CASE WESTERN RESERVE UNIVERSITY | CASE COMPREHENSIVE CANCER CENTER

NCI Comprehensive Cancer Center
A Cancer Center Designated by the National Cancer Institute

STUDY NUMBER: CASE 4623

ClinicalTrials.gov NCT #:

Protocol Date: 5/16/2023

STUDY TITLE: **Short contact protocols to reduce pain during treatment of actinic keratoses with 10% ALA gel red-light photodynamic therapy (PDT)**

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SPONSOR: Investigator-initiated study
Edward Maytin M.D. Ph.D.

SUPPORT/FUNDING: Biofrontera, Inc.
120 Presidential Way, Suite 330

Woburn, MA 01801

SUPPLIED AGENTS AND DEVICES:

(1) Ameluz gel, 10% aminolevulinic acid, an FDA-approved drug;
(2) BF-RhodoLED®, an FDA-approved light source

Title: Short contact protocols to reduce pain during treatment of actinic keratoses with 10% ALA gel-red light photodynamic therapy (PDT)

Principal Investigator: Edward V. Maytin, M.D. Ph.D.

PRINCIPAL INVESTIGATOR SIGNATURE:

_____ Date: _____

SUMMARY OF CHANGES

Protocol Date	Section	Change
		Initial IRB approval

STUDY SCHEMA

Timeline of procedures for patients enrolled in the study.

	VISIT 1 PDT treatment session #1	VISIT 2 Photos of skin redness	VISIT 3 PDT treatment session #2	VISIT 4 Final lesion counts
Scheduled visit: → Procedure: ↓	**Day 1**	**Day 3 ±1 day**	**Week 8 ±1week**	**Month 3-6**
Sign informed consent	X			
Examine patient, mark each lesion with a pen, and count the lesions	X		X	X
Take photographs in professional studio (frontal view and side views) to document inflammation.	X	X		
Take photographs using clinic iPhone camera to document lesion counts (pen-marks)	X		X	X
Apply topical ALA gel and incubate for 10, 20, or 60 min prior to turning on the light source (as per assigned study arms A, B, or C, respectively)	X		X	
Illuminate with red light for 20, 10, or 10 min (as per assigned study arms A, B, or C, respectively)	X		X	
Record patient-reported pain level (VAS)	X		X	
Give the patient a questionnaire to fill out at home (to describe side-effects on each of the 6 days post-PDT)	X		X	
Provide patient with a hat, sunscreen and aftercare instructions	X		X	
Patient satisfaction survey				X

PROTOCOL SUMMARY

Protocol Number/Title	*Short contact protocols to reduce pain during treatment of actinic keratoses with 10% ALA gel red-light photodynamic therapy (PDT)*
Study Phase	Investigator-initiated study
Brief Background/Rationale	Ameluz 10% ALA gel is a topical FDA-approved photosensitizer that is used in combination with red light (BF-RhodoLED® unit, an FDA-approved device) to treat actinic keratoses. Red light illumination is typically administered after a 3-hour incubation with the ALA gel, and is associated with an undesirable side effect, stinging pain. The proposed clinical study will test the hypothesis that one or more shorter incubation regimens can provide excellent treatment efficacy while reducing or eliminating the pain that is typically experienced by patients with the conventional 3-hour regimen.
Primary Objective	Primary Endpoint(s) Efficacy Pain
Secondary Objective(s)	Secondary Endpoint(s) Patient satisfaction. AE profile/Safety
Exploratory Objective(s)	
Correlative Objective(s)	None
Sample Size	30 participants
Disease sites/Conditions	Actinic Keratosis
Interventions	Standard PDT using topical aminolevulinate (10% ALA gel) followed by red light (FDA-approved protocol) for actinic keratosis.

ABBREVIATIONS

CCCC	Case Comprehensive Cancer Center
CRF	Case Report Form
CRU	Clinical Research Unit
DSTC	Data Safety and Toxicity Committee
FDA	Food and Drug Administration
ICF	Informed Consent Form
IRB	Institutional Review Board
PRMC	Protocol Review and Monitoring Committee
SOC	Standard of Care
CCF	Cleveland Clinic Foundation
UH	University Hospitals
AK	Actinic Keratosis
ALA	Aminolevulinic acid
PpIX	Protoporphyrin IX

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1.0 Introduction

1.1 Background and Significance

Actinic keratoses (AK), are dysplastic precancerous lesions of the skin with potential to develop into squamous cell carcinoma. AKs are extremely common and are typically observed in sun-damaged skin of Caucasian individuals. It is important to treat AKs because of the possibility that 2-5% of them may evolve into squamous cell carcinoma (SCC). Progression of AK to SCC is even higher in immunosuppressed patients, e.g. organ transplantation recipients. Amongst currently available treatments for widespread AK that include surgical (cryotherapy, scalpel excision) and medicinal approaches (topical 5-fluorouracil, imiquimod, diclofenac cream) approaches, a relatively new modality called photodynamic therapy (PDT) has several distinct advantages [*1*]. PDT is a method in which a topical drug such as aminolevulinic acid (ALA) or methyl-ALA is applied to AK lesions, where it is taken up selectively by precancer cells [*2*]. Within the cells, ALA is converted into a photosensitizer (protoporphyrin IX, PpIX) within mitochondria, whereupon a strong visible light is used to activate the PpIX, triggering photochemical reactions such as the generation of reactive oxygen species that kill the precancer cells. PDT excels as a modality to treat widespread areas of field cancerization, and it has the additional advantages of minimal down time and rapid recovery, lack of scarring, lack of pigmentary changes, and repeatability [*3*]. However, a limiting factor with all current versions of aminolevulinate-based PDT is *stinging pain* that patients often have to endure during illumination [*4*]. This pain is proportional to the amount of the target photosensitizer (PpIX) that is present in the skin prior to the start of illumination. Pain is a frequent occurrence in patients who receive PDT with standard protocols currently approved by the FDA, all of which required an ALA incubation period of many hours, i.e., 14-18 hr with Levulan Kerastick (20% ALA), 3 hr with Metvixia (16% methyl-ALA), and 3 hr for Ameluz (10% ALA nanoemulsion). Metvixia and Ameluz, also require that an occlusive dressing be applied to enhance penetration. Metvixia is no longer available in the US, leaving Ameluz and Levulan as the only two viable options for dermatologists providing PDT in the United States. Ameluz has generated significant interest due its properties as lipid-based nanoemulsion that exhibits superior uptake into AK lesions [*5*].

