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PHARMACOLOGY REVIEW(S)

**DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH**

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

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Indication: Mild to moderate actinic keratosis
Applicant: Biofrontera Bioscience GmbH
Review Division: Dermatology and Dental Products
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1 Executive Summary

1.1 Introduction

The sponsor has submitted a 505(b)(1) NDA application for Ameluz (aminolevulinic acid HCl) Gel, 10% with BF-RhodoLED light administration. Aminolevulinic acid (ALA) is a photodynamic therapy photosensitizer. Photodynamic therapy (PDT) requires 3 components: (1) a photosensitizer, (2) light with a sufficient amount of energy at a suitable spectrum of wavelengths, and (3) oxygen. In PDT light energy is transferred through the photosensitizer to oxygen, leading to the formation of reactive oxygen species (ROS). ROS oxidize cell membranes and other cellular compounds, causing necrosis or apoptosis of targeted cells.

The sponsor has developed Ameluz, in combination with red light photodynamic therapy (PDT) using the BF-RhodoLED® lamp, for (b) (4) treatment of actinic keratoses (AKs) of mild to moderate severity on the face and scalp.

1.2 Brief Discussion of Nonclinical Findings

ALA is a delta-amino acid and occurs as an endogenous molecule of the heme biosynthesis pathway in almost every cell in humans, animals and plants. The hydrochloride salt is used as the drug substance in the to-be-marketed Ameluz gel, 10% formulation. ALA is used in this topical drug product as a photodynamic therapy photosensitizer. ALA functions as a pro-drug and is metabolized to the photoactive substance protoporphyrin IX (PpIX) in mitochondria. PpIX is a natural metabolite of ALA within the heme pathway. PpIX can be activated by the absorption of energy at several different wave lengths, ranging from blue to red light.

The sponsor has demonstrated that there is a negligible systemic increase in plasma levels of ALA above background endogenous levels and no increase in PpIX plasma levels (which is a biomarker of systemically absorbed ALA pharmacology) under maximal clinical use conditions for Ameluz. The need for reproductive toxicity studies and a systemic carcinogenicity study were waived based on the level of systemic exposure demonstrated under maximal clinical use conditions for Ameluz. Maternal use of Ameluz is not expected to result in fetal exposure to the drug and breastfeeding is not expected to result in exposure of the child to the drug due to the negligible systemic absorption of ALA following topical administration of Ameluz under clinical maximal use conditions. The need for a dermal carcinogenicity study for Ameluz was waived due to the clinical conditions of use (single application followed by another single application after 3 months, if needed).

The target organ of toxicity identified in a 14 day repeat dose intravenous dog toxicity study was the liver.

A repeat dose dermal minipig study was conducted with once monthly topical administration of ALA gel, 10% or vehicle gel for 3 months with and without red light exposure. The results from this study were the expected effects based on the

pharmacologic mechanism of photodynamic therapy. Mild to moderate erythema and eschar formation were noted at ALA gel, 10% treated sites. The symptoms were more pronounced at ALA gel, 10% treated sites exposed to red light. No increase in local toxicity was noted after repeat dose administration and the recovery process appeared to be quicker from the second application through the fourth application of ALA gel, 10% plus red light treated sites. Histopathological evaluation of treated skin sites 28 days after the last ALA gel, 10% treated site exposed to red light demonstrated complete recovery.

An ICH battery of genotoxicity studies were conducted with ALA HCl. ALA HCl revealed no evidence of mutagenic or clastogenic potential based on the results of three in vitro genotoxicity tests (Ames assay, HPRT test in V79 cells and Human lymphocyte chromosomal aberration assay) and one in vivo genotoxicity test (mouse micronucleus assay). The in vitro genotoxicity studies were conducted without red light exposure. There is literature data that indicates a low genotoxicity potential of ALA when combined with UVA light exposure. The observed DNA damage is probably caused by the oxidative free radicals formed when ALA derived PpIX is exposed to light of the correct wavelength. This is the desired pharmacologic effect that is utilized for the treatment of actinic keratosis lesions.

ALA gel, 10% without red light exposure was not a dermal irritant or ocular irritant in rabbits. ALA gel, 10% without red light exposure was not a sensitizer in the murine LLNA assay.

The toxicity profile of ALA gel, 10% has been adequately characterized by the nonclinical studies conducted by the sponsor. The toxicity profile elicited by ALA gel, 10% in the presence of red light exposure was what is anticipated for PDT.

1.3 Recommendations

1.3.1 Approvability

NDA 208081 for Ameluz with BF-RhodoLED light administration is approvable from a Pharmacology/Toxicology perspective provided that the recommended changes in the label described in Section 1.3.3 are incorporated into the label.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

The need for reproductive toxicity studies and a systemic carcinogenicity study were waived because it has been demonstrated that there is a negligible systemic increase in plasma levels of ALA above background endogenous levels and no increase in PpIX plasma levels (which is a biomarker of systemically absorbed ALA pharmacology) under maximal clinical use conditions for Ameluz. The need for a dermal carcinogenicity study for Ameluz was waived due to the clinical conditions of use (single application followed by another single application after 3 months, if needed). The information in Sections 8.1 and 8.2 of the label will be minimal and utilize wording similar to what is suggested

in the PLLR guidance document for no systemic absorption of the drug product. Also, there will be no Section 8.3 because there is no concern about possible treatment related effects on fertility due to the negligible systemic absorption of ALA.

It is recommended that the underlined wording be inserted into and the ~~strikeout~~ wording be deleted from the Ameluz label reproduced below. The pharmacologic class designation for aminolevulinic acid for the treatment of actinic keratosis is prophylin precursor. Recommended revisions for the nonclinical information contained in Section 8 of the label are made below. Refer to the clinical review for recommended revisions for the clinical information contained in Section 8 of the label. A clean copy of the suggested wording for the nonclinical sections of the label is provided in Appendix 1.

HIGHLIGHTS OF PRESCRIBING INFORMATION

INDICATIONS AND USAGE

AMELUZ® Gel, a porphyrin precursor, in combination with photodynamic therapy using the BF-RhodoLED® lamp, is indicated for the treatment of actinic keratosis of mild to moderate severity of the face and scalp (b) (4) (1).

FULL PRESCRIBING INFORMATION

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary

(b) (4)

There are no available data on AMELUZ use in pregnant women to inform a drug associated risk. Animal reproduction studies were not conducted with ALA. Systemic absorption of ALA in humans is negligible following topical administration of AMELUZ under maximal clinical use conditions [See Clinical Pharmacology (12.3)]. It is not expected that maternal use of AMELUZ will result in fetal exposure to the drug.

8.2 Lactation

Risk Summary

(b) (4)

The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for AMELUZ® and any potential adverse effects on the breastfeeding child from AMELUZ® or from the underlying maternal condition.

No data are available regarding the presence of ALA in human milk, the effects of ALA on the breast fed infant or on milk production. However, breastfeeding is not expected to result in exposure of the child to the drug due to the negligible systemic absorption of ALA in humans following topical administration of AMELUZ under maximal clinical use conditions [See *Clinical Pharmacology* (12.3)].

CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Photoactivation following topical application of AMELUZ occurs when (b) (4) aminolevulinic acid (ALA) (prodrug) (b) (4) is metabolized to protoporphyrin IX (PpIX), a photoactive compound which accumulates in the skin (b) (4). (b) (4) When exposed to red light of a suitable wavelength and energy, PpIX is activated resulting in an excited state of porphyrin molecules. In the presence of oxygen, reactive oxygen species are formed which causes damage to cellular components, and eventually destroys the (b) (4) cells. AMELUZ (b) (4) photodynamic therapy of AK lesions utilizes (b) (4) photoactivation of (b) (4)

(b) (4)

NONCLINICAL TOXICOLOGY**13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility**

Long-term studies to evaluate the carcinogenic potential of AMELUZ or ALA have not been performed.

