COVER PAGE

STATISTICAL ANALYSIS PLAN

Study ALA-AK-CT018

NCT05060237

A non-randomized, open-label, multicenter study to evaluate the safety and tolerability of BF-200 ALA (Ameluz®) in the expanded field-directed treatment of actinic keratosis on the face and scalp with photodynamic therapy (PDT)

Statistical Analysis Plan
for
final analysis

Version 2.0

Study:	A non-randomized, open-label, multicenter study to evaluate the safety and tolerability of BF-200 ALA (Ameluz®) in the expanded field-directed treatment of actinic keratosis on the face and scalp with photodynamic therapy (PDT)
Study-ID:	ALA-AK-CT018
Sponsor / Contact:	Biofrontera Bioscience GmbH Hemmelrather Weg 201 51377 Leverkusen, Germany
Evaluation:	[redacted]
[redacted]	[redacted]
Version:	2.0 of 05Jul2023 (final)
Previous versions:	1.0 of 15Nov2021 (final)

Revision history

Version	Author	Date	Reason for Revision
2.0	[redacted]	05Jul2023	- General: update of SAP to protocol version 3.0 - Update of SAP according to DRM decisions - Analysis of concomitant medications corrected - Analysis of concomitant medications given as pain relieving measures prior illumination added - Complete AE section: details added for clarification - AE tables for AESIs and application site reaction added - Subgroup analyses added for NRS-11 analysis - Graphical presentation of vital signs added - Section 4.7: clarification which data will be presented - Linguistic improvements - Update of Appendix A corresponding to main part and inaccuracies corrected

Table of Contents

List of Abbreviations

In the following abbreviations are listed as used within this statistical analysis plan or which might occur within the tables, listings and graphs outputs:

AE	Adverse event
AESI	Adverse event of special interest
ADaM	Analysis Data Model
AK	Actinic keratosis
ALA	5-aminolevulinic acid
ATC	Anatomical therapeutic chemical
BCC	Basal cell carcinoma
BMI	Body mass index
CRF	Case report form
CS	Clinically significant
ICF	Informed consent form
ICH	International council of harmonisation
IMP	Investigational medicinal product
ITT	Intention-to-treat
MedDRA	Medical dictionary for regulatory activities
N	Number of subjects
NCS	Not clinically significant
NMSC	Non-melanoma skin cancer
PDT	Photodynamic therapy
PT	Preferred term
PTAE	Pre-treatment adverse event
SAE	Serious adverse event
SAF	Safety analysis set
SAP	Statistical analysis plan
SCC	Squamous cell carcinoma
SD	Standard deviation
SDTM	Study Data Tabulation Model
SOC	System organ class
TEAE	Treatment emergent adverse event
TLG	Tables, listings, graphs

1 General

This Statistical Analysis Plan (SAP) was defined by the Sponsor and the responsible Statistician. It is based upon the Study Protocol (Version 3.0 of 21Apr2022) and contains detailed description of the statistical methods described therein.

The SAP describes prospectively the analyses to be performed on study data. It was finalized prior to enrolment of first subject.

1.1 Study Design

This is a non-randomized, open-label, multicenter study to evaluate the safety and tolerability of BF-200 ALA in the treatment of actinic keratosis (AK) located on the face and scalp with photodynamic therapy (PDT) when using the BF-RhodoLED® XL lamp. Each subject will receive the content of 3 tubes (6 g) of BF-200 ALA for an expanded field-directed treatment. Approximately 10 sites in the US will participate in this study. Each site should dose between 5 and 15 subjects. No site should dose more than 20 subjects unless prior approval of the sponsor is obtained. The maximum number should in no case exceed 25 subjects.

The clinical study will consist of:

- a screening visit (within 14 days before treatment), at which eligibility of subjects for study participation will be assessed (Visit 1)
- a baseline/treatment visit, at which eligible subjects will receive PDT with application of the content of 3 tubes of BF-200 ALA (Visit 2)
- two interim visits for safety assessment 7 days (± 2 days) (Visit 3) and 14 days (± 2 days) (Visit 4) post PDT
- a final clinic visit, 28 (± 7) days post PDT, at which safety and tolerability assessments will be performed (Visit 5).

