ± SD)
Tissue pathologically changed	Strong fluorescence	Vital, solid and proliferative tumor	36	48.5 ± 34.6
		Infiltrating tumor	36	29.2 ± 31.1
	Weak fluorescence	Vital, solid and proliferative tumor	34	34.7 ± 31.1
		Infiltrating tumor	34	38.8 ± 29.4
Tumor margin	Strong fluorescence	Vital, solid and proliferative tumor	24	17.1 ± 26.1
		Infiltrating tumor	24	56.5 ± 28.7
	Weak fluorescence	Vital, solid and proliferative tumor	34	25.9 ± 30.2
		Infiltrating tumor	34	43.3 ± 32.3

a: A tissue that is judged as tumor-positive even when it is not fluorescing is defined as the "tissue that was pathologically changed", and a site that cannot be classified as seemingly normal or tumor tissue when it is not fluorescing after resection of pathologically changed tissue is defined as the "tumor margin".

b: A proliferative tumor tissue that is vital (not necrotic) and solid tumor with high tumor cell density is defined as the "vital, solid and proliferative tumor", and a tissue with low tumor cell density that includes infiltrating tumor cells as the "infiltrating tumor".

The median overall survival until death or final observation day, the secondary endpoint, was 7.9 months (95% CI, [4.5, 13.2]). Tumor recurred in 20 of 36 subjects within 6 months.

For the safety, the incidence of adverse events were 55.0% (22 of 40 subjects). Adverse events occurring in ≥ 5 subjects during the observation period are shown in Table 15. Only one subject died of primary disease progression up to 28 days after surgery and no death due to adverse event was reported. Serious adverse events up to 28 days after surgery occurred in 4 subjects (hemiparesis in 2 subjects, subcutaneous retention of spinal fluid and hemiplegia in 1 subject, and fever and pneumonia in 1 subject).

Table 15. Adverse events occurring in ≥ 5 subjects during the observation period (safety analysis set)

Adverse Events	Number of subjects with the event (incidence [%])
Nerve disorder–motor	8 (20.0)
Language disorder	7 (17.5)
Subcutaneous retention of spinal fluid	6 (15.0)
Vision	5 (12.5)

4.(iii).A.(2).5) Phase III study (5.3.5.2-4, Study No. MC-ALS.32/GLI, 20 to 20, Reference data)

An open-label, uncontrolled study was conducted at 24 foreign medical institutions to evaluate the safety and efficacy of 5-ALA HCl orally administered at a single dose of 20 mg/kg to patients with primary malignant glioma (WHO grade III/IV) (target sample size of 160 subjects).

5-ALA HCl (1.5 g) was dissolved in water (50 mL) and administered 3 hours (range, 2-6 hours) prior to induction of anesthesia for surgery. Tumor sites were resected under white light and blue light excitation light conditions ($\lambda = 380-440$ nm). The time to avoid exposure to strong light sources after administration of 5-ALA HCl was 48 hours.

Of 245 enrolled subjects, 2 subjects who were not given the investigational product were excluded, and the other 243 subjects were included in the safety analysis set. Of the 243 subjects, 18 subjects were excluded from the FAS because they were not classified as WHO grade III/IV by histological examination and 6 subjects were also excluded due to recurrent malignant glioma, while the other 219 subjects were included in the FAS for efficacy.

For the efficacy endpoints, the Kaplan-Meier estimate of 12-month survival rate in all subjects was 59.2% (95% CI, [50.6, 66.8]) and the median survival was 14.7 months.

For the safety, the incidence of adverse events was 51.9% (126 of 243 subjects). Adverse events occurring in ≥ 5 subjects during the observation period are shown in Table 16. Death occurred in 3 subjects up to 30 days after surgery. Serious adverse events were reported in 49 subjects. Serious adverse events were reported in 24 subjects up to 48 hours after surgery. Of these, adverse events occurring in ≥ 2 subjects included hemiparesis in 11 subjects, aphasia and hemiplegia in 5 subjects each, and post-procedural bleeding in 3 subjects.

Table 16. Adverse events occurring in ≥ 5 subjects during the observation period (safety analysis set)

Adverse Events	Number of subjects with the event (incidence [%])
Hemiparesis	23 (9.5)
Aphasia	18 (7.4)
Post-procedural complication	9 (3.7)
post-procedural bleeding	9 (3.7)
Headache	8 (3.3)
Hemiplegia	8 (3.3)
Deep venous thrombosis	7 (2.9)
Thrombocytopenia	6 (2.5)
Seizure	5 (2.1)
Hemianopia	5 (2.1)

4.(iii).B Outline of the review by PMDA

4.(iii).B.(1) Clinical positioning

The applicant explained clinical positioning of 5-ALA HCl as follows:

Malignant glioma is a broad term referring to highly malignant tumor that is classified into glioma. It generally falls into the category of WHO grade III or IV, and is a poor-prognostic brain tumor. The standard treatment for malignant glioma is microscopic resection of tumor and the survey results of the Committee of Brain Tumor Registry of Japan indicates that the 5-year survival rate is improved if the rate of complete resection of malignant glioma is high (*Neurologia Medical-Chirurgia*. 2009;49:Supplement). Therefore, prognosis is improved by resecting tumor as completely as possible while preserving neurological functions. However, malignant glioma infiltrates into normal tissues and grows, consequently, the border between normal and tumor tissues is unclear. As a result, tumors requiring resection may not be able to be identified during surgery. Since residual tumors cause recurrence, an intraoperative procedure for differentiating tumor from normal tissues has been sought. Intraoperative diagnosis by means of MRI (intraoperative MRI) is performed as a useful procedure but currently available at limited medical institutions, indicating that intraoperative MRI is still not common in Japan. Furthermore, the surgery needs to include an about 1-hour interruption for MRI scans even though intraoperative MRI is available at the medical institution. In some cases, a patient is required to undergo MRI twice or three times during surgery, resulting in the prolonged duration of surgery, which is a problem.

On the other hand, 5-ALA is converted into PPIX characterized by emission of red fluorescence in cells after excitation with blue light (*J Photochem Photobiol B*. 1998;45:160-9). PBG deaminase activity involved in PPIX synthesis is higher in tumor cells than in normal cells while ferrochelatase activity related to conversion of PPIX to heme is lower in tumor cells than in normal cells, leading to accumulation of abundant PPIX in tumor cells. The first report on the clinical study of intraoperative diagnosis for malignant glioma using 5-ALA was published in 1998 (*Neurosurgery*. 1998;42:518-26). The results of several studies of intraoperative diagnosis of malignant glioma using 5-ALA marketed as a reagent have been reported also in Japan. The foreign phase II studies (Studies MC-ALS.28/GLI and MC-ALS.30/GLI) have confirmed that 5-ALA HCl has high diagnostic performance of malignant glioma (WHO grade III/IV). The foreign phase III study (Study MC-ALS.3/GLI) has confirmed superior

results of the rate of subjects without residual tumor and the 6-month progression free survival in the 5-ALA group to those in the control group (conventional white-light resection). In the Japanese phase III study (Study NPC-07-1), the positive predictive rate of tissue fluorescence in fluorescent sites per subject was 65.8%, particularly in areas of strong fluorescence, the rates were 100% and 85.7% in patients with primary and recurrent malignant glioma, respectively. Consequently, tumor tissues can be specifically visualized by the use of 5-ALA HCl. Diagnosis with 5-ALA HCl has advantages of real-time identification of the presence or absence of residual tumors, which can reduce the frequency of intraoperative MRI requiring interruption of surgery also in medical institutions where intraoperative MRI is available.

Based on the above, the applicant considered that the clinical significance of photodynamic diagnosis with 5-ALA HCl is supported by its advantages outweighing those with conventional surgery under white light.

PMDA considers as follows:

The results of Japanese and foreign clinical studies suggested the efficacy of 5-ALA HCl in Japanese patients with malignant glioma [see “4.(iii).B.(3) Efficacy”]. Real-time identification of the presence or absence of residual tumor is expected to improve the rate of complete resection of malignant glioma and prognosis also in clinical practice in Japan. Therefore, it is meaningful to provide 5-ALA HCl in clinical practice in Japan.

4.(iii).B.(2) Use of foreign clinical studies

The applicant provided the following explanation about the appropriateness of the use of the results of the foreign clinical studies in addition to the Japanese phase III study (Study NPC-07-1) to evaluate the efficacy and safety of 5-ALA HCl:

The Committee of Brain Tumor Registry of Japan investigated the epidemiology of malignant glioma and reported that gliomas accounted for 25.8% of all primary brain tumors that developed during the period of 1984 to 2000. Of malignant gliomas, glioblastoma, anaplastic astrocytoma, and anaplastic oligodendroglioma accounted for 9.1%, 4.7%, and 0.2%, respectively, of primary brain tumors (*Neurologia Medical-Chirurgia*. 2009; 49:Supplement). In the US, the Central Brain Tumor Registry of the United States (CBTRUS 2012) reported that glioma accounted for 33.0% of primary brain tumor. Of malignant gliomas, glioblastoma, anaplastic astrocytoma, and anaplastic oligodendroglioma accounted for 16.3%, 2.0%, and 0.6%, respectively, of primary brain tumors (*CBTRUS Statistical Report: Primary Brain and Central Nervous System Tumors Diagnosed in the United States in 2004-2008*, Central Brain Tumor Registry of the United States). Although the incidence of glioblastoma in the US was slightly higher than that in Japan, the incidence of malignant glioma in Japan and the US did not substantially differ from each other. In both Japanese and foreign studies, central nervous system tumors were histopathologically classified based on the histogenesis (cell-derived) and cellular atypism (differentiation and malignancy) in accordance with the WHO classification. Therefore, no differences

in pathological diagnoses between Japan and other countries were considered to be found. Both Japanese and foreign guidelines for treatment of malignant glioma recommend that patients with primary brain tumor undergo tumor resection that is as complete as possible, followed by radiotherapy and chemotherapy as the postoperative treatment, and that patients with recurrent tumor also undergo surgical tumor resection first (*National Comprehensive Cancer Network [NCCN] Clinical Practice Guidelines in Oncology-v.1*. 2009, the Japan Neurosurgical Society and the Japanese Society of Pathology ed., *General rules for clinical and pathological studies on brain tumors*. 3rd edition. Kanehara & Co., Ltd; 2010:232-44). The results of Japanese and foreign clinical studies (Studies NPC-07-1 and MC-ALS.20/BV) showed no substantial differences in plasma 5-ALA and PPIX concentrations. Based on the above, the applicant considered that the results of foreign clinical studies can be used to evaluate the efficacy and safety of 5-ALA HCl in Japanese patients with malignant glioma.