To date, the problem of PDT treatment-associated pain has become a serious impediment to the full implementation and acceptance of PDT by the general population of dermatology patients. Stories about painful experiences during PDT are shared online or by word of mouth, and tend to generate fear and reluctance to undergo PDT. Another issue is lack of convenience. Traditional PDT can require many hours of waiting time in the office, which tends to discourage patients and physicians from embracing PDT as enthusiastically as they should, given its potential benefits. A third issue is the requirement for occlusion when using Ameluz and red light. While the latter protocol was originally designed to achieve maximal AK clearance for a small number of AK in a defined area, it is not ideal for broad field treatment of AK above the neck, particularly on the face where occlusive dressings are very inconvenient.

1.2 Scientific rationale for the proposed study

The proposed pilot study will simultaneously address the 3 current drawbacks of PDT using Ameluz and red light, which are: (1) pain during illumination, (2) lengthy incubation periods, and (3) the inconvenience of plastic wrap occlusion.

The rationale for this study is based upon recent findings and observations from another version of PDT technology for actinic keratosis, namely, 5-ALA (Levulan) and blue light, in which a short-incubation regimen was developed and shown to provide excellent efficacy while generating minimal pain during illumination [6, 7]. The principle exploited by those studies is that conversion of ALA to PpIX begins as soon as ALA is applied to the lesions [8]. Thus PpIX can be photoactivated, even at low levels, by illumination with blue or red light, thereby destroying mitochondria and triggering a somewhat delayed yet highly effective inflammatory-immunological response to eliminate the lesions [9]. Previously, in developing a 'painless blue light protocol', our group compared two Levulan/blue light protocols in a bilateral comparative format (each patient serving as his/her own control). On one side, AK lesions were treated using the shortest drug incubation time that was known to produce significant lesion clearance (1 h ALA incubation), followed by illumination for 16 min 40 sec (as in the standard FDA-approved blue light protocol). This produced a pain level, on average, ~5 on a 1-10 visual-analog scale. On the contralateral side of the face, AK lesions were treated with immediate (simultaneous) illumination, in which 20% ALA solution was applied and then blue light was shone for either 30 min, 45 min, or 60 min. In all three of these timed cohorts, patients reported very low pain levels (~1 out of 10), and yet they developed erythema and lesion clearance rates at 3 month that were similar (statistically non-inferior) to the 'traditional' 1 hr ALA incubation period [7]. This study demonstrated that good treatment efficacy can still be obtained even when pain is minimized using an ALA short-contact approach.

In the current study, we will seek to translate the findings observed with 5-ALA/blue light (above), into a regimen with 10% ALA gel and red light, and assess its feasibility.

1.3. Rationale for the experimental design.

The experimental strategy is to examine short-contact Ameluz/red light protocols in a well-established PDT clinic, to explore pain reduction and treatment efficacy using an FDA-approved PDT drug (Ameluz) and light source (BF-RhodoLED® red light panel) under conditions that have been modified to be sub-maximal (i.e., using shorter than usual drug-incubation times). Our hypothesis is that patient comfort will be greatly improved, yet an acceptable treatment efficacy will be maintained. We plan to invite patients who are already scheduled to undergo PDT treatment in Dr. Maytin's clinic for actinic keratoses of the face to participate in the study.

*The following **scientific questions** will be asked and addressed in a manner consistent with scientific principles as well as practical considerations.*

(1) Will the application of the nanoemulsion (10% ALA gel), in the absence of occlusion, still achieve significant inflammation and lesion clearance?

(2) Will shortened incubation times of Ameluz still achieve significant inflammation and lesion clearance?

(3) Will the new test regimens achieve reduced pain during illumination?

(4) Will the new test regimens be safe?

Consideration and discussion about the rationale *for how we developed the proposed short-contact protocols, in relation to each scientific question, is provided below*:

(1) Will the nanoemulsion (ALA gel) still work in the absence of occlusion?
Answer: Because the 10% ALA gel has much better penetration than 5-ALA/Kerastick that was shown to work without occlusion, it seems highly likely that 10% ALA gel will penetrate lesions and accumulate sufficient PpIX even in the absence of occlusion.

(2) Will short 10% ALA gel incubation times still achieve significant inflammation and lesion clearance?
Answer: For comparing the efficacy of 10% ALA-gel-red light treatment, the benchmark is the 3-hr incubation regimen approved by the FDA, which uses 10% ALA gel (under occlusion), followed by 8 min (37 J/cm2) of red light [*10, 11*]. However, we can also point to a study by Nestor et al. that compared Ameluz and Levulan directly, and showed equivalent AK lesion clearance when each agent was applied for 1 hour (without occlusion) followed by a standard 1000 sec exposure to blue light [*12*]. Those results indicate that short 10% ALA gel incubation times without occlusion can still achieve significant inflammation and lesion clearance. To develop this idea further for 10% ALA gel and red light, we have extrapolated from another study using blue light PDT, our clinical trial [*7*] in which the duration of blue light illumination was drastically shortened but yet achieved surprising efficacy. That work involved several different study arms. In one arm, the original 14-18 hr 20% ALA solution incubation, was shortened to 1 hr ALA incubation followed by a standard 16 min 40 sec blue light illumination. Based upon those results, we believe that the Ameluz incubation time can be reduced from 3 hr to 1 hr, followed by a standard 10 min (37 J/cm2) of red light to achieve a reasonable therapeutic effect. In a different study arm from Kaw et al, the 20% ALA solution incubation time was shortened to zero, i.e., ALA was applied and then blue light was turned on for 30 minutes to provide a simultaneous incubation/illumination. Pain was minimized, yet the amount of AK lesion clearance achieved by the simultaneous regimen was remarkably similar (statistically non-inferior) to AK clearance seen after the 1-hour ALA incubation regimen. For the red light protocol proposed here, we will choose a total Ameluz incubation time of 30 min, and perform simultaneous incubation/illumination using two slight variations. In **Regimen A**, there will be a 10 min ALA incubation time followed by 20 min (75 J/cm2) of red light. **Regimen B** will have a 20 min incubation time followed by 10 min (37 J/cm2) of red light. As a third arm (control), **Regimen C** will have a 1 hr incubation followed by 10 min (37 J/cm2) of red light, which is comparable to the arm of the Kaw study that used 1 hr incubation followed by ~16 min (10 J/cm2) of blue light. By modeling our test conditions after Kaw et al [*7*], we expect to observe outcomes similar to that study (i.e., equivalent inflammation, equivalent AK lesion clearance rates, and reduced pain). The biological rationale for simultaneous ALA incubation/illumination is that PpIX is continuously produced and consumed, thereby producing therapeutic effects over a wide range and with a wide safety margin.