1.2 Study Objective

The objective of the study is to evaluate the safety and tolerability of BF-200 ALA PDT, utilizing BF-RhodoLED® XL illumination, in the treatment of mild to severe actinic keratosis on the face and scalp in an expanded field-directed treatment.

1.3 Sample Size

In agreement with FDA about 100 subjects are considered sufficient to evaluate the safety profile after application of the content of 3 tubes of BF-200 ALA. All subjects will receive BF-200 ALA. No formal sample size calculation was conducted.

1.4 Study Duration

For each subject the duration of the study is expected to be approx. 6 weeks. The overall study duration is estimated to be about 6.5 months. The duration of the overall study or the subject recruitment period may vary. The end of study will be defined as last subject last visit.

1.5 Definitions

- “illumination area” is the area effectively illuminated by one BF-RhodoLED® XL lamp in one illumination session
- “treatment field” is the part of the skin to which the investigational medicinal product (IMP) is applied
- “treatment area” describes a region of the body in which the treatment field is located, distinguishing between face (including forehead, excluding eyes, nostrils, and mouth) and scalp (bald scalp, excluding ears)
- “target lesions” are all AK lesions (mild to severe) located within the treatment field at Visit 1

2 Endpoints

The endpoints of the study are:

- Frequency and severity of adverse events (AEs), serious AEs (SAEs), and treatment-emergent adverse events (TEAEs). TEAEs are defined as all AEs with onset or worsening after treatment with IMP up to Visit 5
- Duration of TEAEs including the breakdown of severity category (mild, moderate, severe)
- Assessment of new lesions (AK, non-melanoma skin cancer (NMSC) such as basal cell carcinoma (BCC), squamous cell carcinoma (SCC) or Bowens disease and melanoma) if they occur inside the treatment field
- Assessment of new lesions (AK, NMSC such as BCC, SCC or Bowens disease and melanoma) if they occur around the treatment field at a distance of < 10 cm
- Application site skin reactions during and post PDT, assessed by the investigator
- Application site discomfort during and post PDT, reported by the subjects
- Application site pain during illumination, as assessed by the subjects using an 11-point numeric rating scale
- Changes in vital signs
- Safety laboratory data
- Physical examination data
- Neurological assessments

3 Statistical Analysis Sets

3.1 Consented Set

The consented set will consist of all subjects who have provided informed consent to participate in the study.

3.2 Safety Analysis Set (SAF)

The safety analysis set (SAF) will include all subjects who undergo at least one of the following treatment procedures: preparation of the treatment field, application of BF-200 ALA, or illumination. If it is not certain whether a subject has received any of the treatment procedures, the subject will be included in the SAF.

3.3 Assignment of Analysis Sets to Analysis

As safety and tolerability are the objectives of the study, all endpoints are analyzed based on the SAF. Disposition of subjects will be presented based on the consented set.

3.4 Additional Subgroup Analysis

Selected safety analyses will be conducted overall and stratified by age groups, sex, skin type, and treatment area:

- Age groups:
  - ≥18 - ≤65
  - ≥66 - ≤74
  - ≥75
- Sex:
  - male
  - female
- Skin type subgroups:
  - Fitzpatrick skin type I-III
  - Fitzpatrick skin type IV-VI
- Treatment area:
  - Face
  - Scalp
  - Face and Scalp

Additionally, related TEAEs will be analyzed stratified for subjects with major PDs (defined during DRM, which possibly have an impact on the safety results) and subjects without major PDs.

4 Statistical Evaluation

Last assessment before administration of IMP will be used as baseline value. Change from baseline will be calculated as post-baseline value(s) – baseline value. If no baseline value is available, no change from baseline will be calculated.

No replacement of missing values will be done.

Unscheduled visits will only be listed and not included in any tables except table “Subjects per visit”. Data listings of laboratory assessments will present study days. The day of treatment will be identified as Study Day 1. The day before treatment will be identified as Day -1.

All analyses will be conducted by treatment area (face / scalp / face and scalp) and overall for the safety analysis set if not stated otherwise. Selected safety analyses will be conducted overall and stratified by age groups, sex, skin type, and treatment area.