(b) (4)

(b) (4) .
(b) (4) revealed no evidence of mutagenic or
clastogenic potential based on the results of three in vitro genotoxicity tests (Ames
assay, HPRT test in V79 cells and Human lymphocyte chromosomal aberration assay)
and one in vivo genotoxicity test (mouse micronucleus assay). These genotoxicity
studies were conducted without exposure to light. (b) (4) There is a
literature report that indicates that (b) (4) may cause genotoxic effects in the presence (b) (4)
in the absence of activating light. These genotoxic effects are likely (b) (4)
(b) (4) caused by the formation of reactive oxygen species (b) (4)

(b) (4)

Animal fertility studies have not been conducted with ALA because of the negligible systemic absorption of ALA in humans following topical administration of AMELUZ under maximal clinical use conditions.

2 Drug Information

2.1 Drug

CAS Registry Number
15451-09-2

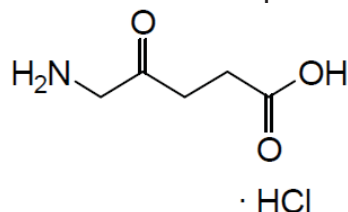
Generic Name
5-Aminolevulinic acid HCl, ALA HCl, 5-ALA, ALA

Code Name
BF-200 ALA, MC 506/1, MC 506

Chemical Name
5-Amino-4-oxopentanoic acid hydrochloride

Molecular Formula/Molecular Weight
 $C_5H_9NO_3 \cdot HCl$ / MW=167.59

Structure or Biochemical Description



Pharmacologic Class
Porphyrin precursor, Photodynamic therapy photosensitizer

2.2 Relevant INDs, NDAs, BLAs and DMFs

Pre-IND 115412 (ALA gel, 10%; actinic keratosis; DDDP; no formal IND was submitted by the sponsor prior to NDA submission)

NDA 21415 (Metvixia {methyl ALA cream, 16.8%}; actinic keratosis; DDDP; Sponsor – Gladerma Labs LP; approved July 27, 2004; withdrawn effective November 12, 2015)

2.3 Drug Formulation

The qualitative and quantitative composition of the drug product is provided in the following table.

Components	Amount per		Function	Quality standards
	1 gram [mg]	percentage [%]		
5-Aminolevulinic acid hydrochloride	100.000	10.000	Drug substance	In-house
Xanthan gum	(b) (4)		(b) (4)	Ph.Eur / USP-NF
Soybean phosphatidylcholine				In-house / DMF (b) (4)
Polysorbate 80				Ph.Eur / USP-NF
Triglycerides, medium-chain				Ph.Eur / USP-NF
Isopropyl alcohol				Ph.Eur / USP-NF
(b) (4)				Ph.Eur / USP-NF
(b) (4)				Ph.Eur / USP-NF
Propylene glycol				Ph.Eur / USP-NF
Sodium benzoate				Ph.Eur / USP-NF
Purified water				Ph.Eur / USP-NF
Total				

* current edition of the respective pharmacopoeial monograph

** related to (b) (4)

Reviewer's comments: During development of the ALA gel, the composition of the formulation was slightly modified (b) (4)

(b) (4) in the earlier formulation (Formulation A), but were replaced (b) (4)
 in the intended commercial formulation (Formulation B). (b) (4)

There are no nonclinical toxicity concerns for the slight changes in excipients for the ALA gel.

2.4 Comments on Novel Excipients

There are no novel excipients.

2.5 Comments on Impurities/Degradants of Concern

Three potential impurities have been identified for the ALA gel. The (b) (4) impurities are two esters of ALA and do not pose any additional toxicity concern compared to ALA. The specification for

these two impurities in Ameluz is set at NMT (b) (4) % each, which is acceptable from a Pharmacology/Toxicology perspective.

The third impurity is identified as (b) (4) and is formed under stressed conditions (b) (4). The specification for this impurity in Ameluz is set at NMT (b) (4) %. This specification is acceptable from a Pharmacology/Toxicology perspective. There will be no systemic exposure to this impurity due to the negligible systemic absorption of ALA from Amulez demonstrated under maximal clinical use conditions.

The sponsor also conducted a dermal irritation study in rabbits and a LLNA test in mice to assess the irritation and sensitization potential of the ALA gel formulation after enrichment with the three impurities. No skin irritation or sensitization potential was observed in these studies.

2.6 Proposed Clinical Population and Dosing Regimen

For the photodynamic therapy of actinic keratoses with Ameluz, apply Ameluz to single lesions or an entire field affected by multiple lesions (b) (4) occlude treated area and illuminate with BF- RhodoLED® light after 3 hours.

Treated lesions or fields that have not completely resolved after 3 months can be retreated with Amulez and BF-RhodoLED® light.

2.7 Regulatory Background

The following meetings were conducted with the sponsor under Pre-IND 115412. The sponsor did not submit an IND prior to submitting the NDA submission.

- 1) Pre-IND meeting conducted on July 11, 2012
- 2) Pre-NDA meeting conducted on October 8, 2014

3 Studies Submitted

3.1 Studies Reviewed

Pharmacology

- 1) Comparison of the photodynamic induction of cell death in a squamous cell carcinoma cell line by different red light sources (Study No. ALA-AK-PT032)

Safety Pharmacology

- 1) 5-Aminolevulinic acid, hydrochloride: Effect on hERG tail currents recorded from stably transfected HEK 293 cells (Study Number A1273 or ALA-AK-PT036)
- 2) Examination of the influence of MC 506 (5-aminolevulinic acid hydrochloride) on several cardiovascular parameters and the respiration in anesthetized beagle dogs following intravenous administration (Study Number (b) (4) 10115/1/96)

Pharmacokinetics/Toxicokinetics

- 1) The in vitro percutaneous absorption of radiolabelled [¹⁴C]-5-ALA through human skin (Study Number 27258 or ALA-AK-PT002)
- 2) The in vitro percutaneous absorption of a nanoemulsion formulation radiolabelled [¹⁴C]-5-ALA through human skin (Study Number 780288 or ALA-AK-PT005)

General Toxicology

- 1) 14-Day subchronic toxicity study of MC 506/1 (5-aminolevulinic acid hydrochloride) by intravenous administration to beagle dogs (Study Number (b) (4) 11664/98)
- 2) 3-Month local tolerance and toxicity study of BF-200 ALA in minipigs following repeated dermal administration (once monthly) (Study Number 24945 or ALA-AK-PT017)

Genetic Toxicology

- 1) Mutagenicity study of MC 506 (5-aminolevulinic acid HCl) in the *Salmonella Typhimurium* reverse mutation assay (in vitro) (Study Number 10112/96)
- 2) Mutagenicity study of MC 506 In mammalian cells (V79) in the in vitro gene mutation assay (HPRT Test) (Study Number 10113/96)
- 3) In vitro assessment of the clastogenic activity of MC 506 (5-aminolevulinic acid HCl) in cultured human peripheral lymphocytes (Study Number 10114/96)
- 4) Micronucleus test of 5-ALA in bone marrow cells of the NMRI mouse following oral administration (Study Number 17551/03)

Special Toxicology

- 1) Dermal tolerability study in rabbits (Study Number ALA-AK-PT001)
- 2) Dermal tolerability study in rabbits (Study Number ALA-AK-PT012)
- 3) Local lymph node assay in mice (LLNA/IMDS) (Study Number ALA-AK-PT007)
- 4) Local lymph node assay in mice (LLNA/IMDS) (Study Number ALA-AK-PT013)
- 5) Acute eye irritation/corrosion test of BF-200 ALA gel 10% in rabbits (Study Number ALA-AK-PT027)

3.2 Studies Not Reviewed

These nonclinical studies were not reviewed because they did not provide additional pivotal nonclinical data to support the safety of ALA gel, 10%.