(3) Will the new test regimen achieve reduced pain during illumination?
Answer: The likelihood is extremely high that the test regimen (or at least regimens A and B) will be nearly painless. There is now substantial evidence in the literature, showing that if visible light is delivered continuously while PpIX levels are low, then PpIX does not build up to high enough levels within pre-cancer cells to trigger pain. Instead, the PpIX is activated and destroyed as soon as it is produced. Without high PpIX concentrations in pre-cancer cells, there is no PpIX

concentration gradient and therefore no PpIX leakage from epithelial pre-cancer cells into neighboring nerve endings. The latter is the main cause of pain during traditional PDT [*13*].

(4) Will the new test regimen be safe?
Answer: Yes, because all test conditions (drug concentration, drug incubation time, illumination time) are either at or below the FDA-approved levels for Ameluz paired with the BF-RhodoLED® red light source.

The possible benefit of this study will be the demonstration of suggestive evidence for a new ALA-PDT regimen using Ameluz/red light for actinic keratosis, that provides a therapeutic result equivalent to current regimens, yet minimizes the pain that patients must endure.

2.0 OBJECTIVES

2.1 Primary Objective

- To demonstrate that a short-contact PDT protocol with Ameluz and red light generates less pain during illumination than the standard FDA-approved protocol currently used.
- To test the hypothesis that a short-contact PDT protocol with Ameluz and red light will produce lesion clearance outcomes that are statistically non-inferior to the traditional, painful protocol.

2.2 Secondary Objective(s)

- To test the hypothesis that a short-contact PDT protocol with Ameluz and red light will generate an inflammatory response.
- To test the hypothesis that a short-contact PDT protocol with Ameluz and red light will be better tolerated and provide higher patient satisfaction than the currently practiced protocol.

3.0 STUDY DESIGN

3.1 Study protocol description

The objective of this study is to test three new PDT regimens for actinic keratoses (AK) of the face that promise to greatly reduce the pain normally associated with the procedure. The new regimens, called A, B, and C, respectively, will use a photosensitizer (aminolevulinic acid, 10% ALA gel, Ameluz®) and an activating red light illumination source (BF-RhodoLED® red light panel, 635 nm) from Biofrontera, Inc. Both ALA gel and the 635 nm light source from Biofrontera are currently FDA-approved for broad-area PDT treatment of AK. The approved FDA treatment (standard regimen) specifies that ALA gel be applied topically and left on for 3 hours, followed by exposure to red light for 10 minutes. Here, the timing for topical ALA gel incubation will be shortened, to test the hypothesis that pain experienced during red light illumination can be significantly reduced while still preserving the efficacy of the procedure in terms of lesion clearance (pain and efficacy are primary endpoints). Safety and patient satisfaction will also be assessed (secondary endpoints). Timing for the 3 regimens will be as follows: ***A***, ALA gel for 10 min, then red light for 20 min while ALA gel is left incubating. ***B***, ALA gel for 20 min, then red light for 10 min while ALA gel is left incubating. ***C***, ALA gel for 60 min, then red light for 10 min. We hypothesize that each of the new regimens will cause significantly less pain than the

current standard regimen for ALA gel/red light PDT, and that a lesion clearance response will be achieved that is similar (i.e., statistically non-inferior) to the standard treatment regimen.

3.2 Number of subjects

A total of 30 patients will be needed to complete the study.

3.3 Replacement of subjects

If a study subject withdraws for any reason, up to 3 months after the start of this study, then a new subject can be recruited in his/her place.

3.4 Expected duration of therapy, and duration of subject participation

The total duration of the subject participation in this study will be approximately 4 months and will include 3-4 clinic visits and a screening procedure (either in person or via telephone interview).

4.0 PATIENT SELECTION

4.1 Inclusion Criteria

Males or females, must be at least 18 years of age, with a minimum of 10 actinic keratoses lesions on the face. A total of 10 subjects will be enrolled for each of the study arms A, B, and C. The study will be conducted at the Cleveland Clinic.

4.1.2 **Female subjects must not become pregnant during the study**
The effects of 5-aminolevulinic acid (Ameluz®) on the human fetus are unknown. For this reason, women of child-bearing potential must agree to use contraception. *However, it should be noted that the vast majority of patients with chronic sun-induced AK lesions are beyond the age of menopause*. Should a woman become pregnant or suspect that she is pregnant while she is participating in this study, she should inform the treating physician immediately.

4.1.3 **Subjects must be able to understand and willing to sign a written informed consent document**.

4.2 Exclusion Criteria

4.2.1 Pregnant or nursing.
4.2.2 Using any topical treatment on their AKs; must stop at least one month prior.
4.2.3 Currently undergoing treatment for other cancers with medical or radiation therapy.
4.2.4 Patients with a known hypersensitivity to 5-aminolevulinic acid or any component of the study material.
4.2.5 Patients with history of a photosensitivity disease, such as porphyria cutanea tarda.

4.3 Inclusion of Women, Children, and Minorities

Men and women at least 18 years of age of any ethnic group are eligible for this trial, as long as they fulfill the eligibility criteria.