Taking account of the explanation of the applicant, PMDA has concluded that there is no difference in intrinsic or extrinsic ethnic factors that may rule out the use of foreign clinical study results to evaluate the efficacy and safety of 5-ALA HCl.

4.(iii).B.(3) Efficacy

4.(iii).B.(3).1) Efficacy of 5-ALA HCl in Japanese clinical studies

(a) Study design

The Japanese phase III study (Study NPC-07-1) was an open-label, uncontrolled study in a small number of subjects and the applicant explained the study design as follows:

The Japanese phase III study (Study NPC-07-1) was conducted in subjects with primary and recurrent malignant glioma (WHO grade III/IV) as an uncontrolled study to evaluate the positive predictive rate of tissue fluorescence (the percentage of subjects with positive tumor cell identification in all biopsies taken from areas of any fluorescence) after single oral administration of 5-ALA HCl at 20 mg/kg, an approved dose in foreign countries. The dosage regimen employed in the Japanese study was based on the following reports: Adverse drug reactions occurred following oral dose of 5-ALA HCl at 30 to 60 mg/kg in photodynamic therapy of gastrointestinal cancer, familial adenomatous polyposis, and oral cancer (*Gut*. 1995;36:67-75, *Eur J Cancer*. 31A[7/8]. 1995:1160-5, *Cancer*. 1996;78:1374-83). In addition, the diagnostic performance of 5-ALA HCl was favorable and no safety problems were found following oral administration of 5-ALA HCl at 20 mg/kg to subjects with glioblastoma multiforme (*J Neurosurg*. 2000;93:1003-1013). Thus, it was considered that the investigation at doses of >20 mg/kg was unnecessary in the foreign phase I/II study (Study MC-ALS.8-I/GLI). Following oral administration of 5-ALA HCl at 0.2, 2 and 20 mg/kg in the foreign phase I/II study (Study MC-ALS.8-I/GLI), the extent and quality of fluorescence were improved with increasing doses at tumor sites, suggesting that the maximum dose of 20 mg/kg was confirmed to be the most effective and adequate. Also in Japan, intraoperative diagnosis of malignant glioma is performed using 5-ALA HCl that is available on the market as a reagent, and several studies conducted using 5-ALA HCl at 20 mg/kg, an approved dose in foreign countries, have been reported.

PMDA considers as follows:

Based on foreign clinical study results showing the efficacy and safety of 5-ALA HCl at 20 mg/kg in patients with malignant glioma (WHO grade III/IV) and on the fact that Japanese clinical study was conducted at 20 mg/kg, an approved dose in foreign countries, it is appropriate to design a study to evaluate the efficacy of 5-ALA HCl at 20 mg/kg in Japanese patients with malignant glioma (WHO grade III/IV). Furthermore, in order to evaluate a diagnostic agent that is intended to identify tumor lesions, it is important to confirm that target sites are appropriately detected by the diagnostic agent while untargeted sites are not detected. However, it is difficult to specify in protocol that biopsies be collected from non-fluorescent areas in all subjects because such areas are more likely to include normal brain tissues compared to fluorescent areas. The significance of the use of 5-ALA HCl in surgical resection of malignant glioma lies in that tumor resection is as complete as possible. If high tumor detection rate is secured in fluorescent sites, the use of 5-ALA HCl is clinically helpful. Therefore, it is acceptable to choose the positive predictive rate of tissue fluorescence in fluorescent sites as the primary endpoint. Given that the foreign phase III study (Study MC-ALS.3/GLI) suggested that prognoses were improved by surgical resection of malignant glioma using 5-ALA HCl, that photodynamic diagnosis with 5-ALA is performed in clinical settings in Japan, and that there is no approved drug or medical device that allows real-time visualization of malignant glioma in the surgical field, it is difficult to conduct a comparative study of 5-ALA HCl in patients in Japan. Furthermore, taking into account that there is only a limited number of patients with malignant glioma for whom 5-ALA HCl is indicated, it was inevitable to conduct an uncontrolled study in patients with malignant glioma although the number of patients enrolled was small.

(b) Efficacy of 5-ALA HCl in Japanese Phase III study

In Japanese phase III study (Study NPC-07-1), the lower limit of 95% confidence interval of the positive predictive rate of tissue fluorescence in fluorescent sites per subject as the primary endpoint was below 53%, the threshold limit value set when the study was designed. Therefore, PMDA asked the applicant to explain the reason why the lower limit of 95% confidence interval was below the threshold limit value and to explain whether the results of the relevant study showed clinical significance of 5-ALA HCl taking the rationale for the threshold limit value into consideration.

The applicant explained as follows:

In Japanese and foreign clinical studies, ≤ 3 biopsy samples each from areas of strong and of weak fluorescence, a total of 6 biopsy samples per subject were collected. The percentage of patients with positive tumor cell identification in all biopsies taken from areas of any fluorescence was defined as the positive predictive rate of tissue fluorescence in fluorescent sites per subject. However, 6 samples were not always collected from all subjects in the foreign clinical study. Therefore, the threshold limit value of the positive predictive rate of tissue fluorescence in fluorescent sites per subject for the Japanese phase III study (Study NPC-07-1) was set based on the positive predictive rate of tissue fluorescence

per biopsy sample taken from fluorescent sites in foreign clinical studies. The positive predictive rate of tissue fluorescence per biopsy sample taken from fluorescent sites was 96.2% in patients with primary malignant glioma in the foreign clinical study (Study MC-ALS.28/GLI) and 96.6% in patients with recurrent malignant glioma in the foreign clinical study (Study MC-ALS.30/GLI). Considering these values, the expected lowest positive predictive rate of tissue fluorescence per biopsy sample taken from fluorescent sites was set at 90%, expecting a positive predictive rate of $\geq 95\%$ in 1 sample. Furthermore, if 6 samples each are collected from all the subjects, the positive predictive rate of tissue fluorescence in fluorescent sites per subject is calculated as $(0.90)^6 = 0.531$. Therefore, the threshold limit value of the lower limit of 95% confidence interval for the positive predictive rate of tissue fluorescence was set at 53%.

The positive predictive rate of tissue fluorescence in fluorescent sites per subject in the FAS, the primary endpoint for the Japanese phase III study (Study NPC-07-1) was 65.8% (95% CI, [48.6, 80.4]), and 63.6% (95% CI, [40.7, 82.8]) in subjects with primary malignant glioma and 68.8% (95% CI, [41.3, 89.0]) in subjects with recurrent malignant glioma, showing that the lower limit of 95% CI was below 53% in both groups. The results were separately evaluated for areas of strong and of weak fluorescence, the positive predictive rate of tissue fluorescence in areas of strong fluorescence was 100% (95% CI, [84.6, 100.0]) in the subjects with primary malignant glioma and 85.7% (95% CI, [57.2, 98.2]) in subjects with recurrent malignant glioma, and the lower limit of 95% confidence interval of the primary malignant glioma group exceeded 73% ($0.90^3 = 0.729$), which was the positive predictive rate expected when 3 samples were collected from areas of strong fluorescence in each subject. For areas of weak fluorescence, the positive predictive rate was 63.6% (95% CI, [40.7, 82.8]) in the subjects with primary malignant glioma and 68.8% (95% CI, [41.3, 89.0]) in subjects with recurrent malignant glioma. As a result, in both groups, the lower limit of 95% confidence interval was below 73% ($0.90^3 = 0.729$), which was the positive predictive rate expected when 3 samples were collected from areas of weak fluorescence in each subject. Therefore, the lower limit of 95% confidence interval of the positive predictive rate of tissue fluorescence in fluorescent sites per subject, the primary endpoint for the Japanese phase III study (Study NPC-07-1), was below 53% because the positive predictive rate of tissue fluorescence in fluorescent sites per subject and its origin, the positive predictive rate of tissue fluorescence per biopsy sample taken from areas of weak fluorescence were lower than expected.

In areas of strong fluorescence, tumor tissues can be specifically visualized at almost 100% of the positive predictive rate. On the other hand, normal cells are scattered in areas of weak fluorescence. The smaller number of tumor cells is considered to yield weaker fluorescence, resulting in lower positive predictive rate than that in areas of strong fluorescence. However, since not a few tumor cells are detected even in areas of weak fluorescence, it would be possible to confidently resect tumors also in the tumor margins, where resection cannot be determined confidently under conventional white light, as long as there is no risk of neurological dysfunction resulting from the tumor resection in the area. It has been reported that the purpose of treatment for malignant glioma is to suppress tumor regrowth and that

it is important to reduce residual tumor cells to the lowest extent possible through surgical tumor resection, followed by postoperative radiotherapy and/or chemotherapy (*Brain tumor surgery*. 14-18. Medicus Shuppan Publishers; 2006). The secondary endpoint for the Japanese phase III study (Study NPC-07-1), the positive predictive rate of tissue fluorescence per biopsy sample taken from fluorescent sites, was 77.2% in areas of weak fluorescence, therefore, 5-ALA HCl has clinical significance because 5-ALA HCl allows residual tumor cells to be visualized and resected. Based on the above, the results of clinical studies indicate the clinical significance of 5-ALA HCl.