Secondary Pharmacology

- 1) The impact of ALA PDT on cultured dorsal root ganglion sensory neurons (Study Number ALA-AK-PT022)

- 2) ATP-release from keratinocytes after ALA-PDT (Study Number ALA-AK-PT023)

Pharmacokinetics/Toxicokinetics

- 1) Influence of BF-200 on porphyrin synthesis after ALA application to HaCat cells (Study Number ALA-AK-PT004)
- 2) Evaluation of 5-aminolevulinic acid induced protoporphyrin IX fluorescence in nude mouse skin comparison of BF-200 ALA versus Metvix® (Study number ALA-AK-PT009)
- 3) Comparative investigations into the penetration behavior of BF-200 ALA in an ex-vivo porcine skin model (Study number ALA-AK-PT014)
- 4) Penetration behavior of BF-200 in human skin (Study number ALA-AK-PT020)
- 5) ATP release from keratinocytes after ALA-PDT (Study number ALA-AK-PT023)
- 6) Influence of uptake inhibitors on porphyrin synthesis after ALA application to HaCat cells (Study number ALA-AK-PT024)
- 7) Characterization of the ALA-uptake stimulation by BF-200 (Study number ALA-AK-PT025)
- 8) Formation of PpIX in cultured cells after incubation with ALA or MAL (Study number ALA-AK-PT026)
- 9) Time course of the BF-200 induced increase in membrane penetration (Study number ALA-AK-PT028)
- 10) Epidermal penetration and protoporphyrin IX formation of two different 5-aminolevulinic acid formulations in ex vivo human skin (Study number ALA-AK-PT037)

General Toxicology

- 1) 7-Day dose-range-finding study for a 14-day subchronic toxicity study of MC 506/1 (5-aminolevulinic acid hydrochloride) by intravenous administration to beagle dogs (Study Number (b) (4) 11652/98)

Special Toxicology

- 1) Acute dermal irritation/corrosion test (Patch test) of BF-200 ALA Gel 10% in rabbits (Study Number ALA-AK-PT018)
- 2) BF-200 ALA Gel 10%: Skin sensitization: Local lymph node assay in NMRI mice (Study Number ALA-AK-PT019)
- 3) BF-200 ALA Gel 10%: Skin sensitization: Local lymph node assay in NMRI mice (Study Number ALA-AK-PT029)
- 4) Acute dermal irritation/corrosion test (Patch test) of BF-200 ALA Gel 10% in rabbits (Study Number ALA-AK-PT030)

3.3 Previous Reviews Referenced

None

4 Pharmacology

4.1 Primary Pharmacology

Study 1

Comparison of the photodynamic induction of cell death in a squamous cell carcinoma cell line by different red light sources (Study Number ALA-AK-PT032)

A431 Squamous Cell Carcinoma (SCC) cells were treated in vitro with the vehicle, 0.6 nM, or 6.0 nM ALA for 1 or 3 hours, followed by illumination with BF-RhodoLED lamp (630 nm) at 3, 6, 15, or 37 J/cm², Aktilite CL128 lamp (635 nm) at 3, 6, 15, or 37 J/cm², or PhotoDyn 750 lamp (above 590 nm continuous) at 7.5, 15, 75, or 170 J/cm². Cell viability in response to PDT treatment was assessed. The results showed that both LED systems (Aktilite CL128 and BF-RhodoLED lamps) were equally effective in the induction of SCC cell death, while the broad spectrum light system (PhotoDyn 750 lamp) was significantly less efficient in the induction of SCC cell death.

4.2 Secondary Pharmacology

N/A

4.3 Safety Pharmacology

Study 1

5-Aminolevulinic acid, hydrochloride: Effect on hERG tail currents recorded from stably transfected HEK 293 cells (Study Number ALA-AK-PT0036)

The potential effects of ALA on hERG (human-ether-a-go-go related gene) potassium channels stably expressed in HEK 293 (human embryonic kidney) cells were evaluated in vitro. Treatment with 6 mM ALA did not change the hERG tail current amplitude. However, the reference compound, E-4031 at 100 nM caused significant inhibition (97%) of hERG tail current amplitude in this study.

Study 2

Examination of the influence of MC 506 (5-aminolevulinic acid hydrochloride) on several cardiovascular parameters and the respiration in anesthetized beagle dogs following intravenous administration (Study Number ALA-AK-PT (b) (4) 10115-1-96)

Five female dogs were treated with the vehicle (5.7% sodium monohydrogenphosphate) and with increasing dose levels (5, 15, and 45 mg/kg) of ALA HCl by intravenous administration. The intravenous administration of test article was carried out at intervals of 30 minutes in a dark room with a light source that did not emit any light at <635 nm. The heart rate, peripheral arterial blood pressure, and central venous pressure were measured continuously and recorded immediately before and after dosing as well as 5,

15 and 30 min after administration for periods of approximately 30 sec. The pulmonary arterial blood pressure, left ventricular blood pressure, dP/dt max, and respiratory rate and volume were recorded continuously but only evaluated immediately before and after the administration as well as 5, 15, and 30 min after administration. The cardiac output and the blood gases were measured immediately before and after 5, 15, and 30 min after administration. There were no treatment-related effects on systolic and diastolic blood pressure, heart rate, cardiac output or stroke volume, left ventricular pressure, dP/dt max, central venous pressure, blood gas analysis (pH, pO₂ and pCO₂), respiratory rate and volume following treatment with 5 or 15 mg/kg ALA HCl. Only a very slight decrease in the peripheral arterial blood pressure, systolic left ventricular pressure, and dP/dt max was noted immediately after treatment with 45 mg/kg ALA HCl. All parameters reached the pre-dosing levels at 5 min post-dose.

Reviewer's comments: No significant treatment related effects were noted in the in vivo safety pharmacology study in anesthetized Beagle dogs at a single intravenous dose up to 45 mg/kg ALA HCl. In addition, no effects were noted in the in vitro hERG assay at a high concentration of 6 mM ALA HCl.

It is anticipated that no systemic safety pharmacology signals of concern will be noted under clinical use conditions of Ameluz based on the endogenous levels of ALA and the negligible additional systemic exposure noted after topical treatment with Ameluz under maximal clinical use conditions (refer to Section 5 below). The sponsor did not conduct any additional safety pharmacology studies for Ameluz which is acceptable.

5 Pharmacokinetics/ADME/Toxicokinetics

Study 1

The in vitro percutaneous absorption of radiolabelled [¹⁴C]-5-ALA through human skin (Study Number ALA-AK-PT002)

Following topical application of [¹⁴C]-5-ALA in 1%, 3%, and 10% gel formulations to full-thickness human skin in vitro, the unabsorbed dose at 24 hours was 96.97%, 100.16%, and 101.23% of the applied dose, respectively. The absorbed dose of [¹⁴C]-5-ALA was 0.30% (0.60 µg equiv./cm²), 0.09% (0.54 µg equiv./cm²), and 0.09% (1.80 µg equiv./cm²), respectively. The dermal delivery of [¹⁴C]-5-ALA was 1.30% (2.65 µg equiv./cm²), 0.55% (3.38 µg equiv./cm²), and 0.36% (7.53 µg equiv./cm²), respectively. The mass balance of [¹⁴C]-5-ALA was complete with 100.13%, 102.03%, and 102.02% recovered, respectively.

Study 2

The in vitro percutaneous absorption of a nanoemulsion formulation radiolabelled [¹⁴C]-5-ALA through human skin (Study Number ALA-AK-PT005)

Following topical application of 5-ALA gel, 10% containing [¹⁴C]-5-ALA to full-thickness human skin in vitro, the unabsorbed dose at 1, 3, 12, and 24 h was 98.52%, 89.77%,

96.36%, and 92.46% of the applied dose, respectively. The absorbed dose of [^{14}C]-5-ALA at 1, 3, 12, and 24 h was <0.01% (0.06 $\mu\text{g equiv./cm}^2$), 0.31% (6.33 $\mu\text{g equiv./cm}^2$), 0.06% (1.23 $\mu\text{g equiv./cm}^2$), and 0.38% (7.65 $\mu\text{g equiv./cm}^2$), respectively. The dermal delivery of [^{14}C]-5-ALA at 1, 3, 12, and 24 h was 0.21% (4.31 $\mu\text{g equiv./cm}^2$), 2.04% (41.33 $\mu\text{g equiv./cm}^2$), 0.23% (4.57 $\mu\text{g equiv./cm}^2$), and 0.91% (18.33 $\mu\text{g equiv./cm}^2$), respectively. Mass balance of [^{14}C]-5-ALA was essentially complete (92.11% - 98.96%).