5.0 REGISTRATION

5.1 Recruitment

This study will be introduced to patients who meet eligibility criteria during their routine PDT visits and will either be provided a copy of the consent form to take home and review or give permission to be contacted for more information regarding the study. If the study coordinator is available, they will briefly meet with the patient to provide copy of informed consent form and explain study design, and the patient will be instructed to review consent form and call study coordinator with any questions and/or if they would like to move forward with participation. If the study coordinator is not available to meet with the patient during their routine visit, they will be notified and will call the patient to explain the study, and will also send a copy of the consent form via mail or email to the patient to review. If the study is not introduced to the patient, but they are eligible, the study coordinator can send a copy of our recruitment letter to patient via mail or MyChart to be followed up by a phone call. Recruitment will also be aided by IRB-approved advertisements posted in the Dermatology waiting room or other approved locations.

5.2 Consent:

Once the patient reviews the copy of the consent form that was provided by the study coordinator, either in person or via mail or email and the patient expresses interest in participating he/she will be scheduled for the first study visit. Once the patient arrives in clinic, the study will be explained once again, along with the chance to ask additional questions, and if the patient indicates continuing willingness then he/she will sign the consent form and be considered enrolled.

5.3 Randomization:

Upon enrollment, each patient will be to assigned to one of the three treatment regimens (A, B, or C) using a block-randomization scheme that will be generated by staff in the Research Pharmacy (Investigational Drug Service) of the Cleveland Clinic; this assignment will insure an equal distribution of study patients across the 3 groups.

6.0 TREATMENT PLAN

6.1. Overall descriptive narrative

At the **first visit (Visit #1)** (Day 1), informed consent will be obtained before starting any study procedures. AK lesions will be counted by Dr. Maytin and his personally-supervised CCLCM medical student researcher using clinical criteria that include presence of a visible lesion with erythema (pink or red), presence of scale, and a gritty consistency by feel (palpation). Each identified lesion will be marked with a black pen. All the marked lesions will then be counted. As a back-up, the patient's face will be photographed with all the pen-marks in place, for later independent confirmation of lesions counts.

Photographs will be taken two ways. First, documentation of the pen-marked lesions on the face will be taken using CCF i-Phones that are routinely used in our clinic; these digital photographs will be transferred and stored directly in each study patient's electronic (Epic) record.

Second, for photographic documentation of inflammation, the patient will also be photographed by a professional photographer using standardized lighting and multiple views to document the degree of inflammation at baseline.

The skin will be degreased with an alcohol wipe, lesions gently abraded with fine sandpaper, and a thin layer of ALA gel (Ameluz®) will be applied to the entire face. No occlusive dressing will be used. For patients in group ***A***, the ALA gel will be left on for 10 min, and then positioned in front of the red light and exposed for 20 min; this is a (10 + 20) regimen. Patients in group ***B*** and group ***C*** will receive a (20 + 10) regimen or (60 + 10) regimen, respectively.

Pain will be assessed by asking the patient to rate their perceived pain level on an 11-point VAS scale (0= no pain, 10 = intolerable pain). Patients will be asked to report their VAS score at 1 minute after red light begins, and at the 5 minute mark (when the RhodoLED beeps). They will be asked for a final VAS score immediately after the light is turned off.

To assess side effects, patients will receive a 6-day questionnaire to record their side-effects daily during the coming week, and which asks them to confirm that they completely avoided any sun exposure during the first two days after PDT. Patients will also receive a standard set of instructions for home aftercare that includes Aquaphor emollient, a topical steroid if necessary for comfort, and instructions to wear sunscreen and avoid sunlight for 48 hr.

At the **second visit (Visit #2)** (Day 3 ± 1 day), the patient will be photographed by our professional photographer using standardized lighting and multiple views, to document the degree of inflammation at 3 days after red light PDT.

At the **third visit (Visit #3)** (Week 8 ± 1 week), lesions will again be marked and counted. Photographs will be taken using CCF iPhones that are routinely used to document the marked lesions on the face. These digital photographs will be transferred and stored directly in each study patient's electronic (Epic) record.

If any remaining lesions are identified, the skin will be degreased with an alcohol wipe, lesions gently abraded with fine sandpaper, and a thin layer of ALA gel (Ameluz®) will be applied to the entire face. No occlusive dressing will be used. For patients in group ***A***, the ALA gel will be left on for 10 min, and then positioned in front of the red light and exposed for 20 min; this is a (10 + 20) regimen. Patients in group ***B*** and group ***C*** will receive a (20 + 10) regimen or (60 + 10) regimen, respectively.

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To assess side effects, patients will receive a 6-day questionnaire to record their side-effects daily during the coming week. They will also receive a standard set of instructions for home aftercare that includes Aquaphor emollient, a topical steroid if necessary for comfort, and instructions to wear sunscreen and avoid sunlight for 48 hr.

The **fourth (final) visit (Visit #4)** will occur at 3-6 months after Visit 1, for final lesion counts, photographs using department iPhone, and administration of a patient satisfaction questionnaire.

(See section 11.0 for more details about the Visit Calendar)

6.2 Duration of Therapy

Each PDT therapy session (Visit 1 and Visit 3) will last for no more than 2 hours, depending upon which arm of the study the patient is in.

6.3 Duration of Follow Up

Patients will be followed until the end of Visit 4, which must occur within a follow-up window of 3-6 months after Visit 1. Thus the maximum duration of F/U is 6 months.

7.0 DOSING DELAYS / DOSE MODIFICATIONS: n/a

8.0 ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

The following is a list of AEs and the reporting requirements associated with observed AEs.

The clinical course of each event will be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period will necessitate follow-up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation will be recorded and reported immediately.

8.1 Adverse Events and Potential Risks

8.1.1 *Ameluz®*

Aminolevulinic acid (ALA) is a natural component found in all cells in the human body. Ameluz is 10% ALA suspended in a lipid-based nanoemulsion. The application of ALA is not associated with any risks or side effects.