Given that the positive predictive rate of tissue fluorescence per subject in areas of weak fluorescence in the Japanese phase III study (Study NPC-07-1) was lower than initially expected based on the results of foreign clinical studies, PMDA asked the applicant to explain the differences between Japanese and foreign studies in terms of the laboratory equipment, surgical procedures for brain tumor resection, and criteria for choosing areas of strong and of weak fluorescence.

The applicant responded as follows:

In Japanese and foreign clinical studies (Studies NPC-07-1, MC-ALS.28/GLI, and MC-ALS.30/GLI), there was no significant difference in the performance of light source and filter used.

With regard to the surgical procedure for brain tumor resection, the Japanese phase III study (Study NPC-07-1) and foreign clinical studies (Studies MC-ALS.28/GLI and MC-ALS.30/GLI) did not specify the extent of surgical tumor resection under white light, which was performed prior to fluorescence-guided resection. Therefore, there is the possibility that the extent of surgical tumor resection under white light may have differed depending on the surgeons' subjective decision including their experience, resulting in the differences in the extent of residual tumors and sites collected at areas of weak fluorescence. As a result, the positive predictive rate of tissue fluorescence per biopsy sample taken from areas of weak fluorescence in the Japanese phase III study may have been reduced. No residual tumor² was found in 15 of 38 subjects (39.5%) in the Japanese phase III study and in 21 of 69 subjects (30.4%) in the foreign phase II studies (combined result from Studies MC-ALS.28/GLI and MC-ALS.30/GLI). Among the above-mentioned subjects, biopsy samples not containing positive tumor cells were obtained from 6 of 15 subjects (40.0%) in the Japanese phase III study and from 2 of 21 subjects (9.5%) in the foreign phase II studies. Furthermore, all biopsy samples without positive tumor cells were collected from areas of weak fluorescence. These results suggested that biopsy samples were collected from margins with low tumor cell density more frequently in the Japanese phase III study than those in foreign clinical studies, which may have resulted in lower positive predictive rates of tissue fluorescence per biopsy sample taken from areas of weak fluorescence in the Japanese phase III studies than those in foreign clinical studies.

² Subjects defined as those with 100% tumor resection in the Japanese phase III study (Study NPC-07-1) (Table5)
The same definition applies hereinafter.

With regard to the criteria for areas of strong and weak fluorescence, surgeons subjectively chose areas of strong and weak fluorescence in the Japanese phase III study and the foreign phase II studies. In the foreign phase II study (Study MC-ALS.28/GLI), after choosing areas of strong and of weak fluorescence, the fluorescence intensity was measured by spectrometer and samples were collected. In the Japanese phase III study, photographs of fluorescent images after administration of 5-ALA HCl included in a published literature (*J Neurosurg.* 2000;93:1003-13) was presented to investigators and subinvestigators as reference for areas of strong and of weak fluorescence, but the quantitation of weak fluorescence and the extent of tumor resection prior to sample collection were not pre-defined. As a result, tumor cell density in collected biopsy samples differed and slightly more biopsy samples were determined to be tumor-negative in areas of weak fluorescence in the Japanese clinical study in comparison with those in foreign clinical studies.

As described above, the positive predictive rate of tissue fluorescence in areas of weak fluorescence in the Japanese phase III study was lower than initially expected because of the differences in tumor collecting sites between the Japanese and foreign clinical studies, including the finding about samples collected in areas with lower tumor cell density in the Japanese phase III study. Considering the reason for these differences, the diagnostic performance of 5-ALA HCl in the Japanese and foreign clinical studies is not substantially different from each other.

PMDA considers as follows:

As explained by the applicant, the lower limit of 95% confidence interval of the positive predictive rate in fluorescent sites per subject was below the threshold limit value mainly due to lower positive predictive rates of tissue fluorescence in areas of weak fluorescence than initially postulated for patients with primary and recurrent malignant glioma in the Japanese phase III study. However, the reason why the positive predictive rates of tissue fluorescence in areas of weak fluorescence were lower than those initially postulated is the applicant's speculation and remains unknown.

As for the surgical resection of malignant glioma, on the other hand, the most complete resection possible at tumor margins would be beneficial to patients with malignant glioma as far as the margins may include any tumor cells. The percentage of patients without residual tumor in the Japanese and foreign clinical studies was similar to each other. Judging from the incidences of nervous system disorder and serious nervous system disorder, no significant difference between the Japanese and foreign clinical studies in tumor resection-related effects on cranial nerve function was found, although wider areas of weak fluorescence may have been resected in the Japanese phase III study (Study NPC-07-1) than foreign clinical studies, as indicated by the applicant [see “4.(iii).B.(4).3) Effect on cranial nerve function associated with tumor resection”]. Based on the above findings, the usefulness of 5-ALA HCl for Japanese patients may not always be denied only because the lower limit of 95% confidence interval of the positive predictive rate of tissue fluorescence in fluorescent sites per subject was below the threshold limit value. The following sections will continuously address the review on whether or not the

usefulness of 5-ALA HCl for Japanese patients is expected to be similar to that for foreign patients based on the relevant study results.

4.(iii).B.(3).2 Efficacy of 5-ALA HCl in foreign clinical studies

The applicant explained the efficacy of 5-ALA HCl in foreign clinical studies as follows:

In the foreign phase II studies in patients with primary malignant glioma (Study MC-ALS.28/GLI) and in those with recurrent malignant glioma (Study MC-ALS.30/GLI), the positive predictive rate of tissue fluorescence in fluorescent sites per subject was evaluated. As a result, the positive predictive rate was high in both studies (Tables 10 and 13) and 5-ALA HCl was confirmed to visualize tumor tissues. In the foreign phase III study (Study MC-ALS.3/GLI) using the percentage of subjects without residual tumor on postoperative MRI and 6-month progression-free survival as the primary endpoints, both of the endpoints were significantly high in the 5-ALA group in comparison with those in the control group. Based on the above, it was considered that the rate of complete resection in the 5-ALA group was improved and visualization of malignant tumor tissue using 5-ALA HCl contributed to prognostic improvement.

PMDA considers as follows:

Based on the applicant's response, PMDA concluded that the positive predictive rate of tissue fluorescence and the rate of complete resection, which lead to prognostic improvement, were high in foreign clinical studies.

PMDA asked the applicant to discuss whether or not the same efficacy outcome can be expected in Japanese patients as that in the foreign phase III study (Study MC-ALS-3/GLI), which showed the improvement of prognosis after surgical tumor resection using 5-ALA HCl, taking account of the differences in patient demographics in the Japanese and foreign clinical studies evaluating the positive predictive rate of tissue fluorescence and results of those studies.

The applicant responded as follows:

The foreign phase III study (Study MC-ALS-3/GLI) that indicated the improvement of prognosis was conducted in patients with primary malignant glioma. The patient demographics between the Japanese and foreign clinical studies (Studies MC-ALS-3/GLI, MC-ALS.28/GLI, and NPC-07-1) were generally similar except patient distribution by WHO grade. The results of histopathological diagnosis showed that subjects diagnosed with WHO grade III were 2.3% (4 of 176 subjects) and those with WHO grade IV were 97.2% (171 of 176 subjects) in the foreign phase III study (Study MC-ALS.3/GLI) that evaluated the improvement of prognosis; the subjects diagnosed with WHO grade III and IV were 12.1% (4 of 33 subjects) and 87.9% (29 of 33 subjects), respectively, in the foreign phase II study (Study MC-ALS.28/GLI) that evaluated the positive predictive rate of tissue fluorescence; and the subjects diagnosed with WHO grade III and IV were 27.3% (6 of 22 subjects) and 68.2% (15 of 22 subjects), respectively, in the Japanese phase III study (Study NPC-07-1) in subjects with primary malignant

glioma. The number of subjects diagnosed with WHO grade III was smaller in the foreign clinical studies mentioned above than in the Japanese phase III study (Study NPC-07-1). To evaluate the effect of this difference on the positive predictive rate of tissue fluorescence, the positive predictive rate of tissue fluorescence per biopsy sample taken from fluorescent sites per WHO grade was compared in subjects with primary malignant glioma between the Japanese phase III study (Study NPC-07-1) and the foreign phase II study (Study MC-ALS.28/GLI) instead of the foreign phase III study (Study MC-ALS.3/GLI) in which the positive predictive rate of tissue fluorescence per biopsy sample taken from fluorescent sites was not evaluated. As a result, the positive predictive rate of tissue fluorescence in areas of strong fluorescence in subjects diagnosed with WHO grade III and IV were 100% in both two studies. For areas of weak fluorescence, the positive predictive rate of tissue fluorescence in subjects diagnosed with WHO grade III and IV were 100% (12 of 12 biopsy samples) and 91.0% (71 of 78 biopsy samples), respectively, in the foreign clinical study and 83.3% (15 of 18 biopsy samples) and 75.6% (34 of 45 biopsy samples), respectively, in the Japanese clinical study. Thus, the rates were slightly lower in areas of weak fluorescence than in areas of strong fluorescence and such a tendency was found particularly in the Japanese phase III study (Study NPC-07-1). However, as described in the section [see “4.(iii).B.(3).1.(b) “Efficacy of 5-ALA HCl in Japanese Phase III clinical study”], the diagnostic performance of 5-ALA HCl in the Japanese and foreign studies is not substantially different from each other. Based on the above, the applicant considered that the differences in WHO grade are unlikely to have effects on the positive predictive rate of tissue fluorescence. The percentage of subjects without residual tumor was 63.6% (112 of 176 subjects) in the foreign phase III study (Study MC-ALS.3/GLI) whose protocol specified the inclusion of patients in whom radical resection was possible. On the other hand, in the foreign phase II study (Study MC-ALS.28/GLI) and the Japanese phase III study (Study NPC-07-1) whose protocols did not provide the above specification, the percentage of subjects with primary malignant glioma without residual tumor was 42.4% (14 of 33 subjects) and 45.4% (10 of 22 subjects), respectively, which were similar. As described in the section [see 4.(iii).B.(2) Use of foreign clinical studies], given that there are no significant differences in medical environment between Japan and foreign countries, the improvement of prognosis of malignant glioma that underwent surgical resection using 5-ALA HCl, as shown in the foreign phase III study (Study MC-ALS.3/GLI), can be expected also for Japanese patients.