Human Pharmacokinetic information for Ameluz gel under maximal clinical use conditions

In a maximal clinical use pharmacokinetic study in 12 actinic keratosis patients, plasma levels of ALA and PpIX were assessed up to 24 hours after application of an entire tube (2 g) of Ameluz or vehicle gel, respectively, for 3 hours with subsequent PDT. This represents maximal clinical use conditions as confirmed by the Clinical Pharmacology reviewer (refer to Clinical Pharmacology review if additional details are needed). ALA and PpIX concentrations in plasma were measured through fully validated liquid chromatography-tandem mass spectroscopy (LC-MS/MS) methods. The lower limit of quantification (LLOQ) was 1 ng/mL for each analyte.

Geometric mean baseline concentrations of ALA were 15.80 ng/ml in the vehicle gel treated group compared to 17.28 ng/ml in the Ameluz treated group. There was a negligible increase in the geometric mean plasma concentrations of ALA compared to baseline endogenous levels after topical treatment with Ameluz. A baseline adjusted maximum geometric mean ALA concentration of 21.56 ng/ml [unadjusted C_{max} of 41.18 ng/ml] was reached 3 hours after application. Thereafter, ALA seems to be eliminated quickly from plasma returning to endogenous baseline levels within 10 hours after topical application.

No increase in PpIX plasma concentrations were observed in any of the subjects after topical application of Ameluz compared to baseline in this study.

The results from the maximal use clinical pharmacokinetic study indicate a negligible increase in plasma levels of ALA and no increase in PpIX levels. Since PpIX levels are not increased, there may be no risk of a general light sensitivity of patient skin after topical application of Ameluz.

6 General Toxicology

6.1 Single-Dose Toxicity

N/A

6.2 Repeat-Dose Toxicity

Study 1**14-Day subchronic toxicity study of MC 506/1 (5-aminolevulinic acid hydrochloride) by intravenous administration to beagle dogs (Study number ALA-AK-PT****(b) (4) 11664-98)**

Four groups of 5 male and 5 female dogs received 0 (0.9% NaCl), 3, 9, or 27 mg/kg ALA HCl (MC 506/1) intravenously once daily for 14 days. Three males and three females were sacrificed on day 15 and the remaining animals were observed for 14 additional days. The experiment was conducted in a dark room with a light source that did not emit any light below 635 nm.

The treatment with all ALA doses led to vomiting and increased salivation. In addition, a reduced body weight and food consumption, and dose dependent changes in several clinical chemistry parameters were noted at 9 and 27 mg/kg/day ALA.

Starting at 3 mg/kg/day ALA, an intrahepatic cholestasis was noted which exhibited a dose-dependent increase in severity. The finding, which is considered to be related to the treatment with ALA, was only marginal at the low dose. The NOAEL was 3 mg/kg/day in this study.

Within the 14-day treatment-free recovery period, the intrahepatic cholestasis had completely recovered at the low dose. However, the treatment related intrahepatic cholestasis was not completely reversible at 9 or 27 mg/kg/day ALA.

ECG recordings were done on test days 1 and 14 in all animals (before and 5 minutes after drug administration) and on test day 28 for animals scheduled for the recovery period. No treatment related effects on ECG parameters were noted in this study.

Blood samples were collected for toxicokinetics analysis from animals in the low- and high-dose groups at 1 minute, 2, 4, 8, and 24 hours post dose on Days 1 and 14.

One minute after administration mean peak values of 10063.7 and 7174.9 µg ALA/L in plasma were determined in the low dose animals for males and females, respectively (range of individual values: 4852.3 to 10721.0 µg/L). In the high dose animals, mean peak values of 59317.4 and 71643.6 µg ALA/L were noted in plasma of males and females, respectively (range of individual values: 43741.0 to 89971.9 µg/L). Plasma elimination half-life for ALA HCl was 50.5 to 68.3 minutes. Protoporphyrin-IX levels were mostly below the limit of quantification, except for high dose males.

The AUC values for ALA and protoporphyrin-IX from this study are provided in the following table (copied from the study report).

AUC _{0-24h} [mg * hour/L]			
5-Aminolevulinic acid		Protoporphyrin-IX	
Group 2: 3 mg/kg <u>male animals</u>		Group 2: 3 mg/kg <u>male animals</u>	
TD 1	11.6	TD 1	-
TD 14	9.0	TD 14	-
<u>female animals</u>		<u>female animals</u>	
TD 1	8.2	TD 1	-
TD 14	10.2	TD 14	-
Group 4: 27 mg/kg <u>male animals</u>		Group 4: 27 mg/kg <u>male animals</u>	
TD 1	71.0	TD 1	0.165
TD 14	85.6	TD 14	0.129
<u>female animals</u>		<u>female animals</u>	
TD 1	80.2	TD 1	-
TD 14	87.7	TD 14	-

- no evaluation possible

Study 2

Study title: 3-Month local tolerance and toxicity study of BF-200 ALA in minipigs following repeated dermal administration (once monthly)

Study no.: 24945 (b) (4) report number) or ALA-AK-PT017 (Sponsor report number)
Study report location: Electronic, SDN 1
Conducting laboratory and location: (b) (4)
Date of study initiation: January 12, 2010
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: ALA Gel, 10%, Batch no. 111A, 98.4% purity
ALA Gel vehicle, Batch No. 20091109

Key Study Findings

Topical treatment with ALA gel, 10% in minipigs, with and without red light illumination, resulted in pronounced erythema 3 hours after illumination which reached levels comparable to severe sunburn. The incidence and severity of the findings were more pronounced for the illuminated ALA gel, 10% treated sites compared to the non-illuminated ALA gel, 10% treated sites. The erythema lasted until the next monthly treatment. Slight eschar formation was noted at ALA gel, 10% treated sites starting on day 3 after treatment. The erythema and eschar formation were considered to be related to the administration of ALA gel, 10% and intensified by the illumination. The skin reaction observed in non-illuminated areas may be due to insufficient light protection during the handling procedures after application. Slight erythema was noted for 2 out of 4 animals on test days 1 and 2 and for 3 out of 4 animals on test day 28 after treatment with vehicle gel. The erythema noted for vehicle gel treated sites may have been caused by the administration technique and subsequent removal of the light-proof dressing.

No edema formation was noted at any of the treated sites.

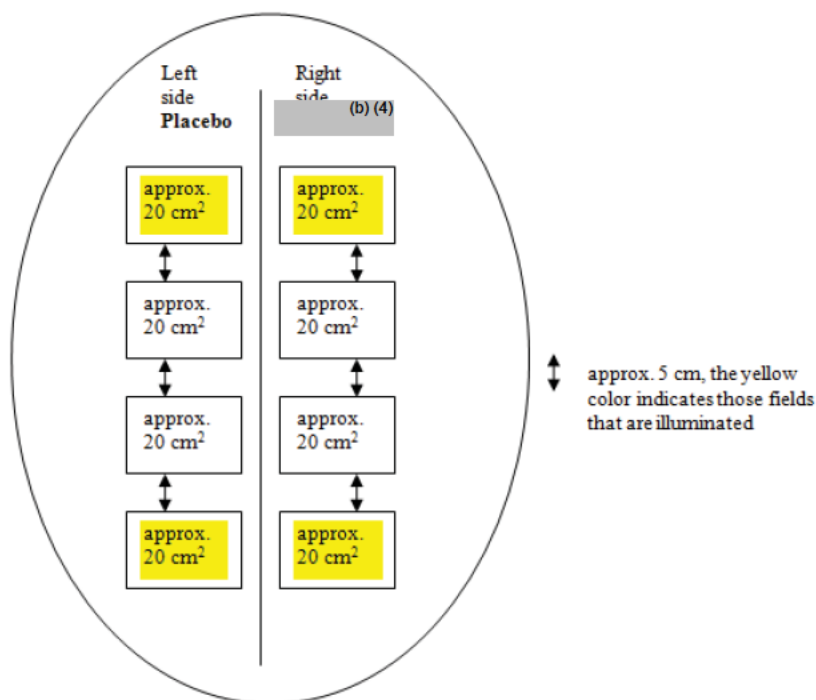
A mild to moderate superficial purulent dermatitis with inflammatory reactions in the dermis was noted at ALA gel, 10% treated sites on test day 88 (3 days after last application). This treatment related effect was generally more pronounced for the illuminated ALA gel, 10% treated skin sites compared to the non-illuminated ALA gel, 10% treated skin sites. An almost complete reversibility of the skin changes was noted after 28 days. The morphological structure of the skin treated with ALA gel, 10% with illumination was comparable to vehicle gel treated skin 28 days after the last application.