8.1.2 *Photodynamic Therapy*

When ALA is converted into PpIX in tumor cells, and illuminated with light, the following signs and symptoms are known to occur as part of the therapeutic response:

(i) Stinging and/or burning sensation: >50%
(ii) Erythema (localized redness): 35%
(iii) Edema, localized: >35%
(iv) Peeling, transient: <50%
(v) Blister formation: rare

8.1.3 *Photodynamic Therapy response (with local cutaneous effects):*

The constellation of transient local symptoms of stinging and/or burning, itching, erythema and edema as a result of topical application of ALA followed by illumination with red light has been observed in clinical studies; for example see ref [*14*]. Stinging and/or burning subsides between 1 min and 24 hours after the light is turned off, and appears qualitatively similar to that perceived by

patients with erythropoietic protoporphyria upon exposure to sunlight. There is no clear drug dose or light dose dependent change in the incidence or severity of stinging and/or burning.

8.1.4 ***Extra-cutaneous adverse experiences reported:***
In previous studies, no non-cutaneous adverse events were found to be consistently associated with Ameluz application followed by red light exposure. No clinically significant patterns of clinical laboratory changes were observed for standard serum chemical or hematologic parameters in any of the controlled clinical trials.

8.1.5 ***Safety in pregnancy:***
No carcinogenicity testing has been carried out using ALA. No evidence of mutagenic effects was seen in four studies conducted with ALA to evaluate this potential. No assessment of effects of ALA on fertility has been performed in laboratory animals. It is unknown what effects systemic exposure to ALA might have on fertility or reproductive function. Therefore, Ameluz is considered as Pregnancy Category C.

8.2 Definitions

8.2.1 *Adverse Events*

An **adverse event** (AE) is any unfavorable or unintended event, physical or psychological, associated with a research study, which causes harm or injury to a research participant as a result of the participant's involvement in a research study. The event can include abnormal laboratory findings, symptoms, or disease associated with the research study. The event does not necessarily have to have a causal relationship with the research, any risk associated with the research, the research intervention, or the research assessments.

Adverse events may be the result of: (a) the interventions and interactions used in the research; (b) the collection of identifiable private information in the research; (c) an underlying disease, disorder, or condition of the subject; and/or (d) other circumstances unrelated to the research or any underlying disease, disorder, or condition of the subject. In general, adverse events that are at least partially the result of (a) or (b) would be considered related to the research, whereas adverse events solely related to (c) or (d) would be considered unrelated to the research.

External adverse events are adverse events experienced by subjects enrolled in multicenter clinical trials at sites other than the site(s) over which the Institutional Review Board has jurisdiction.
Internal adverse events are adverse events experienced by subjects enrolled at the site(s) under the IRB's jurisdiction for either multicenter or single-center research projects.

8.2.2 **The significance of an adverse event** is used to describe the patient/event outcome or action criteria associated with events that pose a threat to a patient's life or functioning (i.e., moderate, severe or life threatening). Based on the National Cancer Institute Guidelines for the Cancer Therapy Evaluation Program, severity can be defined by the following grades of events:

Grades 1 are mild adverse events. (e.g., minor event requiring no specific medical intervention; asymptomatic laboratory findings only; marginal clinical relevance)

Grades 2 are moderate adverse events (e.g., minimal intervention; local intervention; non-invasive intervention; transfusion; elective interventional radiological procedure; therapeutic endoscopy or operation).

Grades 3 are severe and undesirable adverse events (e.g., significant symptoms requiring hospitalization or invasive intervention; transfusion; elective interventional radiological procedure; therapeutic endoscopy or operation).

Grades 4 are life threatening or disabling adverse events (e.g., complicated by acute, life-threatening metabolic or cardiovascular complications such as circulatory failure, hemorrhage, sepsis; life–threatening physiologic consequences; need for intensive care or emergent invasive procedure; emergent interventional radiological procedure, therapeutic endoscopy or operation).

Grades 5 are fatal adverse events resulting in death.

8.2.3 *Serious Adverse Events*

A ***serious adverse event*** (SAE) is any adverse experience occurring at any dose that results in any of the following outcomes:

- Results in ***death.***
- Is a ***life-threatening*** adverse experience. The term life-threatening in the definition of serious refers to an adverse event in which the subject was at risk of death at the time of the event. It does not refer to an adverse event which hypothetically might have caused death if it were more severe.
- Requires ***inpatient hospitalization or prolongation of existing hospitalization***. Any adverse event leading to hospitalization or prolongation of hospitalization will be considered as Serious, UNLESS at least one of the following expectations is met:
    - The admission results in a hospital stay of less than 12 hours OR
    - The admission is pre-planned (i.e., elective or scheduled surgery arranged prior to the start of the study) OR
    - The admission is not associated with an adverse event (e.g., social hospitalization for purposes of respite care.

  However it should be noted that invasive treatment during any hospitalization may fulfill the criteria of "medically important" and as such may be reportable as a serious adverse event dependant on clinical judgment. In addition where local regulatory authorities specifically require a more stringent definition, the local regulation takes precedent.
- Results in ***persistent or significant disability/incapacity***. The definition of disability is a substantial disruption of a person's ability to conduct normal life's functions.
- Is a ***congenital anomaly/birth defect***.
- Is an ***important medical event***. Important medical events that may not result death, be life-threatening, or require hospitalization may be considered a serious adverse experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood disease or disorders, or

convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

8.2.4 Expectedness

Adverse Events can be Expected or Unexpected.

An expected adverse event is an event previously known or anticipated to result from participation in the research study or any underlying disease, disorder, or condition of the subject. The event is usually listed in the Investigator Brochure, consent form or research protocol.

An unexpected adverse event is an adverse event not previously known or anticipated to result from the research study or any underlying disease, disorder, or condition of the subject.

8.2.5 Attribution

Attribution is the relationship between an adverse event or serious adverse event and the study drug. Attribution will be assigned as follows:

- Definite – The AE is clearly related to the study drug.
- Probable – The AE is likely related to the study drug.
- Possible – The AE may be related to the study drug.
- Unlikely – The AE is doubtfully related to the study drug.
- Unrelated – The AE is clearly NOT related to the study drug.

8.3 Reporting Procedures for All Adverse Events

All participating investigators will assess the occurrence of AEs throughout the subject's participation in the study. The clinical course of each event will be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause.