PMDA considers as follows:

Although the number of enrolled subjects classified by WHO grade was different between Japanese and foreign clinical studies, given the positive predictive rate of tissue fluorescence per biopsies by WHO grade, the difference of WHO grade did not significantly affect the evaluation of the positive predictive rate. Therefore, it is concluded that there were no problems in comparison of the results of clinical studies, based on the similarity of intrinsic and extrinsic ethnic factors between Japanese and foreign populations, which were explained by the applicant. In the Japanese phase III study (Study NPC-07-1), the positive predictive rate of tissue fluorescence in fluorescent areas was lower than initially expected based on the results from foreign clinical studies; however, the percentages of subjects without residual

tumor were similar. Considering also that differences in the positive predictive rate of tissue fluorescence by fluorescent site seen in the Japanese and foreign clinical studies had no impact on the rate of complete resection, the effect of 5-ALA HCl to improve prognosis in Japanese patients with malignant glioma can be predicted. Taking into account the applicant's explanation that the reason why slightly more biopsy samples were determined to be tumor-negative in areas of weak fluorescence in the Japanese phase III study (Study NPC-07-1) relative to foreign clinical studies may be because areas of strong or weak fluorescence had been chosen based on the surgeon's subjective determination, measures should be taken to provide surgeons in clinical practice with information on how to appropriately determine the extent of tumor resection using 5-ALA HCl. However, PMDA concluded that the effect of 5-ALA HCl to improve prognosis for malignant glioma shown in the foreign phase III study (Study MC-ALS.3/GLI) would be also expected in Japanese patients for the resection of malignant glioma using 5-ALA HCl, provided that the extent of resection in tumor margins is determined in the same way as that in the Japanese phase III study (Study NPC-07-1). As described above, PMDA has determined that surgical tumor resection using 5-ALA HCl is expected to be effective in Japanese patients with malignant glioma. The necessity of post-marketing survey of the improvement of prognosis in Japan and the measures for notifying surgeons in clinical practice of appropriate diagnostic procedures will be determined, based on discussion at the Expert Discussion.

4.(iii).B.(3).3 Efficacy of 5-ALA HCl in recurrent tumor

PMDA asked the applicant to explain the reason why the positive predictive rates of tissue fluorescence in areas of strong fluorescence were lower in recurrent tumors than those in primary tumors in the Japanese and foreign clinical studies (Studies NPC-07-1, MC-ALS.28/GLI, and MC-ALS.30/GLI), and to discuss whether or not the claim is justified that surgical tumor resection using 5-ALA HCl is effective in patients with recurrent tumor.

The applicant responded as follows:

The positive predictive rate of tissue fluorescence per subject and per biopsy sample taken from areas of strong fluorescence was 100% (32 of 32 subjects) and 100% (95 of 95 biopsy samples), respectively, in the foreign phase II study in subjects with primary malignant glioma (Study MC-ALS.28/GLI), and 91.7% (33 of 36 subjects) and 98.2% (161 of 164 biopsy samples), respectively, in the foreign phase II study in subjects with recurrent malignant glioma (Study MC-ALS.30/GLI). Thus, surgical tumor resection using 5-ALA HCl is considered to be also effective in subjects with recurrent malignant glioma similarly to those with primary glioma. Although the details of 3 biopsy samples from 3 subjects with recurrent malignant glioma that were determined as negative are unknown, the positive predictive rate of tissue fluorescence per biopsy sample from subjects with recurrent malignant glioma was 98.2% and equivalent to that from subjects with primary malignant glioma (100%).

The positive predictive rates of tissue fluorescence in areas of strong fluorescence in the Japanese phase III study (Study NPC-07-1) were 100% (22 of 22 subjects) in subjects with primary malignant glioma

and 85.7% (12 of 14 subjects) in subjects with recurrent malignant glioma, showing that the latter was lower than the former. The reason was that 6 biopsy samples from areas of strong fluorescence in 2 of 14 subjects with recurrent tumor were false-positive. According to the investigator's comment about the 2 subjects, it was presumed that non-tumor tissues were fluorescing due to gliosis etc. in one subject and tissues with radiation necrosis were collected from the other. It has been reported that after administration of 5-ALA to patients with recurrent malignant glioma, fluorescence was detected in gliosis in which tissues were converted into reactive astrocytes and sites infiltrated by inflammatory macrophages around radiation necrosis due to radiotherapy of tumor (*Neurosurgery*. 2007;65:1101-4).

In Japanese and foreign clinical studies, there were 5 subjects (2 in Japan and 3 in foreign countries) in whom some tissues in areas of strong fluorescence were determined to be false-positive. Nervous system-related adverse events were reported in the subjects. These are seizure in 1 subject and cerebral infarction (asymptomatic) in the other of the Japanese clinical study; and visual disturbance in 1 subject, sensory disturbance, speech disorder, memory impairment, and cognitive impairment in 1 subject, but none in the remaining subject of the foreign clinical studies. Seizure and visual disturbance resolved while cerebral infarction, sensory disturbance, speech disorder, memory impairment, and cognitive impairment did not. The nervous system-related adverse events in these subjects were not serious and no severe safety problems were found. Based on the above, surgical tumor resection using 5-ALA HCl is considered to be effective in patients with recurrent malignant glioma.

PMDA considers as follows:

As explained by the applicant, in patients with recurrent tumor, unlike those with primary tumor, previous treatment for malignant glioma, which influenced the brain tissues, may affect the diagnostic performance of 5-ALA HCl. The positive predictive rate of tissue fluorescence in areas of strong fluorescence of recurrent tumor tends to be lower than that of primary tumor; however, it is of great significance to resect tumor margins in patients with malignant glioma. As explained by the applicant, false positive tissues in areas of strong fluorescence were found to be not normal though they were not tumor cells. Considering the findings above, PMDA has concluded that 5-ALA HCl is effective for not only primary tumor but also recurrent tumor. The conclusion on the efficacy of 5-ALA HCl in recurrent tumor will be finalized, taking account of comments from the Expert Discussion.

4.(iii).B.(4) Precaution when performing surgical resection of malignant glioma using 5-ALA HCl

4.(iii).B.(4).1 False-positive results

The applicant explained the possibility of false-positive sites detected by the diagnosis with 5-ALA HCl as follows:

A false-positive site may be detected by the diagnosis with 5-ALA HCl possibly because of infiltration of inflammatory cells such as macrophages, fluorescence detected in sites with gliosis, and the disrupted blood-brain barrier due to the past treatment, leading to high uptake of 5-ALA in recurrent tumors.

PMDA considers as follows:

Since non-tumor tissues that are actually unnecessary to resect may be misidentified as a tumor in false-positive sites through the diagnosis with 5-ALA HCl, adequate attention should be paid to the prevention of serious adverse events caused by resection of tissues. Considering that the site to be resected is not determined only by the diagnosis with 5-ALA HCl and that surgical resection of malignant glioma using 5-ALA HCl was successfully performed in the Japanese and foreign clinical studies, the occurrence of false-positive results shown in these studies is considered not to compromise the usefulness of 5-ALA HCl. The conclusion on the impact of false-positive outcomes on patient's safety and the details of precautions will be finalized, taking account of comments from the Expert Discussion.

4.(iii).B.(4).2) False-negative results

The applicant explained the possibility of false-negative sites identified by the diagnosis with 5-ALA HCl as follows:

Of 45 subjects in the Japanese phase III study (Study NPC-07-1), 3 subjects were excluded from the FAS because no fluorescence in tumor core was detected in the tumor site after administration of 5-ALA HCl although they were determined as malignant glioma by rapid intraoperative cytology. In addition, tumor cells are present in some non-fluorescent areas even in patients with fluorescent tumor sites. In order to confirm that no tumor was found in non-fluorescent sites, the proportions of biopsy samples identified as tumor-positive (positive rate) to biopsy samples collected from areas adjacent to a fluorescent site (non-fluorescence) and to biopsy samples collected from areas distant from a tumor (non-fluorescence) (38 subjects) were determined in the Japanese phase III study (Study NPC-07-1). The biopsy samples were collected only when the risk against the subject was ruled out. As a result, the former was 61.1% (44 of 72 biopsy samples, 95% CI [48.9, 72.4]) and the latter was 47.5% (29 of 61, 95% CI [34.6, 60.7]). These results showed that infiltrating tumor cells was found in non-fluorescent adjacent and distant areas although fluorescence was not detected. This is probably because many normal cells are included in non-fluorescent areas, leading to low tumor cell density which is insufficient for fluorescence detection.

PMDA asked the applicant to, based also on the patient demographics, discuss the reason why fluorescence was not detected in 3 subjects, who were not included in the FAS in the Japanese phase III study (Study NPC-07-1) because their tumor core did not emit fluorescence.

The applicant explained as follows:

The pathological results of 3 subjects in whom fluorescence was not detected in tumor core showed WHO grade II diffuse astrocytoma in 1 subject and WHO grade III anaplastic oligodendroglioma and glioma in 1 subject each. It has been reported that PPIX concentrations in tumor tissues after oral administration of 5-ALA are correlated with malignancy of tumor tissues and intraoperative fluorescence intensity and increase exponentially (*Neurologia medico-chirurgica*. 2001;29:1019-31) and that fluorescence was not detected in some of diffuse astrocytomas after administration of 5-ALA

(*J Neurosurg.* 2006;104:618-20, *Currently Practical Neurosurgery.* 2006;16:989-96). Based on the reports, fluorescence may not have been detected in the tumor core of 1 subject in the Japanese phase III study (Study NPC-07-1) due to low PPIX concentrations in tumor cells. Another report suggested that the percentage of patients with WHO grade III glioma in whom no fluorescence is detected tends to be slightly higher than that of patients with WHO grade IV glioma (*Currently Practical Neurosurgery.* 2006;16:989-96, *Neurol Med Chir(Tokyo).* 2007;47:210-4). Subjects with anaplastic oligodendroglioma and glioma in whom fluorescence was not detected in tumor core were WHO grade III while fluorescence was detected in all subjects with WHO grade IV glioma in the Japanese phase III study (Study NPC-07-1). Based on the above, the package insert will include the caution statement that no fluorescence may be detected in the tumor core in some patients.