Methods

Doses: 0% (vehicle gel) and 10% ALA gel
Frequency of dosing: Once monthly (days 1, 29, 57 and 85)
Route of administration: Topical
Dose volume: Refer to study procedure description below
Formulation/Vehicle: Clinical vehicle
Species/Strain: Minipig / Gottingen
Number/Sex/Group: 4 animals total (2 males and 2 females)
Age: 4 months
Weight: Males: 7.7 and 7.8 kg; females: 7.4 and 7.6 kg
Satellite groups: N/A
Unique study design: Refer to study procedure description below
Deviation from study protocol: None of significance

Study Procedure:

Four 5 x 4 cm sites were assigned on the right and left back of the minipigs (8 treatment sites total) for treatment with either 10% ALA gel on 4 sites on the right side or vehicle gel on 4 sites on the left side. Each site had an area of approximately 20 cm². The sites on each side were serially arranged with a distance of about 5 cm to allow appropriate application of the gel and dressing without contamination or overlap (refer to figure below, copied from study report). Either 2 grams of vehicle gel or 2 grams 10% ALA gel were applied to each 20 cm² site with a 1 mm thickness. The 2 grams of 10% ALA gel treatment corresponds to a total dose per treatment area of 200 mg ALA HCl (10 mg ALA HCl/cm²). This treatment regimen and dose corresponds with the clinical use of Ameluz gel and used repeated monthly applications.



An Aktilite CL128 illumination source (LED lamp emitting red light in the range of 630 nm) was used to illuminate designated treatment sites with a light dose of approx. 37 J/cm². The value was automatically calculated by the lamp software. The distance from skin to lamp was 6 cm (5-8 cm). A spacer was used to assure a reproducible distance for all animals. The animals were restrained during the illumination procedure to guarantee a proper illumination of the individual sites. The treatment sites that were illuminated were the upper and lower treatment sites on each side of the back as indicated in the figure above, the other fields were covered by light-tight dressing during illumination.

Observations and Results

Mortality

Mortality was evaluated once daily. There were no treatment related effects on mortality.

Clinical Signs

Clinical signs were evaluated once daily. The study report indicates that illumination of the skin appeared to be painful. However, a veterinary intervention was not considered necessary.

Body Weights

Body weights were evaluated once weekly. There were no treatment related effects on body weights.

Feed Consumption

Food consumption was evaluated once weekly. There were no treatment related effects on food consumption.

Local Tolerance

The application sites were assessed at the following times.

On days of application: pre-dosing, at the end of the 3-hour occlusion period, at the end of light exposure, and 3 and 6 hours after illumination.

The application sites were assessed once daily thereafter until the end of the study.

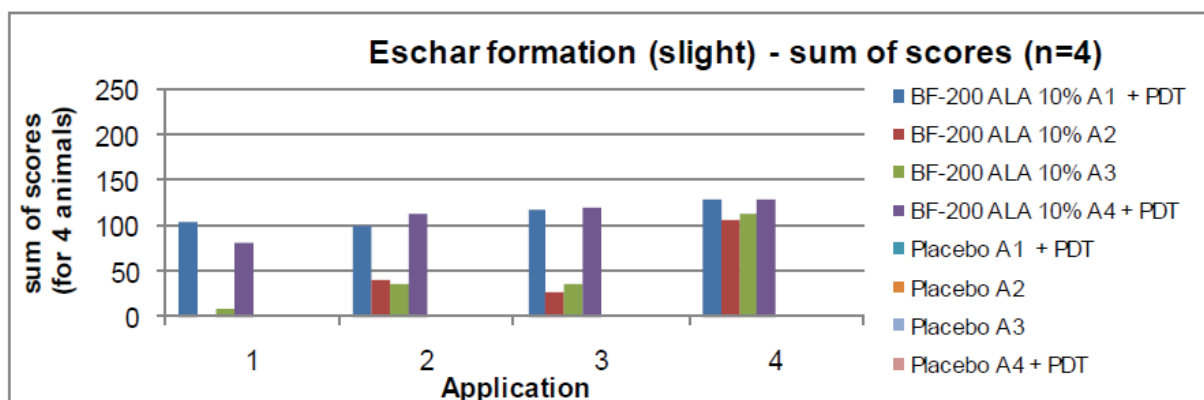
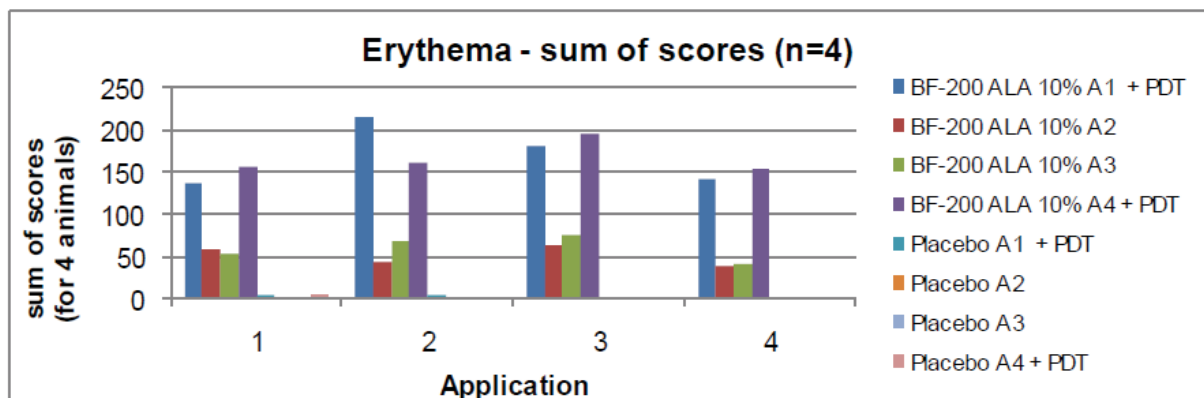
The skin reactions were scored based on the Draize scale.

Treatment with ALA gel, 10% with illumination resulted in pronounced erythema, comparable to severe sunburn, 3 hours after illumination that lasted until the next treatment. Slight eschar formation (scab) was noted starting on test day 3. The eschar was brownish, slightly red with a solid consistency over the complete application area with a thickness of approximately 1 mm. Eschar formation was observed following each administration with a diffuse distribution. However, the incidence decreased slightly with time until the next administration. The incidence and severity of these findings were more pronounced for application sites 1 and 4 (ALA gel, 10% with illumination) compared to application sites 2 and 3 (ALA gel, 10% without illumination). These changes are considered to be related to the administration of the test item and intensified by the illumination. Reduced erythema noted on non-illuminated ALA gel, 10% treated sites may have been caused by insufficient light protection which could not be absolutely guaranteed due to the handling procedure during and after application.

Very slight erythema was noted for two of 4 animals on test days 1 and 2 and for 3 animals on test day 29 after treatment with vehicle gel. The study report indicates that the effects noted for vehicle gel are considered to be possibly caused by the administration and securing of the lightproof dressing. This seems like a reasonable explanation for the effects noted at the vehicle gel treated sites.

No edema formation was noted at any treatment site.

An overview of the erythema and eschar formation is provided in the following figure (copied from the study report).



Histopathology

Adequate Battery

No, only the treatment site was evaluated in this study. However, this is appropriate for this study.