The investigator is responsible for ensuring that all adverse events observed by the investigator or reported by the subject which occur after the subject has signed the informed consent are fully recorded in the subject's case report form, subject's medical records, and/or any other institutional requirement. Source documentation must be available to support all adverse events.

A laboratory test abnormality considered clinically relevant (e.g., causing the subject to withdraw from the study), requiring treatment or causing apparent clinical manifestations, or judged relevant by the investigator, should be reported as an adverse event.

The investigator will provide the following for all adverse events:

- Description of the event
- Date of onset and resolution
- Grade of toxicity
- Attribution of relatedness to the investigational agent
- Action taken as a result of the event
- Outcome of event

In this study, descriptions and grading scales found in the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 available at http://ctep.cancer.gov will be utilized for AE reporting.

Investigative sites will report adverse events to their respective IRB according to the local IRB's policies and procedures in reporting adverse events.

8.4 Serious Adverse Event Reporting Procedures

Serious adverse events that occur beginning with the signing of the informed consent, during treatment, or within 30 days of the last dose of treatment must be reported to the CCF Principal Investigator.

Reports of all serious adverse events (including follow-up information) will be submitted to the CCF IRB per the guidelines located in the CCF IRB Standard Operating Procedures under IRB-60 in manual. Copies of each report and documentation of IRB notification and response will be filed in the regulatory binder.

The following four types of events will be reported to the Cleveland Clinic IRB:

1. Adverse events which are serious, unexpected, and related or possibly related to participation in the research.
2. Serious adverse events that are expected in some subjects, but are determined to be occurring at a significantly higher frequency or severity than expected.
3. Other unexpected adverse events, regardless of severity, that may alter IRB analysis of the risk versus potential benefit of the research and, as a result, warrant consideration of substantive changes in the research protocol or informed consent process/document.
4. Unanticipated Problems involving risks to subjects or others or any serious or continuing noncompliance with this policy or the requirements or determinations of the IRB.

The study team will notify Biofrontera of any serious adverse events via email to [redacted] within 24 hours of study team awareness.

9.0 PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the commercial agents administered in this study can be found in Section 8.0.

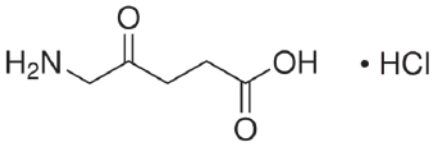
9.1. ***Ameluz®***: The following information about ***Ameluz®*** is cited from the package insert:

Chemical Name: 5-aminolevulinic acid (ALA)

Other Names: *delta*-aminolevulinic acid

Classification: Topical agent

Molecular Formula: The chemical name for ALA HCl is 5-amino-4-oxopentanoic acid hydrochloride (MW = 167.59). The structural formula is represented below:



Mode of Action: Photoactivation following topical application of AMELUZ occurs when aminolevulinic acid (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 cells. AMELUZ photodynamic therapy of AK lesions utilizes photoactivation of topically applied AMELUZ resulting from BF-RhodoLED® illumination, which provides a red light of narrow spectrum and a light dose of approximately 37 J/cm^2.

Metabolism: ALA is converted to PpIX within mitochondria. PpIX is then destroyed upon illumination with the incident light (see above).

Product description: AMELUZ (aminolevulinic acid hydrochloride) topical gel, 10% is a non-sterile white-to-yellowish gel. The gel formulation contains a nanoemulsion.
Aminolevulinic acid, a porphyrin precursor, is a white to off-white crystalline solid. It is readily soluble in water, methanol, and dimethylformamide. Its chemical name is 5-amino-4-oxo-pentanoic acid hydrochloride, molecular weight is 167.59 and molecular formula is C5H9NO3·HCl.

Each gram of AMELUZ contains 100 mg of aminolevulinic acid hydrochloride (equivalent to 78 mg aminolevulinic acid) as the active ingredient and the following inactive ingredients: xanthan gum, soybean phosphatidylcholine, polysorbate 80, medium-chain triglycerides, isopropyl alcohol, dibasic sodium phosphate, monobasic sodium phosphate, propylene glycol, sodium benzoate and purified water.

Product Supply: AMELUZ gel is supplied in an aluminum tube with a white, high density polyethylene (HDPE) screw cap. Each tube contains 2 g of gel.

Storage requirements and stability: AMELUZ has a shelf-life of 24 months and should be stored in a refrigerator, 2°C – 8°C (36°F – 46°F). Excursions permitted to 15°C – 30°C (59°F – 86°F).
After opening, AMELUZ can be stored for up to 12 weeks in a refrigerator at 2°C – 8°C (36°F – 46°F) if the tube is tightly closed.

Route of administration: AMELUZ® will be applied topically to the skin, as directed by the manufacturer. Specifically, using glove protected fingertips or a spatula, AMELUZ gel will be applied using sufficient amount of gel to cover the entire treatment area with 1 mm thickness, including approximately 5 mm of the surrounding skin. Application area should not exceed 20 cm2 and no more than 2 grams of AMELUZ (one tube) should be used at one time. The gel can be applied to healthy skin around the lesions. Application near mucous membranes such as the eyes, nostrils, mouth, and ears (keep a distance of 1 cm from these areas) should be avoided. The study subject will be advised to avoid sunlight until he/she will return to the clinic for the illumination phase of the treatment.

Drug Procurement: AMELUZ® will be supplied by Biofrontera, in the amount of one tube of AMELUZ® gel per study patient.

Drug Accountability: The investigator or designated study personnel are responsible for maintaining accurate dispensing records of the study drug. All study drugs must be accounted for, including study drug accidentally or deliberately destroyed. Under no circumstances will the investigator allow the investigational drug to be used other than as directed by the protocol. If appropriate, drug storage, drug dispensing, and drug accountability may be delegated to the pharmacy section of the investigative site.