PMDA considers as follows:

The following information is important: (1) there are some patients with malignant glioma in whom no fluorescence may be detected in the tumor core after administration of 5-ALA HCl, consequently, the diagnosis with 5-ALA HCl cannot provide additional information to detect tumor and the resection range has to be decided in the same way as those for the conventional surgery under white light; and (2) subjects in whom fluorescence can be detected in the tumor core also may have tumor in non-fluorescent areas, and thus it cannot be ruled out that tumor tissues to be resected may unnecessarily remain unresected in such subjects if the diagnosis with 5-ALA HCl is overestimated. However, in the foreign phase III study (Study MC-ALS.3/GLI), increases in the percentage of subjects without residual tumor and 6-month progression-free survival were shown in the 5-ALA group versus the control group and the percentage of subjects without residual tumor in the Japanese phase III study (Study NPC-07-1) was similar to that in foreign clinical studies. Based on the results, the limitation of diagnosis due to false-negative results following 5-ALA HCl administration cannot rule out the usefulness of 5-ALA HCl. The detailed caution statement will be finalized, taking account of comments from the Expert Discussion.

4.(iii).B.(4).3 Effect on cranial nerve function associated with surgical tumor resection

Cranial nerve impairment is critical for surgical resection of malignant glioma. For this reason, PMDA asked the applicant to show the details of adverse events related to important cranial nerve impairment after surgery supported by diagnosis with 5-ALA HCl in the Japanese phase III study (Study NPC-07-1) and foreign clinical studies (Studies MC-ALS.28/GLI, MC-ALS.3/GLI, and MC-ALS.30/GLI). Furthermore, with respect to the finding that positive predictive rate of tissue fluorescence in areas of weak fluorescence per subject in the Japanese phase III study (Study NPC-07-1) was lower than that in the foreign clinical studies, PMDA asked the applicant to explain whether or not there is a possibility that larger tissues resected in the Japanese phase III study (Study NPC-07-1) than that in the foreign clinical studies may have resulted in damages to important cranial nerve function.

The applicant explained as follows:

The incidence of adverse events classified as “nervous system disorders” in the system organ class

(SOC) was 51.1% (23 of 45 subjects) in the Japanese clinical study (Study NPC-07-1), 61.1% (22 of 36 subjects) in the foreign phase II study (Study MC-ALS.28/GLI), 43.8% (88 of 201 subjects) in the foreign phase III study (Study MC-ALS.3/GLI), and 32.5% (13 of 40 subjects) in the foreign phase II study (Study MC-ALS.30/GLI), showing no significant difference. Adverse events classified as nervous system disorders of which incidence was higher in the Japanese clinical study than in the foreign clinical studies were headache (26.7% [12 of 45 subjects]), cerebral infarction (6.7% [3 of 45 subjects]), hemiparesis (6.7% [3 of 45 subjects]), and hemiplegia (4.4% [2 of 45 subjects]). With regard to hemiparesis and hemiplegia that are adverse events related to motor function, the incidence of motor dysfunction in the foreign clinical studies (Studies MC-ALS.28/GLI, MC-ALS.3/GLI, and MC-ALS.30/GLI) was 19.4% (7 of 36 subjects), 12.4% (25 of 201 subjects), and 20.0% (8 of 40 subjects), respectively. Therefore, adverse events related to motor function including hemiplegia did not occur more frequently in the Japanese clinical study than in the foreign clinical studies. The incidence of hearing disorders of “ear and labyrinth disorders” (SOC) in the foreign clinical studies (Studies MC-ALS.28/GLI, MC-ALS.3/GLI, and MC-ALS.30/GLI) was 0% (0 of 36 subjects), 2.5% (5 of 201 subjects), and 0% (0 of 40 subjects), respectively, and the incidence of visual impairment of “eye disorders” (SOC) was 19.4% (7 of 36 subjects), 12.9% (26 of 201 subjects), and 12.5% (5 of 40 subjects), respectively, while hearing disorders and visual impairment in the Japanese clinical study was both 0% (0 of 45 subjects).

Of serious adverse events reported in the foreign clinical studies (Studies MC-ALS.28/GLI, MC-ALS.3/GLI, and MC-ALS.30/GLI), “nervous system disorders” (SOC) was occurred in 19.4% (7 of 36 subjects), 14.9% (29 of 201 subjects), and 7.5% (3 of 40 subjects), respectively, visual impairment occurred in 0.5% (1 of 201 subjects) in Study MC-ALS.3/GLI. Serious adverse event reported in the Japanese phase III study (Study NPC-07-1) was only hydrocephalus in 2.2% (1 of 45 subjects), which was lower than that in the foreign clinical studies.

Based on the above, the applicant considered that there was no possibility that surgery in the Japanese clinical study (NPC-07-1) affected important cranial nerve function more seriously than surgery in foreign clinical studies.

PMDA asked the applicant to present the differences in the incidence of adverse events related to important cranial nerve function between the 5-ALA and control groups in the foreign phase III study (Study MC-ALS.3/GLI), and to explain whether or not adverse effect on important cranial nerve function tended to occur more frequently in the 5-ALA group than in the control group.

The applicant explained as follows:

As adverse events related to important cranial nerve function, the occurrence of “nervous system disorders” (SOC), hearing disorders, and visual impairment was compared between the 5-ALA group (201 subjects) and the control group (173 subjects). For “nervous system disorders” (SOC), the

incidence of adverse events was 43.8% (88 of 201 subjects) in the 5-ALA group and 50.9% (88 of 173 subjects) in the control group, which was similar to each other. Of the adverse events of “nervous system disorders” (SOC), those with a higher incidence were motor dysfunction reported in 12.4% (25 of 201 subjects), speech disorder reported in 11.9% (24 of 201 subjects), and seizure reported in 10.9% (22 of 201 subjects) in the 5-ALA group; and speech disorder reported in 13.3% (23 of 173 subjects), motor dysfunction reported in 11.6% (20 of 173 subjects), and seizure 8.7% (15 of 173 subjects) reported in the control group. Thus, the occurrence of “nervous system disorders” (SOC) was similar in the two groups. Serious adverse events of “nervous system disorders” (SOC) were reported in 14.4% (29 of 201 subjects) in the 5-ALA group and 12.7% (22 of 173 subjects) in the control group, showing similar incidence. However, the incidence of aphasia was 3.5% (7 of 201 subjects) in the 5-ALA group and 0.6% (1 of 173 subjects) in the control group, and the incidence of seizure was 6.0% (12 of 201 subjects) and 2.9% (5 of 173 subjects), respectively, showing the slightly higher incidence in the 5-ALA group. The incidence of visual impairment was 12.9% (26 of 201 subjects) in the 5-ALA group and 7.5% (13 of 173 subjects) in the control group, showing the slightly higher incidence in the 5-ALA group, and that of abnormal auditory perception was 2.5% (5 of 201 subjects) and 1.2% (2 of 173 subjects), respectively. Of vision disorders and hearing disorders, the serious event was visual impairment reported in 0.5% (1 of 201 subjects) in the 5-ALA group. However, the incidence of events reported as serious adverse events related to important cranial nerve function was too low to detect clinical differences. Therefore, it could not be decided that these results showed adverse effects on important cranial nerve function in the 5-ALA group of the foreign phase III study (Study MC-ALS.3/GLI).

PMDA considers as follows:

Study results did not tend to show that the incidence of adverse events in Japanese subjects was higher than that in foreign subjects or that adverse events in Japanese subjects were more serious than those in foreign subjects. Therefore, PMDA determined that no results were obtained that surgery in the Japanese phase III study (Study NPC-07-1) had adverse effects on important cranial nerve function more seriously than surgery in foreign clinical studies. For the comparison between the 5-ALA and control groups in the foreign phase III study (Study MC-ALS.3/GLI), it cannot be determined that adverse events related to the central nervous system and the occurrence of serious adverse events related to the central nervous system are significantly different between the two groups at present. Therefore, considering the efficacy of 5-ALA HCl [see “4.(iii).B.(3) Efficacy”], PMDA concluded that the effect of surgical resection of malignant glioma using 5-ALA HCl in Japan on important cranial nerve function is clinically acceptable. However, since the above review was based on small-scale studies, it is necessary to continue to collect information concerning postoperative nerve function after market launch and to evaluate the effect on important cranial nerve function in surgical resection of malignant glioma using 5-ALA HCl in patients with different backgrounds. The evaluation of the effect on cranial nerve function in the Japanese phase III study (Study NPC-07-1) compared with foreign clinical studies and in the 5-ALA group compared with the control group of the foreign phase III study (Study MC-ALS.3/GLI) will be finalized, taking account of comments from the Expert Discussion.

4.(iii).B.(5) Safety

4.(iii).B.(5).1 Effect on liver function

The applicant explained the increasing risk of 5-ALA-induced liver function tests raised as follows:

Liver function tests raised was reported in 50% to 70% of subjects in the Japanese and foreign clinical studies following a single oral dose of 5-ALA HCl at 20 mg/kg. Many of the subjects showed liver function tests raised 7 (or 14) days after administration. However, many of abnormal liver function test found in the Japanese phase III study (Study NPC-07-1) were grade 1 or 2 in accordance with the Common Terminology Criteria for Adverse Events (CTCAE). The percentage of subjects with CTCAE grade ≥ 3 was 0% (0 of 45 subjects) for AST, 2.3% (1 of 43 subjects) for ALT 28 days after administration, and 2.2% (1 of 45 subjects), 2.3% (1 of 44 subjects), 4.5% (2 of 44 subjects), and 2.3% (1 of 43 subjects) for γ -GTP 3, 7, 14 and 28 days after administration, respectively. γ -GTP of CTCAE grade 4 occurred only in 2.3% (1 of 43 subjects) 28 days after administration. These incidences were similar to those of abnormal liver function test found following administration of 5-ALA HCl at 20 mg/kg in foreign clinical studies. In the foreign phase III study (Study MC-ALS.3/GLI), AST, ALT and γ -GTP 24 hours after administration of 5-ALA HCl were higher in the 5-ALA group than in the control group and reached the peak 24 hours or 7 days after surgery. However, all of the increased values were transient and many of them were CTCAE grade ≤ 2 . The occurrence of abnormal liver function test 7 days after surgery and after tended to be similar to that in the control group. Therefore, the effect on liver function after administration of 5-ALA HCl should be monitored carefully but is unlikely to preclude the administration of 5-ALA HCl to patients scheduled to undergo surgical resection of malignant glioma.