From all animals punch biopsies (diameter approx. 4 mm) were taken at each of the sites at the following times: day 88 (3 days after the last administration), day 99 (14 days after the last administration), day 114 (28 days after the last administration and before sacrifice). Tissue samples obtained from punch biopsies (in total 27 sections per animal) and at necropsy (in total 9 sections per animal) were examined histologically after preparation of paraffin sections and hematoxylin-eosin staining.

Peer Review

No

Histological Findings

A mild to moderate superficial purulent dermatitis with inflammatory reactions in the dermis on test day 88 (3 days after the last administration) was noted at ALA gel, 10% treated sites. The intensity of these changes was decreased on test day 99 (14 days after the last administration) and even further on test day 114 (28 days after the last administration, before sacrifice). The effects observed for ALA gel, 10% treated sites

with illumination appeared to be generally more pronounced compared to the skin sections without illumination. An almost complete reversibility of the skin changes was noted after 28 days. The morphological structure of the skin treated with ALA gel, 10% and illuminated was comparable to vehicle-treated skin 28 days after the last application. No obvious differences were noted between males and females.

Dosing Solution Analysis

Not provided.

7 Genetic Toxicology

7.1 In Vitro Reverse Mutation Assay in Bacterial Cells (Ames)

Study title: Mutagenicity study of MC 506 (5-aminolevulinic acid HCl) in the *Salmonella Typhimurium* reverse mutation assay (in vitro)

Study no.: 10112/96

Study report location: Electronic , SDN 1

Conducting laboratory and location:

(b) (4)

Date of study initiation: 2-7-97

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: 5-aminolevulinic acid HCl, Batch#
960315/2, 98.84%

Key Study Findings

Aminolevulinic acid HCl was not mutagenic in the Ames test, under the conditions of this experiment.

Methods

- Strains: *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537
- Concentrations in definitive study: 100, 316, 1000, 3160, 10000 µg/plate (± S9; S9 derived from Aroclor 1254 induced rat liver homogenate); 3 plates/dose, 2 experiments
- Basis of concentration selection: Dose range finding study performed with concentrations of 0.316, 1.0, 3.16, 10, 31.6, 100, 316, 1000, 3160 and 10000 µg/plate in all tester strains. No precipitate or cytotoxicity was noted at concentrations up to 10000 µg/plate.
- Negative control: Phosphate buffered saline
- Positive control: Refer to table below
- Formulation/Vehicle: Phosphate buffered saline
- Incubation & sampling time: Plates were incubated at 37 ± 2°C for 2 days after treatment. Plates were counted for colony formation by hand after completion of the incubation period.

Positive control substances table is provided below.

a) without metabolic activation	
1. sodium azide ⁴ in H ₂ O (10 µg/plate)	TA 1535, TA 100
2. 2-nitro-9H-fluorene ⁴ in DMSO (10 µg/plate)	TA 98
3. 9-amino-acridine ⁴ in ethanol (100 µg/plate)	TA 1537
4. methyl methane sulfonate ⁴ , MMS in DMSO (1300 µg/plate)	TA 102
b) with metabolic activation	
2-aminoanthracene ⁴ in DMSO (2 µg/plate)	TA 98, TA 100, TA 102, TA 1535, TA 1537

4 (b) (4)

Study Validity

A test article was considered to be positive if it produced at least a 2-fold increase in the mean revertants per plate for tester strains TA98, TA100 or TA102 or if it produced at least a 3-fold increase in mean revertants per plate for tester strains TA1535 or TA1537. This increase in the mean number of revertants per plate had to be accompanied by a dose response to increasing concentrations of the test article.

Solvent control mean reversion frequencies fell within established ranges. Positive control results were appropriate showing a mean reversion frequency that was three times or more greater than the mean reversion frequency of the solvent control plates. The dose range selected for the definitive study was appropriate according to ICH guidelines, even though the high dose of 10000 µg/plate was higher than the ICH recommended high dose of 5000 µg/plate.

Results

The test article produced a negative response in the presence and absence of S-9 activation. All of the tester strains treated with the test article exhibited a mean reversion frequency that was similar to the corresponding solvent control.

7.2 In Vitro Assays in Mammalian Cells

Study 1

Study title: Mutagenicity study of MC 506 In mammalian cells (V79) in the in vitro gene mutation assay (HPRT Test)

Study no.:	10113/96
Study report location:	Electronic , SDN 1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	2-10-97
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	5-aminolevulinic acid HCl, Batch# 960315/2, 98.84%

Key Study Findings

Aminolevulinic acid HCl was negative in the hypoxanthine guanine phosphoribosyltransferase (HPRT) test with and without S9 activation, under the conditions of this assay.

Methods

Cell line:	V79 cells (derived from lung tissue of fetal hamsters)
Concentrations in definitive study:	Concentrations of 312.5, 625, 1250, 2500 and 5000 µg/ml aminolevulinic acid HCl (± S9; S9 derived from Aroclor 1254 induced rat liver homogenate) were evaluated in two independent experiments
Basis of concentration selection:	Dose range finding study performed with concentrations of 5, 10, 50, 100, 500, 1000 and 5000 µg/ml aminolevulinic acid HCL (± S9). Significant cytotoxicity was noted at 5000 µg/ml. Concentrations selected for

the definitive study utilized a high dose of 5000 µg/ml even though significant cytotoxicity was noted at this concentration.

Negative control: Phosphate buffered saline

Positive control: Ethyl metanesulfonate dissolved in DMSO (-S9): 700 µg/ml
9,10-dimethyl-1,2-benzanthracene dissolved in DMSO (+S9): 30 µg/ml

Formulation/Vehicle: Phosphate buffered saline

Incubation & sampling time: Cell cultures were exposed to test article for 24 hours in the absence of S9 and for 4 hours in the presence of S9. Cells were processed by standard techniques for this assay and stained appropriately for measurement of mutation frequency.

Study Validity

A test article was considered to be positive if it produced at least a 2-fold increase in the mutation frequency. This increase in the mutation frequency had to be accompanied by a dose response to increasing concentrations of the test article.

Solvent control mean reversion frequencies fell within established ranges. Positive control results were appropriate for this assay. The dose range selected for this assay was appropriate according to ICH guidelines.

Results

No significant increase in mutation frequency was noted in the analyzed cultures. Moderate cytotoxicity was noted at 2500 µg/ml aminolevulinic acid HCL (± S9) and significant cytotoxicity was noted at 5000 µg/ml aminolevulinic acid HCL (± S9).

Study 2

Study title: In vitro assessment of the clastogenic activity of MC 506 (5-aminolevulinic acid HCl) in cultured human peripheral lymphocytes

Study no.: 10114/96

Study report location: Electronic , SDN 1

Conducting laboratory and location: (b) (4)

Date of study initiation: 2-10-97

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: 5-aminolevulinic acid HCl, Batch# 960315/2, 98.84%

Key Study Findings

Aminolevulinic acid HCl was negative in the human blood peripheral lymphocyte assay with and without S9 activation, under the conditions of this assay.

Methods

Cell line:	Human peripheral blood lymphocytes
Concentrations in definitive study:	Concentrations of 250, 500, 1000, 2000 and 4000 µg/ml aminolevulinic acid HCl (± S9; S9 derived from Aroclor 1254 induced rat liver homogenate, 4 hour exposure) and Concentrations of 125, 250, 500, 1000 and 2000 µg/ml aminolevulinic acid HCl (-S9, 24 hours exposure) were evaluated in two independent experiments
Basis of concentration selection:	Dose range finding study performed with concentrations of 5, 10, 50, 100, 500, 1000 and 5000 µg/ml aminolevulinic acid HCL (± S9, 4 hour exposure; -S9, 24 hour exposure). Significant cytotoxicity was noted at 5000 µg/ml (± S9, 4 hour exposure) and cytotoxicity was noted at 1000 µg/ml (-S9, 24 hours exposure). Concentrations selected for the definitive study utilized a high dose of 4000 µg/ml (± S9, 4 hour exposure) and 2000 µg/ml (-S9, 24 hours exposure).
Negative control:	Phosphate buffered saline
Positive control:	Mitomycin C (-S9): 0.2 µg/ml for 4 hour exposure, 0.1 µg/ml for 24 hour exposure Cyclophosphamide (+S9): 20 µg/ml for 4 hour exposure
Formulation/Vehicle:	Phosphate buffered saline
Incubation & sampling time:	Cell cultures were incubated with test article ± S9 for 4 hours and harvested 20 hours after treatment initiation. Cell cultures were incubated with test article –S9 for 24 hours and then harvested for analysis. Slides were prepared from the harvested cultures and stained with 5% Giemsa solution for the analysis of mitotic index and chromosomal aberrations.