Drug Destruction: AMELUZ® can be stored for 12 weeks in a refrigerator at 2°C – 8°C (36°F – 46°F) if the tube is tightly closed

9.2. ***Light Sources for PDT***

BF-RhodoLED® lamps are red light sources with a narrow spectrum around 635 nm that deliver a light dose of approximately 37 J/cm^2 within 10 minutes. PDT using AMELUZ® in combination with BF-RhodoLED® lamp is indicated for lesion-directed and field-directed treatment of actinic keratoses (AKs) of mild-to-moderate severity on the face and scalp.

10.0 CORRELATIVE / SPECIAL STUDIES:

Study patients will be identified only by patient Study ID number, and their data stored in a password-encrypted REDCap database accessible only be members of the study team.

Privacy and confidentiality: Medical information gathered in this study will be limited to what is recorded in the routine clinical PDT treatment note, along with any study-related notes entered into the electronic medical record (EPIC). Patients must consent to have photographs taken of their facial lesions at each visit, using a standard departmental photographic consent. Consent to allow use of those images in medical publications or presentations that result from the study is voluntary, as the patient will indicate in a checkbox.

11.0 STUDY PARAMETERS AND DETAILED STUDY CALENDAR

Details of 10% ALA application and red light treatment were described in detail under Section 6.0, "Treatment Plan". The timing of visits is summarized below and in section 11.5 ("Calendar").

11.1 **PDT treatment (Visit 1)**

All AK lesions are measured clinically and photographed. The clinician applies 10% ALA gel to entire face, without occlusion, and allows it to incubate if for either 10 min, 20 min, or 60 min, depending upon the study arm assignment. The patient will then be exposed to red light (BF-RhodoLED® device) for either 10 min or 20 min, as per the assigned study arm.

11.2 **Post-treatment assessment of side-effects (Visit 2)**

The patient will return at day 3 (±1 day) for facial photographs to document their erythema reaction. They will also be asked about their side-effects, and insure that they know to fill out the side-effects questionnaire during the 6 days post-PDT.

11.3 **PDT treatment (Visit 3)**

This will occur 8 weeks (±1 week) after Visit 1. All AK lesions will be measured clinically and photographed and if any remain, then the same PDT treatment regimen used at Visit 1 will be employed again here for a second round of treatment.

11.4 **Final observation (Visit 4)**

This will occur 3-6 months after Visit 1. The subject will return for final skin exam, lesion counts, and photography. A patient satisfaction questionnaire will also be administered at this visit. The large scheduling visit window is designed to maximize the likelihood of patient compliance, since many patients with AK are retirees who spend many months traveling and spend their winters in warmer climates far from Cleveland.

11.5 **Study Calendar**

CALENDAR. Timeline of procedures for patients enrolled in the study.

	VISIT 1 PDT treatment session #1	**VISIT 2 Photos of skin redness**	**VISIT 3 PDT treatment session #2**	**VISIT 4 Final lesion counts**
Scheduled visit: → Procedure: ↓	**Day 1**	**Day 3 ±1 day**	**Week 8 ±1 week**	**Month 3-6**
Sign informed consent	X			
Examine patient, mark each lesion with a pen, and count the lesions	X		X	X
Take photographs in our professional studio (frontal view and side views) to document inflammation.	X	X		
Take photographs using clinic iPhone camera to document lesion counts (pen-marks)	X		X	X

Apply topical ALA gel and incubate for 10, 20, or 60 min prior to turning on the light source (as per assigned study arms A, B, or C, respectively)	X		X	
Illuminate with red light for 20, 10, or 10 min (as per assigned study arms A, B, or C, respectively)	X		X	
Record patient-reported pain level (VAS)	X		X	
Give the patient a questionnaire to fill out at home (to describe side-effects on each of the 6 days post-PDT)	X		X	
Provide patient with a hat, sunscreen and aftercare instructions	X		X	
Patient satisfaction questionnaire				X

12.0 MEASUREMENT OF EFFECT

Endpoints to be evaluated are as follows: Pain reported by the patients during illumination will be measured on a subjective 11-point visual-analog scale (VAS). Treatment response will be evaluated by clinical exam and follow-up photographs at the two follow-up visits.
Endpoints will be:

12.1 Pain during illumination (VAS scale 0-10) at 1 min, 5 min, and 10 min
12.2 100% lesion clearance rates
12.3 75% lesion clearance rates
12.4 Visual differences observed in the before-and-after photos
12.5 Subject satisfaction questionnaire
12.6 Adverse Events- Patients' side effects log (6 days post-PDT) and Investigator assessment.

Regarding lesions clearance rates (items 12.2 and 12.3), the final efficacy outcome will be defined as the lesion counts after two treatments, and these final counts are what the estimated enrolled sample sizes are based on. Lesion status after 1 PDT treatment will also be analyzed, but not considered in the analyses of noninferiority.

13.0 RECORDS TO BE KEPT / REGULATORY CONSIDERATIONS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 8.0 (Adverse Events: List and Reporting Requirements).

13.1 Data Reporting

The OnCore Database will be utilized, as required by the Case Comprehensive Cancer Center, to provide data collection for both accrual entry and trial data management. OnCore is a Clinical Trials Management System housed on secure servers maintained at Case Western Reserve University. OnCore properly used is compliant with Title 21 CFR Part 11.

Access to data through OnCore is restricted by user accounts and assigned roles. Once logged into the OnCore system with a user ID and password, OnCore defines roles for each user which limits access to appropriate data. User information and password can be obtained by contacting the OnCore Administrator at [redacted].

OnCore is designed with the capability for study setup, activation, tracking, reporting, data monitoring and review, and eligibility verification. This study will utilize electronic Case Report Form completion in the OnCore database. A calendar of events and required forms are available in OnCore.

13.2 Regulatory Considerations

The study will be conducted in compliance with ICH guidelines and with all applicable federal (including 21 CFR parts 56 & 50), state or local laws.

13.2.1 Written Informed consent

Provision of written informed consent must be obtained prior to any study-related procedures. The Principal Investigator will ensure that the subject is given full and adequate oral and written information about the nature, purpose, possible risks and benefits of the study as well as the subject's financial responsibility. Subjects must also be notified that they are free to discontinue from the study at any time. The subject should be given the opportunity to ask questions and allowed time to consider the information provided.
The original, signed written Informed Consent Form must be kept with the Research Chart in conformance with the institution's standard operating procedures. A copy of the signed written Informed Consent Form must be given to the subject.