Hepatic dysfunction including serious events was reported in Japanese and foreign clinical studies and the toxicity studies showed the effect on the liver [see “3.(iii).B *Outline of the review by PMDA*”]. Based on the results, PMDA asked the applicant to consider whether or not to take measures such as including periodic liver function tests to the Important Precautions section, and hepatic dysfunction in the Clinically significant adverse reactions section in the package insert.

The applicant responded as follows:

Serious hepatic dysfunction was found in 2 subjects in the Japanese clinical study. A causal relationship with 5-ALA HCl was denied in one of them and raised liver function test reported as a serious adverse event in the other was not considered to be related to 5-ALA HCl. In foreign clinical studies, no serious hepatic dysfunction was reported in any of 526 subjects given 5-ALA HCl. The percentage of subjects with liver function test raised of CTCAE grade ≥ 4 in the foreign phase III study (Study MC-ALS.3/GLI) was 1.1% (2 of 184 subjects) for ALT and 2.7% (5 of 183 subjects) for γ -GTP, all of which occurred 7 days after administration of 5-ALA HCl. None of the events were considered to be related to 5-ALA HCl. Based on the above, it is not necessary to include hepatic dysfunction in the Clinically significant adverse reactions section, but periodic liver function tests will be added to the Important Precautions section in the package insert (draft) because of concerns about the effect of 5-ALA on the liver function shown in toxicity studies.

PMDA considers as follows:

Although abnormal liver function test reported in both Japanese and foreign clinical studies was transient, serious hepatic dysfunction was reported in 2 subjects in the Japanese phase III study (Study NPC-07-1) and the event in one of them was not denied to have causal relationship with 5-ALA HCl. Also in the foreign phase I/II study for dose-finding of 5-ALA HCl (Study MC-ALS.8-I/GLI), abnormal γ -GTP, ALT, and AST values were reported in 14% to 57% of subjects and were considered to be related to 5-ALA HCl. Furthermore, PPIX-induced hepatic dysfunction was found in nonclinical studies (rat, dog). Based on the above, it is appropriate to include hepatic dysfunction in the Clinically significant adverse reactions section in the package insert. Besides, it should be advised that liver function tests be performed for a certain period of time after administration of 5-ALA HCl to carefully monitor the occurrence of hepatic dysfunction. Detailed precautions will be decided, taking account of comments from the Expert Discussion. In addition, information on the effect of 5-ALA HCl on liver function should be collected via the post-marketing surveillance.

4.(iii).B.(5).2) Photosensitivity

Since photosensitivity was confirmed in toxicity studies of 5-ALA HCl [see “3.(iii).B.(2) Phototoxicity”], the package insert (draft) specifies the avoidance of exposure to strong light sources (e.g., light in the operating room, direct sunlight or bright intensive indoor light) for at least 24 hours post-dose. Considering that plasma PPIX concentrations decreased to the baseline at 48 hours after administration of 5-ALA HCl in the foreign clinical pharmacology study (Study MC-ALS.20/BV) [see “4.(ii).A.(4) Pharmacodynamic evaluation”] and that photodermatitis occurred in the foreign clinical study, PMDA asked the applicant to explain the time of onset of photosensitivity in the foreign clinical study and the presence or absence of findings suggesting phototoxicity in the skin and subcutaneous tissues in the Japanese phase III study (Study NPC-07-1), and whether or not 24 hours of avoidance of exposure to strong light sources is sufficient to be included as a precaution in the package insert (draft).

The applicant responded as follows:

The plasma PPIX concentration or minimum erythema dose (MED) at sites of UV irradiation (immediate and delayed reaction) was not correlated with the time of light avoidance in the foreign clinical pharmacology study (Study MC-ALS.20/BV). Photosensitivity reaction or photodermatitis was found in 2 of 562 subjects (3 events) in the foreign clinical studies (1 subject, 2 events in Study MC-ALS.8-I/GLI; 1 subject, 1 event in Study MC-ALS.3/GLI). All of them were mild although 1 event occurred on the day of administration and 2 events at 2 days after administration of 5-ALA HCl. The European labeling specifies the avoidance of exposure of eyes and skins to strong light sources for 24 hours after administration. No safety reports related to phototoxicity of 5-ALA HCl have been submitted since 5-ALA HCl was approved in Europe in September 2007. Taking account of these facts, the exposure of eyes and skins to strong light sources was to be avoided up to 24 hours after administration also in the Japanese phase III study (Study NPC-07-1). No restrictions on exposure to light sources were

provided 24 hours or later after administration. As a result, no adverse event suggesting phototoxicity was confirmed. Based on the above, the applicant decided that it is appropriate for the package insert (draft) to specify 24 hours of avoidance of exposure to strong light sources.

PMDA considers as follows:

Based on the incidences of adverse events related to photosensitivity in Japanese and foreign clinical studies, and considering that the avoidance of exposure to strong light sources for 24 hours is specified in foreign labels and that no post-marketing safety report related to phototoxicity has been submitted, there will be no problems as long as exposure to strong light sources is avoided for 24 hours after administration of 5-ALA HCl. On the other hand, since photosensitivity is an adverse event caused by physicochemical characteristics of PPIX, full precautions are necessary to prevent photosensitivity. Immediate reaction in the skin was investigated in the foreign clinical pharmacology study (Study MC-ALS.20/BV) and the results showed that MED returned to the baseline 48 hours after administration of 5-ALA HCl. Such information should be provided to the clinical practice. The time to avoid exposure to strong light sources and detailed precautions will be finalized, taking account of comments from the Expert Discussion. Post-marketing surveillance also should be conducted to collect information about incidences of adverse events related to photosensitivity of 5-ALA HCl in practical use.

4.(iii).B.(5).3 Other safety

The applicant provided the following explanation regarding the safety of 5-ALA HCl with respect to the above-mentioned adverse events related to liver function and photosensitivity, and the adverse events that occurred in Japanese and foreign clinical studies except those described in the 4.(iii).B.(4).3 Effect on cranial nerve function associated with surgical resection section:

The incidence of adverse events was 93.3% (42 of 45 subjects) in the Japanese phase III study (Study NPC-07-1) and higher than 57.8% (325 of 562 subjects) in combined results in the foreign clinical studies. Particularly nausea and vomiting were frequently reported in the Japanese phase III study (Study NPC-07-1), but their severity was mild or moderate. In Japanese phase III study (Study NPC-07-1), adverse events related to laboratory test results were reported more frequently in comparison with foreign clinical studies. Particularly, events related to hematology and liver function test results including gamma-glutamyl transpeptidase increased, blood amylase increased, C-reactive protein increased, lymphocyte count decreased, neutrophil count decreased, and white blood cell count decreased were reported.

In examination by severity (CTCAE grade 1/2, 3, 4 and 5; 4 rating scores), the incidence of adverse events of CTCAE grade 1/2 was 88.9% (40 of 45 subjects) in the Japanese phase III study (Study NPC-07-1) and 48.4% (272 of 562 subjects) in combined results in the foreign clinical studies, showing a higher incidence in the former. However, the incidence of adverse events of CTCAE grade 3 was 20.0% (9 of 45 subjects) in the Japanese phase III study (Study NPC-07-1) and 22.4% (126 of 562 subjects) in combined results in the foreign clinical studies, and that of CTCAE grades 4 and 5 was 0% (0 of 45

subjects) and 2.2% (1 of 45 subjects), respectively, in the Japanese phase III study (Study NPC-07-1) and lower than that in combined results in the foreign clinical studies (5.0% [28 of 562 subjects] and 3.0% [17 of 562 subjects] for CTCAE grades 4 and 5, respectively). As shown above, the incidence of adverse events was higher in the Japanese phase III study (Study NPC-07-1) than in foreign clinical studies, but many of them occurring in the Japanese phase III study (Study NPC-07-1) were CTCAE grade 1 or 2 while the incidence of adverse events of CTCAE grade ≥ 3 , which have safety problems, was similar to that in the foreign clinical studies. All of adverse events occurring in the Japanese phase III study (Study NPC-07-1) were also reported in foreign clinical studies, showing no great difference in between Japanese and foreign clinical studies. Therefore, 5-ALA HCl at a dose of 20 mg/kg can be used for surgical resection of malignant glioma also in Japan.

PMDA considers as follows:

The results of Japanese and foreign clinical studies indicated no significant difference in the incidences of clinically relevant adverse events after the use of 5-ALA HCl for surgical resection of malignant glioma in the Japanese clinical study. Therefore, the safety of 5-ALA HCl is acceptable. PMDA has concluded that precautions for the safety with respect to the adverse events related to liver function and photosensitivity, and the adverse events except those described in the 4.(iii).B.(4).3 Effect on cranial nerve function associated with surgical resection section has been appropriately specified in the package insert (draft) in accordance with the foreign labels submitted by the applicant. The appropriateness of the conclusion will be determined, taking account of comments from the Expert Discussion.

4.(iii).B.(6) Indication

5-ALA-induced fluorescence was confirmed to be useful for the visualization of malignant tissue, which would contribute to improved completeness of tumor resection in Japanese and foreign clinical studies conducted in patients preoperatively diagnosed with WHO grade III/IV glioma that is malignant glioma. PMDA concluded that the following indications are appropriate.