Study Validity

A test article was considered to be positive for inducing chromosomal aberrations if a significant increase (the difference was considered significant when $p \leq 0.05$) in the

number of cells with chromosomal aberrations is observed at one or more concentrations.

Solvent control mean reversion frequencies fell within established ranges. Positive control results were appropriate for this assay. The dose range selected for this assay was appropriate according to ICH guidelines.

Results

No significant increase in cells with chromosomal aberrations was noted in the analyzed cultures.

7.3 In Vivo Clastogenicity Assay in Rodent (Micronucleus Assay)

Study title: Micronucleus test of 5-ALA in bone marrow cells of the NMRI mouse following oral administration

Study no: 17551/03

Study report location: Electronic, SDN 1

Conducting laboratory and location:

(b) (4)

Date of study initiation: 2-13-04

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: 5-aminolevulinic acid HCl, Batch# M30906, 100%

Key Study Findings

Aminolevulinic acid HCl was negative in the in vivo mouse micronucleus assay, under the conditions of this experiment.

Methods

Doses in definitive study: 0, 400, 800 and 1600 mg/kg aminolevulinic acid HCl

Frequency of dosing: Once

Route of administration: Oral (gavage)

Dose volume: 10 ml/kg

Formulation/Vehicle: 0.8% carboxymethylcellulose

Species/Strain: Mice/NMR1

Number/Sex/Group: 5/sex/group/timepoint

Satellite groups: N/A

Basis of dose selection: Preliminary toxicity study was conducted with oral (gavage) doses of 125, 250, 500, 1000 and 2000 mg/kg aminolevulinic acid HCl in 1 animal/dose. The animal treated with 2000 mg/kg died after 2 days. No mortality was noted at 1000 mg/kg. The high dose was selected as 1600 mg/kg aminolevulinic acid HCl.

Negative control: 0.8% carboxymethylcellulose

Positive control: Cyclophosphamide (27 mg/kg), oral (gavage)

Sampling times: Single oral (gavage) doses of aminolevulinic acid HCl or cyclophosphamide were administered to mice. Bone marrow for analysis of nucleated cells was obtained from control and aminolevulinic acid HCl treated mice at 24 and 48 hours (5/sex/group/timepoint) after dose administration and at 24 hr after dose administration in positive control animals (5/sex). Stained bone marrow slides were scored for micronucleus and the PCE (polychromatic erythrocytes) to NCE (normal chromatic erythrocytes) cell ratio. The micronucleus frequency (expressed as percent micronucleated cells) was determined by analyzing the number of micronucleated PCEs from at least 2000 PCEs per animal. The PCE:NCE ratio was determined by scoring the number of PCEs and NCEs observed while scoring at least 500 erythrocytes per animal.

Study Validity

A test article was considered to be positive if a statistically significant increase in micronucleated PCEs was noted for at least one dose level, and a statistically significant dose-related response were observed.

Solvent control mutant frequencies fell within established ranges. Positive control results were appropriate. Dose range selected for the definitive study was appropriate according to ICH guidelines.

Results

Aminolevulinic acid HCl did not induce any statistically significant increases in micronucleated PCEs at any of the doses tested in this study.

7.4 Other Genetic Toxicity Studies

There is literature data that indicates a low genotoxicity potential of ALA when combined with UVA light exposure¹. The observed DNA damage is probably caused by the oxidative free radicals formed when ALA derived PpIX is exposed to light of the correct wavelength. This is the desired pharmacologic effect that is utilized for the treatment of actinic keratosis lesions.

8 Carcinogenicity

No carcinogenicity study has been conducted with ALA or ALA gel, 10%. Carcinogenicity studies for ALA gel, 10% are waived due to the clinical conditions of use (single application followed by another single application after 3 months, if needed) and the negligible increase in systemic levels of ALA compared to baseline endogenous levels of ALA under maximal clinical conditions of use (refer to Section 5).

9 Reproductive and Developmental Toxicology

No reproductive and developmental toxicology study has been conducted with ALA. Reproductive and developmental toxicity studies for ALA gel, 10% are waived due to the negligible increase in systemic levels of ALA compared to baseline endogenous levels of ALA under maximal clinical conditions of use (refer to Section 5).

10 Special Toxicology Studies

Study 1

Dermal tolerability study in rabbits (Study number ALA-AK-PT001)

Each of five female rabbits received 0.5 g of the ALA gel, 10% (Formulation A) topically over a shaved dorsal skin area of about 6.25 cm² size (80 mg ALA HCl/cm²). An untreated skin area on the counter side served as control. The gel was administered for 4 hours under occlusion and the treated areas were light protected. After the incubation period the gel was wiped off and the skin was evaluated over a period of 14 days post-dosing. No skin reactions were noted at any treatment site.

¹ Fuchs J, Weber S, and Kaufmann R. 2000. Genotoxic potential of porphyrin type photosensitizers with particular emphasis on 5-aminolevulinic acid: Implications for clinical photodynamic therapy. Free Radical Biology & Medicine. 28(4): 537-548.

Study 2**Dermal tolerability study in rabbits (Study number ALA-AK-PT012)**

Each of five female rabbits received 0.5 g of the ALA gel, 10% (Formulation B) or vehicle gel topically applied over a shaved dorsal skin area of about 6.25 cm². The gel was administered for 4 hours under occlusion and the treated areas were light protected. After the treatment period, the gel was wiped off and the skin was evaluated over a period of 7 days post-dosing. No skin reactions were noted at any treatment site, except that in one animal a very slight erythema was noted at the 24 and 48 hour observation time point.

Study 3**Local lymph node assay in mice (LLNA/IMDS) (Study number ALA-AK-PT007)**

The sensitization and/or irritation potential of ALA Gel (Formulation A) was tested using a modified local lymph node assay. Four groups of 6 female mice were treated with vehicle, 1%, 3%, or 10% ALA gel at 25 µL/ear applied to the dorsal part of both ears once daily on three consecutive days. Animals were exposed to a regular 12 hour light/dark cycle. Two additional groups of 6 female mice were treated with vehicle or 10% ALA gel under yellow light and then kept in the dark. After autopsies on day 4, auricular lymph nodes were removed and transferred into physiological saline and weight and cell counts were determined. There were no treatment-related effects on cell counts or weights of the draining lymph nodes, indicating that topical treatment with up to 10% ALA gel did not cause skin sensitization. However, statistically significant increases in ear swelling and ear weights were noted in mice treated with 3% or 10% ALA gel, indicating a skin irritation reaction. No differences were noted in the two 10% ALA gel treated groups (i.e., with and without exposure to yellow light).

Study 4**Local lymph node assay in mice (LLNA/IMDS) (Study number ALA-AK-PT013)**

Two groups of 6 female mice were treated with vehicle gel or ALA gel, 10% (Formulation B). An aliquot (25 µL/ear) of each test article was applied to the dorsal part of both ears once daily on three consecutive days. Animals were sacrificed on day 4. There were statistically significant increases in cell counts and weights of the draining lymph nodes and in ear swelling and ear weights in mice treated with ALA gel, 10% compared to the mice treated with vehicle gel. The results showed that ALA gel, 10% has an irritant potential in mice following dermal application. The study report indicated that the induction of lymph node cell proliferation was likely due to a non-specific activation by the acute inflammation in the ear skin.