13.2.2 Subject Data Protection

In accordance with the Health Information Portability and Accountability Act (HIPAA), a subject must sign an authorization to release medical information to the sponsor and/or allow the sponsor, a regulatory authority, or Institutional Review Board access to subject's medical information that includes all hospital records relevant to the study, including subjects' medical history.

13.2.3 *Accessing Electronic Medical Records for University Hospitals Health System: N/A*

13.2.4 *Retention of records*

The Principal Investigator of The Case Comprehensive Cancer Center supervises the retention of all documentation of adverse events, case report forms, source documents, records of study drug receipt and dispensation, and all IRB correspondence for as long as needed to comply with national and international regulations and the institution in which the study will be conducted, or for the period specified by the sponsor, whichever is longer. No records will be destroyed until the Principal Investigator confirms destruction is permitted.

13.2.5 Audits and inspections

Authorized representatives of the sponsor, a regulatory authority, an Independent Ethics Committee (IEC) or an Institutional Review Board (IRB) may visit the Center to perform audits or inspections, including source data verification. The purpose of an audit or inspection is to systematically and independently examine all study-related activities and

documents to determine whether these activities were conducted, and data were recorded, analysed, and accurately reported according to the protocol, Good Clinical Practice (GCP), guidelines of the International Conference on Harmonization (ICH), and any applicable regulatory requirements.

*13.2.6 **Data Safety and Monitoring Plan***

This protocol will adhere to the policies of the Case Comprehensive Cancer Center Data and Safety Monitoring Plan in accordance with NCI regulations.

14.0 STATISTICAL CONSIDERATIONS

14.1 Primary objective:

We wish to evaluate two primary endpoints, which test the following hypotheses:

Question 1: Pain is significantly reduced in each of the 3 short PDT regimens as compared to the standard regimen.

Question 2: Clinical efficacy (AK lesion clearance) after each of the 3 short PDT regimens is statistically no different than (is non-inferior to) the standard regimen.

14.2 Sample size estimates:

Although there are two primary endpoints, we have powered the study based upon the second endpoint (efficacy) because the first endpoint is already extremely likely to provide acceptable results as an anticipated outcome. Our reasoning is as follows:

For Question 1, evaluating for differences in pain, we will compare the results from our study patients to published results from clinical trials that used ALA 3 hr incubation with occlusion and red light [*5*]. In those studies, on an 11-point VAS scale the mean pain sensation when using the active drug was 5.5 [95% confidence interval (CI) 4.7–6.2] during the first PDT, and 5.8 (95% CI 4.7– 6.9) during the second PDT. When using placebo, the mean pain sensation was 0.9 (95% CI 0.3–1.6) and 0.3 (95% CI 0–0.6), respectively. In our study, for estimating what pain level we might expect in each the two short-duration protocols (regimens A and B), the only estimates available are the VAS pain values reported with a similar short-duration protocols using Levulan and blue light [*7*] in which the mean pain sensation was 0.52 (95% CI 0–1.09). Based on this, we fully expect that the pain reduction with regimens A and B will be substantial and clinically meaningful, regardless of the sample size.

For Question 2, we want to test for non-inferiority between our PDT regimen(s) and, the original 3-hr incubation with occlusion + 10 min red light regimen used for FDA approval [*5, 10*]. For this purpose, we will do the comparisons using our regimen C, in which patients receive a 1 hour incubation without occlusion + 10 min red light. In the original clinical trial of Ameluz and red light, AK clearance rates were reported to be 78 ± 15% after 1 PDT treatment (mean ± SD, n = 31 patients) [*15*]. To come up with an estimate of what might happen with a shorter red light regimen, we cite the study by Nestor et al 2019 which represents a reasonable surrogate for our proposed short (1-hr) red light regimen [*12*]. In that study, Ameluz was incubated for 1 hr without occlusion and then illuminated with blue light; the AK clearance rate on the face in 20 patients using Ameluz was 52.3% after 1 PDT treatment, and 97.1% after 2 PDT treatments. Nestor et al

also examined the AK clearance rate in another 20 patients using Levulan, which was 57.7% after 1 PDT treatment, and 94.9% after 2 PDT treatments. The 1-treatment AK clearance rate (57.7%) resembles a previous study that we performed with Levulan and a 1 hr incubation (Kaw et al), yielding an AK clearance on the face of 61% after 1 PDT treatment. This suggests that responses after blue light or red light are very similar. Therefore, will use the variance for the 1 hr incubation group in the blue light study (Kaw et al) which was 61% ± 30% (mean ± SD; n = 8 patients) [7]. Unfortunately, the Nestor 2019 study did not provide any variance values for their red light data. However, other Ameluz studies in the literature [15] report a typical standard deviation (SD) in the range of 25-30% for the mean AK clearance rate, so we will use 30% as the SD value for our patients in this calculation.

Thus we wish to compare the AK clearance rates for the following two conditions, for statistical non-inferiority:

Group 1 (long 3 hours; control group): 78 ± 15% (mean ± SD, n = 31 patients)
Group 2 (short 1 hours; experimental group): 61% ± 30% (what power will 10 patients give us?)

Statistical calculation of sample size (Dr. Bo Hu, Ph.D): For the non-inferiority design, n=10 patients per group will have 80% power to compare each intervention with the control group, assuming a non-inferiority margin of 25% clearance rate, a true difference of 10% and a standard deviation of 13% (two-sided alpha=0.05).

14.3 Estimate of the accrual rate:

Dr. Maytin treats ~3-4 patients with AK lesions on the arms or legs per week, using red light PDT. Once the IRB protocol is approved, we anticipate that we should be able to recruit one patient per week, or ~3-4 patients/month, with enrollment completion in ~5 months and full study completion within one year.

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Appendix 1: Specifications for Ameluz®
See Appended PDF Ameluz® US-PI

Appendix 2: Specifications for BF-RhodoLED® red-light source
See Appended PDF for BF-RhodoLED® Lamp Manual