[Indication]

Visualization of malignant tissue during surgical resection of malignant glioma

The indication of 5-ALA HCl will be finalized, taking account of comments from the Expert Discussion.

4.(iii).B.(7) Dosage and administration

The applicant explained the justification for the proposed dosage and administration of 5-ALA HCl as follows:

The dose of 5-ALA HCl in the Japanese phase III study (Study NPC-07-1) was set at 20 mg/kg and the positive predictive rate of tissue fluorescence was examined. As a result, the diagnostic performance in Japanese patients with malignant glioma was not practically different from that shown in the foreign clinical studies (Studies MC-ALS.28/GLI and MC-ALS.30/GLI). Therefore, a dose of 20 mg/kg was proposed for 5-ALA HCl in the application. Plasma PPIX concentrations after administration of 5-ALA

HCl in the Japanese phase III study (Study NPC-07-1) showed t_{\max} (mean \pm standard deviation) of 6.17 ± 0.98 hours and $t_{1/2}$ of 4.91 ± 1.90 hours. Based on the findings, it was presumed that when 5-ALA HCl is administered at 3 hours prior to induction of anesthesia, the maximum plasma PPIX concentration will be reached approximately 3 hours after induction of anesthesia and sufficient fluorescence of PPIX can be obtained during surgical tumor resection (5-10 hours). The time from administration of 5-ALA HCl to the start of induction of anesthesia ranged from 2 to 4 hours in 37 of 38 subjects in the Japanese phase III study (Study NPC-07-1) and 32 of 33 subjects in the foreign phase II study (Study MC-ALS.28-I/GLI), showing adequate results in these subjects. Based on the above, the administration timing of 5-ALA HCl was set at 3 hours prior to induction of anesthesia (range, 2-4 hours).

PMDA considers as follows:

Since the efficacy and safety of 5-ALA HCl for visualization of malignant glioma following oral administration of 5-ALA HCl at 20 mg/kg at 3 hours prior to induction of anesthesia (range, 2-4 hours) was demonstrated in Japanese and foreign clinical studies in adult patients with malignant glioma, it is appropriate that the dosage and administration of 5-ALA HCl conforms to the dosage and administration used in the Japanese phase III study (Study NPC-07-1).

[Dosage and administration]

The usual adult dosage is 20 mg/kg of Aminolevulinic Acid Hydrochloride given as an oral solution by dissolving in water at 3 hours (range, 2-4 hours) prior to induction of anesthesia for surgery.

The dosage and administration of 5-ALA HCl will be finalized, taking account of comments from the Expert Discussion.

4.(iii).B.(8) Post-marketing surveillance

The applicant plans to conduct an all cases surveillance in 250 patients for 4 years of the study period with a observation period of 2 weeks after administration of 5-ALA HCl as the post-marketing surveillance to collect patient information; patient demographics including history, the presence or absence of kidney and/or hepatic dysfunction; information of the primary disease including classification of primary/recurrent tumor, pathological diagnosis, and the dose of 5-ALA HCl; efficacy (improved discrimination of tumor and normal tissues by fluorescence guidance); and adverse events.

PMDA considers as follows:

Considering that limited subjects were examined in the Japanese phase III study (Study NPC-07-1), it is generally appropriate to conduct an all cases surveillance, with the observation period designated by the applicant, until a certain number of patients is included in the surveillance. However, if neurological findings at the completion of observation period show worsening of neurological function in the postoperative period versus preoperative period, it is appropriate to monitor the patient over the observation period extended for a certain period and collect information to confirm whether or not

cranial nerve impairment is persistent. For the survey items, it is necessary to appropriately collect information about the incidences of hepatic dysfunction and photosensitivity after administration of 5-ALA HCl and the time to avoid exposure to strong light sources. If the patient undergoes postoperative MRI, as much information as possible should be collected on the rate of complete resection and the progression-free survival, both of which are the endpoints for efficacy. Furthermore, it is necessary to reconsider the planned number of patients to allow the above survey items to be reviewed. The appropriateness of the post-marketing surveillance plan will be determined, taking account of comments from the Expert Discussion.

III. Results of Compliance Assessment Concerning the Data Submitted in the New Drug Application and Conclusion by PMDA

1. PMDA's conclusion on the results of document-based GLP/GCP inspections and data integrity assessment

A document-based compliance inspection and data integrity assessment was conducted in accordance with the provisions of the Pharmaceutical Affairs Act for the data submitted in the new drug application. As a result, there were no particular problems. Thus, PMDA concluded that there should be no problem with conducting a regulatory review based on the submitted application documents.

2. PMDA's conclusion on the results of GCP on-site inspection

GCP on-site inspection was conducted in accordance with the provisions of the Pharmaceutical Affairs Act for the data (5.3.5.2-1) submitted in the new drug application. As a result, it was found that periodic reports of serious adverse drug reactions to medical institutions were delayed by the sponsor. Although issues to be improved were identified, PMDA concluded that there should be no problem with conducting a regulatory review based on the submitted application documents because clinical studies were generally conducted in accordance with GCP.

IV. Overall Evaluation

Based on the submitted data, the efficacy of the product for visualization of malignant tissue during surgical resection of malignant glioma has been demonstrated and the safety of the product is acceptable in view of its observed benefits. With regard to the incidences of hepatic dysfunction and photosensitivity after administration of the product, time to avoid exposure to strong light sources, information about neurological deficits after surgical resection of malignant glioma using the product, rate of complete resection, and progression-free survival, it is necessary to continue to collect information in an appropriate manner via post-marketing surveillance.

The application for the product may be approved if it can be concluded based on comments from the Expert Discussion that there are no particular problems.

Review Report (2)

March 5, 2013

I. Product Submitted for Registration

[Brand name]	(a) Alabel for Oral Administration 1.5 g and (b) Alaglio for Oral Administration 1.5 g (which are to be changed to (a) Alabel Oral 1.5 g and (b) Alaglio Oral 1.5 g, respectively)
[Non-proprietary name]	Aminolevulinic Acid Hydrochloride
[Name of applicant]	(a) Nobelpharma Co., Ltd. and (b) SBI Pharmaceuticals Co., Ltd.
[Date of application]	July 5, 2012

II. Content of the Review

The outline of the comments from the Expert Discussion and the subsequent review by the Pharmaceuticals and Medical Devices Agency (PMDA) is described in the following sections. The expert advisors for the Expert Discussion were nominated based on their declarations etc. concerning the product submitted for registration, in accordance with the provisions of the “Rules for Convening Expert Discussions etc. by Pharmaceuticals and Medical Devices Agency” (PMDA Administrative Rule No. 8/2008 dated December 25, 2008).

1. Clinical positioning

The results of Japanese and foreign clinical studies suggested the efficacy of the product (5-ALA HCl) in Japanese patients with malignant glioma. It is expected that the diagnosis with 5-ALA HCl can provide useful information for determining the extent of resection, thereby improving the rate of complete resection of malignant glioma and the prognosis in clinical practice in Japan similarly to foreign clinical studies. Therefore, the expert advisors supported PMDA's conclusion that it is meaningful to provide 5-ALA HCl in clinical practice in Japan.

2. Efficacy

In Japanese phase III study (Study NPC-07-1), the lower limit of 95% confidence interval of the positive predictive rate of tissue fluorescence in fluorescent sites per subject, the primary endpoint, was below the threshold limit value that had been set when the study was designed based on the foreign study results. However, it may be preferable for patients with malignant glioma to undergo tumor resection that is as complete as possible. Given that the percentages of patients without residual tumor in the Japanese and foreign clinical studies were similar to each other and that no significant difference in the effect of surgical tumor resection on the cranial nerve function is found in the Japanese and foreign clinical studies, the efficacy of 5-ALA HCl used for the resection can be expected in Japanese patients with primary and recurrent malignant glioma similarly to that confirmed in foreign clinical studies,

including contribution to prognostic improvement. The expert advisors supported the above conclusion of PMDA.

3. Precaution when performing surgical resection of malignant glioma using 5-ALA

(1) False-positive and false-negative results

The Expert Discussion highlighted the following conclusion by PMDA: Considering that the site to be resected is not determined only by the diagnosis with 5-ALA HCl for surgical resection of malignant glioma and that surgical resection of malignant glioma using 5-ALA HCl was successfully performed in the Japanese and foreign clinical studies, the usefulness of 5-ALA HCl cannot be compromised even if false-positive sites as shown in these studies are detected by the diagnosis with 5-ALA HCl also in clinical settings. However, the package insert needs to include a caution statement that full attention should be paid to the prevention of serious adverse events caused by resection of tissues that are actually unnecessary to resect.

The expert advisors gave their comment as follows: (1) it is usual to determine the range of resection considering the nerve function and diagnostic procedures other than the diagnosis with 5-ALA HCl and the usefulness of 5-ALA HCl cannot be compromised by the false-positive result itself, although it is important to take into account that false-positive sites are detected by the diagnosis with 5-ALA HCl; (2) it is well known that false-positive sites are caused by the diagnosis with 5-ALA HCl particularly for recurrent patients in clinical practice; and (3) false-positive sites caused by the diagnosis with 5-ALA HCl will not be a significant problem because a site of any abnormality may be positive although the site does not include tumor cells. Consequently, the expert advisors reached the conclusion that the false-positive sites caused by the diagnosis with 5-ALA HCl will not be clinically significant problems if information about false-positive sites detected by the diagnosis with 5-ALA HCl is provided and the determination of resection range using the results from other diagnostic procedures and the neurological function is advised in the package insert.

The Expert Discussion also addressed the following conclusion by PMDA: Considering that the percentage of subjects without residual tumor and the 6-month progression-free survival were improved in the 5-ALA HCl group relative to the control group in the foreign phase III study (Study MC-ALS.3/GLI) and that the percentage of subjects without residual tumor in the Japanese clinical study was similar to that in foreign clinical studies, the limitation of diagnosis due to false-negative results caused by 5-ALA HCl administration does not rule out the usefulness of 5-ALA HCl; however, precautions about false-negative results should be given.