Study 5

Acute eye irritation/corrosion test of BF-200 ALA gel 10% in rabbits (Study number ALA-AK-PT027)

Three young adult rabbits received 0.1 mL ALA gel, 10% (Formulation B) in the right eye and 0.1 mL vehicle gel as control in the left eye. After placing the gels into the conjunctival sac of the assigned eye of each animal, the lids are gently held together for about one second in order to prevent loss of the material. Room light was minimized for up to 24 h after instillation to avoid phototoxic effects by ambient light. The eyes were rinsed with saline at 24 hours post-dosing and additionally treated with fluorescein to render changes on the eye ball's surface visible. The eyes were examined prior to administration and 1, 24, 48, 72 hours and 7 days post application. No ocular irritation was observed in either ALA gel, 10% or vehicle gel treated animals in this study. ALA gel, 10% was non-irritating to rabbit eyes.

11 Integrated Summary and Safety Evaluation

Aminolevulinic acid (ALA) is a delta-amino acid and occurs as an endogenous molecule of the heme biosynthesis pathway in almost every cell in humans, animals and plants. The hydrochloride salt is used as the drug substance in the to-be-marketed Ameluz (ALA HCl) gel, 10% formulation. ALA is used in this topical drug product as a photodynamic therapy photosensitizer. Photodynamic therapy (PDT) requires 3 components: (1) a photosensitizer, (2) light with a sufficient amount of energy at a suitable spectrum of wavelengths, and (3) oxygen. In PDT light energy is transferred through the photosensitizer to oxygen, leading to the formation of reactive oxygen species (ROS). ROS oxidize cell membranes and other cellular compounds, causing necrosis or apoptosis of targeted cells. ALA functions as a pro-drug and is metabolized to the photoactive substance protoporphyrin IX (PpIX) in mitochondria. PpIX is a natural metabolite of ALA within the heme pathway. PpIX can be activated by the absorption of energy at several different wave lengths, ranging from blue to red light.

The sponsor has developed Ameluz gel in combination with red light photodynamic therapy (PDT) using the BF-RhodoLED® lamp, for the (b) (4) treatment of actinic keratoses (AKs) of mild to moderate severity on the face and scalp. The sponsor has demonstrated that there is a negligible systemic increase in plasma levels of ALA above background endogenous levels and no increase in PpIX plasma levels (which is a biomarker of systemically absorbed ALA pharmacology) under maximal clinical use conditions for Ameluz gel.

The need for reproductive toxicity studies and a systemic carcinogenicity study were waived based on the level of systemic exposure demonstrated under maximal clinical use conditions for Ameluz. Maternal use of Ameluz is not expected to result in fetal exposure to the drug and breastfeeding is not expected to result in exposure of the child to the drug due to the negligible systemic absorption of ALA following topical administration of Ameluz under clinical maximal use conditions. The need for a dermal

carcinogenicity study for Ameluz was waived due to the clinical conditions of use (single application followed by another single application after 3 months, if needed).

The sponsor conducted a 14 day repeat dose intravenous dog toxicity study. Intravenous doses of 0 (0.9% NaCl), 3, 9, or 27 mg/kg/day ALA HCl were administered in this study. The target organ of toxicity noted in high dose animals was the liver.

The sponsor conducted a repeat dose dermal minipig study with once monthly topical administration of ALA gel, 10% or vehicle gel for 3 months with and without red light exposure. Vehicle gel was topically administered to 4 designated treatment sites (20 cm² each) to the left of dorsal side of each minipig and ALA gel, 10% was administered to 4 designated treatment sites (20 cm² each) to the right of the mid-dorsal side of each minipig. Two female and two male minipigs were treated in this study with topical administration of 2 grams of test article on each designated treatment site. Four of the treatment sites were exposed to red light after topical application of test article and four of the treatment sites were not exposed to red light after topical application of test article.

The results from this study demonstrated the expected toxicity at the treatment site based on the pharmacologic mechanism of photodynamic therapy. Four administrations of ALA gel, 10% with one month intervals demonstrated local toxicity of erythema and eschar formation which was mostly of mild to moderate intensity at ALA gel, 10% treated sites. The symptoms were more pronounced at ALA gel, 10% treated sites exposed to red light. No increase in local toxicity was noted after repeat dose administration and the recovery process appeared to be quicker from the second application through the fourth application. Histopathological evaluation of treated skin sites 28 days after the last ALA gel, 10% treatment with exposure to red light demonstrated complete recovery.

An ICH battery of genotoxicity studies were conducted with ALA HCl. ALA HCl revealed no evidence of mutagenic or clastogenic potential based on the results of three in vitro genotoxicity tests (Ames assay, HPRT test in V79 cells and Human lymphocyte chromosomal aberration assay) and one in vivo genotoxicity test (mouse micronucleus assay). The in vitro genotoxicity studies were conducted without red light exposure. There is literature data that indicates a low genotoxicity potential of ALA when combined with UVA light exposure. The observed DNA damage is probably caused by the oxidative free radicals formed when ALA derived PpIX is exposed to light of the correct wavelength. This is the desired pharmacologic effect that is utilized for the treatment of actinic keratosis lesions.

ALA gel, 10% without red light exposure was not a dermal irritant or ocular irritant in rabbits. ALA gel, 10% without red light exposure was not a sensitizer in the murine LLNA assay.

The toxicity profile of ALA gel, 10% has been adequately characterized by the nonclinical studies conducted by the sponsor. The toxicity profile elicited by ALA gel,

10% in the presence of red light exposure was what is anticipated for PDT. This NDA is approvable from a Pharmacology/Toxicology perspective. No nonclinical postmarketing requirement is recommended for this NDA.

12 Appendix/Attachments

Clean copy of recommended wording for Nonclinical sections of the label

HIGHLIGHTS OF PRESCRIBING INFORMATION

INDICATIONS AND USAGE

AMELUZ Gel, a porphyrin precursor, in combination with photodynamic therapy using the BF-RhodoLED[®] lamp, is indicated for the treatment of actinic keratosis of mild to moderate severity of the face and scalp (b) (4) (1).

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary

There are no available data on AMELUZ use in pregnant women to inform a drug associated risk. Animal reproduction studies were not conducted with ALA. Systemic absorption of ALA in humans is negligible following topical administration of AMELUZ under maximal clinical use conditions [See *Clinical Pharmacology* (12.3)]. It is not expected that maternal use of AMELUZ will result in fetal exposure to the drug.

8.2 Lactation

Risk Summary

No data are available regarding the presence of ALA in human milk, the effects of ALA on the breast fed infant or on milk production. However, breastfeeding is not expected to result in exposure of the child to the drug due to the negligible systemic absorption of ALA in humans following topical administration of AMELUZ under maximal clinical use conditions [See *Clinical Pharmacology* (12.3)].

CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Photoactivation following topical application of AMELUZ occurs when aminolevulinic acid (ALA) (prodrug) is metabolized to protoporphyrin IX (PpIX), a photoactive compound which accumulates in the skin. When exposed to red light of a suitable wavelength and energy, PpIX is activated resulting in an excited state of porphyrin molecules. In the presence of oxygen, reactive oxygen species are formed which causes damage to cellular components, and eventually destroys the (b) (4) cells.

AMELUZ photodynamic therapy of AK lesions utilizes photoactivation of the active substance after topical application of AMELUZ and subsequent illumination with BF-RhodoLED® that provides a red light of narrow spectrum (light dose of approximately 37 J/cm²).

(b) (4)

NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Long-term studies to evaluate the carcinogenic potential of AMELUZ or ALA have not been performed.

ALA revealed no evidence of mutagenic or clastogenic potential based on the results of three in vitro genotoxicity tests (Ames assay, HPRT test in V79 cells and Human lymphocyte chromosomal aberration assay) and one in vivo genotoxicity test (mouse micronucleus assay). These genotoxicity studies were conducted without exposure to light. There is a literature report that indicates that ALA may cause genotoxic effects in the presence as well as in the absence of activating light. These genotoxic effects are likely caused by the formation of reactive oxygen species.

Animal fertility studies have not been conducted with ALA because of the negligible systemic absorption of ALA in humans following topical administration of AMELUZ under maximal clinical use conditions.

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/s/

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