The expert advisors gave their comment as follows: (1) Since glioblastoma tissue images differ depending on the site and tumors may include low-grade tissues that do not emit 5-ALA-induced fluorescence, tissues with 5-ALA-induced fluorescence are not determined as normal tissues in clinical practice; (2) false-negative results caused by 5-ALA are well known in clinical practice and it is

appropriate to continue to provide information also in the package insert of 5-ALA HCl. Consequently, the expert advisors reached the conclusion that the detection of false-negative sites itself is not a clinically significant problem if information about false-negative sites detected by the diagnosis with 5-ALA HCl is provided in the package insert.

Based on the conclusion from the Expert Discussion, PMDA instructed the applicant to include in the package insert a caution statement to the effect that resection range should be decided based on the results of other diagnostic procedures and the nerve function, considering false-negative and/or false-positive sites caused by the diagnosis with 5-ALA HCl. The applicant took appropriate action accordingly.

(2) Effect on cranial nerve function associated with surgical resection

The expert advisors supported the conclusion by PMDA: the effect of resection of malignant glioma using 5-ALA HCl on important cranial nerve function is acceptable considering the efficacy of 5-ALA HCl because the results of Japanese and foreign clinical studies showed no significant difference in the effect of tumor resection on the cranial nerve function.

The Expert Discussion also addressed PMDA's conclusion that it is necessary to continue to collect information concerning postoperative neurological function also after market launch. The expert advisors gave their comment as follows: (1) Since adverse events related to the central nervous system were not attributed to administration of 5-ALA HCl but caused by surgical procedure, it is less meaningful to focus these events in the post-marketing surveillance; (2) it is difficult to extract only the significance of the diagnosis with 5-ALA-induced fluorescence in evaluation of postoperative malignant glioma; and (3) it is appropriate to investigate the effect of 5-ALA HCl on postoperative neurological function in clinical settings. Consequently, the expert advisors reached the conclusion that detailed information on the occurrence of adverse events related to postoperative neurological function should be collected if they are found after surgery.

4. Safety

(1) Effect on liver function

The expert advisors supported the following conclusion by PMDA: It is necessary to additionally include "hepatic dysfunction" in the Clinically significant adverse reactions section in the package insert (draft), to conduct liver function tests for a certain period after administration of 5-ALA HCl, and to advise caution to the effect that careful monitoring should be conducted if hepatic dysfunction occurs, considering that a causal relationship of abnormal liver function test or serious hepatic dysfunction reported in Japanese and foreign clinical studies to 5-ALA HCl could not be ruled out and PPIX-induced hepatic impairment was found in nonclinical studies (rat, dog).

Based on the above conclusion from the Expert Discussion, PMDA asked the applicant to revise the

package insert and the applicant took appropriate action.

(2) Photosensitivity

Since no adverse events suggesting phototoxicity were reported in the Japanese phase III study where the subjects were required to avoid strong light sources (light in operating room, direct sunlight or bright intensive indoor light) for 24 hours after administration of 5-ALA HCl, there will be no critical problems if exposure to strong light sources is avoided. In contrast, the foreign clinical pharmacology study (Study MC-ALS.20/BV) was conducted to examine immediate reaction in the skin, as a result, minimum erythema dose (MED) at sites of UV irradiation returned to the baseline 48 hours after administration of 5-ALA HCl. Also, adverse events related to photosensitivity that occurred in the foreign clinical studies include those which developed 2 days after administration of 5-ALA HCl. Based on these findings, the appropriateness of the caution statement about the time to avoid the exposure to strong light sources was discussed.

The expert advisors gave the following comment: Given the actual use of 5-ALA in clinical settings, there will be no clinically significant problems if exposure to strong light sources is avoided up to 24 hours after administration of 5-ALA HCl because the risk for photosensitivity caused by 5-ALA HCl seems to be lower than the known risk of other drugs. In contrast, the other comment was as follows: Since patients with malignant glioma are not expected to receive strong light for several days after surgery in clinical practice, no clinically significant problems will be caused if the package insert, from the perspective of the safety, specifies that exposure of the patient to strong light sources should be avoided up to 48 hours after administration of 5-ALA HCl. Consequently, the expert advisors reached the conclusion that the package insert specifies that exposure of the patient to strong light sources should be avoided up to 48 hours to ensure the safety.

Based on the above conclusion from the Expert Discussion, PMDA asked the applicant to revise descriptions in the package insert to specify the time to avoid exposure to strong light sources for 48 hours, and the applicant took appropriate action accordingly.

(3) Other safety

The expert advisors supported the following conclusion by PMDA: The results of Japanese and foreign clinical studies indicated no marked difference in the incidences of adverse events after administration of 5-ALA HCl and the difference is clinically acceptable. Furthermore, precautions about the safety except adverse events related to liver function, photosensitivity, and cranial nerve function are appropriately provided in the package insert (draft) in accordance with the foreign package insert submitted by the applicant.

5. Indication

The expert advisors supported PMDA's conclusion that the indication proposed as follows is appropriate

because 5-ALA-induced fluorescence was confirmed to be useful for the visualization of malignant tissue, which would contribute to improved completeness of tumor resection in Japanese and foreign clinical studies.

[Indication]

Visualization of malignant tissue during surgical resection of malignant glioma

6. Dosage and administration

The expert advisors supported the following conclusion by PMDA: Based on the foreign clinical study results and the clinical use in Japan, 5-ALA HCl was orally administered at 20 mg/kg 3 hours prior to induction of anesthesia (range, 2-4 hours) in the Japanese phase III study and the efficacy and safety of 5-ALA HCl for the visualization of malignant glioma was demonstrated. Therefore, it is appropriate to establish the dosage and administration of the product as follows.

[Dosage and administration]

The usual adult dosage is 20 mg/kg of Aminolevulinic Acid Hydrochloride for an oral solution prepared by dissolving in water 3 hours (range, 2-4 hours) prior to induction of anesthesia for surgery.

7. Post-marketing surveillance

The Expert Discussion addressed the following conclusion by PMDA: considering that the number of subjects and patient background were limited in the Japanese phase III study and that the efficacy exceeding the pre-defined threshold limit value was not found in the primary endpoint of the Japanese phase III study, it is necessary to confirm the efficacy of 5-ALA HCl in Japan after market launch and it is appropriate to collect information about the rate of complete resection and the progression-free survival that were established as the endpoints in foreign clinical studies, in addition to the survey items proposed by the applicant for the post-marketing surveillance in all the patients in whom 5-ALA HCl is used.

The expert advisors gave their comment as follows: (1) Although it is difficult to extract only the significance of the fluorescence-guided diagnosis in evaluation of postoperative malignant glioma, it can be agreed to study the rate of complete resection and the progression-free survival; (2) 5-ALA HCl is merely a diagnostic agent to improve the rate of tumor resection, and even if tumor tissues can be identified by the diagnosis with 5-ALA HCl, tumors cannot be often resected in the regions of nerve function or the rate of complete resection is not always improved; (3) since the post-marketing surveillance does not include the control and has difficulties in matching the evaluation period between medical institutions, it is impossible to evaluate the effect of 5-ALA HCl on the rate of complete resection and the progression-free survival; (4) the usefulness of 5-ALA HCl was realized in the case where resection of a site that was not determined under white light but was additionally determined by the diagnosis with 5-ALA HCl; and (5) it is common to confirm the presence or absence of a fluorescent

site while taking biopsies, as required, to identify malignant tissue, thereby determining the extent of resection in the surgical resection of malignant glioma using 5-ALA HCl. Consequently, the expert advisors reached the conclusion that in order to evaluate the usefulness of 5-ALA HCl, it is beneficial to collect information on the presence or absence of tissues that are to be additionally resected by the diagnosis with 5-ALA HCl and on the presence or absence of tumor cells in biopsies taken from the tumor margins in fluorescence guidance using 5-ALA HCl.

The expert advisors supported PMDA's conclusion that it is necessary to collect information about the incidences of hepatic dysfunction and photosensitivity after administration of 5-ALA HCl as other items to investigate.

Based on the above conclusion from the Expert Discussion, PMDA asked the applicant to reconsider the plan of post-marketing surveillance (draft).

The applicant responded as follows:

Through the post-marketing surveillance, the applicant will collect information about the presence or absence of tissues that were additionally decided to resect based on the diagnosis with 5-ALA HCl although they were not determined to resect under white light. To study the presence or absence of tumor cells in biopsies that are taken from fluorescence sites of tissues resected in tumor margins under 5-ALA-induced fluorescence guidance, the applicant will collect information about them by collecting biopsies taken from fluorescent tissue resected and from non-fluorescent tissues that were not resected. Furthermore, the applicant will collect information about the time to prevent exposure to strong light sources after administration of 5-ALA HCl.

PMDA accepted the applicant's response.

III. Overall Evaluation

As a result of the above review, PMDA concludes that the product may be approved after modifying the indication, and dosage and administration statements as shown below.

The re-examination period is 10 years, neither the drug substance nor the drug product is classified as a poisonous drug or a powerful drug, and the product is not classified as a biological product or a specified biological product.

[Indication]

Visualization of malignant tissue during surgical resection of malignant glioma

[Dosage and administration]

The usual adult dosage is 20 mg/kg of Aminolevulinic Acid Hydrochloride for an oral solution prepared by dissolving in water. The solution should be administered orally 3 hours (range, 2-4 hours) prior to induction of anesthesia for surgery.

[Conditions for approval]

The applicant is required to conduct a post-marketing drug use-results survey over a certain period of time until the data about a certain number of patients are accumulated after market launch, covering all patients treated with the product, in order to understand background information about patients treated with the product because the product is an orphan drug and subjects for Japanese clinical studies are extremely few. At the same time, the data about the safety and efficacy of the product should be collected and necessary measures should be taken to ensure that the product is properly used.