Calculation of a duration in days (e.g. duration of AE [days]) is performed as follows: stop date - start date + 1 [day]. Details of calculation of AE duration by severity are specified in section 4.4.1: Duration and severity of AEs.

Duration of illumination [min] is calculated excluding interruptions. Calculation is performed as follows: stop time - start time - duration of interruptions. (Duration of interruptions will be set to 0 if illumination was not interrupted.)

All planned analyses are of exploratory nature without any formal statistical hypotheses.

A detailed description of the planned tables, listings and graphs is given in Appendix A (version 2.0).

Visit terminology

The notation displayed in table 1 will be used for table, listing and graph (TLG) presentation of visits.

Table 1: Visits and timepoints

Notation used in the protocol	**Notation used for TLG presentation**
Visit 1: Screening (≤ 14 days prior to PDT)	Visit 1
Visit 2: PDT (Baseline/ treatment)	Visit 2
Visit 3: Safety Interim Visit (7 days (± 2 days) post-PDT)	Visit 3
Visit 4: Safety Interim Visit (14 days (± 2 days) post-PDT)	Visit 4
Visit 5: Final Visit (28 days (± 7 days) post-PDT)	Visit 5
Unscheduled visit	Unscheduled visit

4.1 Dispositions of Subjects and Analysis Sets

Disposition of subjects and analysis sets

Descriptive analysis of subjects will be presented overall and per study site.

The disposition of subjects and analysis sets (Consented Set, Screening Failures, SAF), study visits, inclusion and exclusion criteria, and the status at study termination, together with reason for discontinuation, will be shown.

4.2 Demographics and Other Covariates

Demographic data and baseline characteristics

Demographic data (age, sex, race and ethnicity), body measures (body height [cm], body weight [kg]) and skin type Fitzpatrick (Type I / Type II / Type III / Type IV / Type V-VI) and Fitzpatrick skin type subgroups (Type I-III, Type IV-VI) will be tabulated.

Reproductive potential and pregnancy test

Details regarding reproductive potential and pregnancy tests will be listed.

Treatment area and assessment of AK lesions

Total area of AK target lesions (≥ 4 mm) (calculated) [cm²] and total number of AK target lesions within the treatment field at baseline will be tabulated per subject. In addition, for AK target lesions ≥ 4 mm, location, diameter and automatically calculated lesion area [mm²] as well as severity will be tabulated per lesion.

Medical history and concomitant medical conditions

The proportion of subjects with relevant medical history / concomitant medical conditions will be tabulated. For medical skin history, the proportion of subjects with

- first diagnosis of AK
- AK lesions diagnosed in the past located within the selected treatment area(s)
- diagnoses of NMSC or melanoma in the past
- other relevant skin diseases

will be tabulated.

Medical History and concomitant diseases will not be coded.

Concomitant medication

Concomitant medications (CMs) will be coded by WHO-DD. Concomitant medications will be tabulated by anatomical therapeutic chemical (ATC) level 2, ATC level 3, and preferred name.

Concomitant medications given as pain relieving measures prior illumination will be displayed by anatomical therapeutic chemical (ATC) level 2, ATC level 3, and preferred name.

Concomitant procedures will be listed only.

4.3 Study Drug Administration

PDT details (duration of incubation [min], duration of illumination [min], number and duration [min] of interruptions) will be displayed using basic statistics. Furthermore, it will be tabulated whether preparation of treatment fields, application of IMP and illumination was done according to protocol, and if illumination was performed with BF-RhodoLED® XL.

4.4 Analysis of Endpoints

4.4.1 Adverse Events

AEs will be coded by using the Medical dictionary for regulatory activities (MedDRA, MedDRA version available at the day of database closure). If an AE occurs in multiple treatment areas, it is reported separately for each treatment area within the eCRF. Thus, some AEs are counted repeatedly for the analysis.

Analysis will focus on severity and duration of TEAEs. TEAEs are defined as all AEs with onset or worsening after treatment with IMP up to Visit 5 (Final visit). AEs with start on the day of PDT which are unrelated or unlikely related to IMP belonging to AE category 'NEW LESION' and with action taken 'PDT treatment' will be classified as pre-treatment emergent adverse events. If unclear due to incomplete start date/time of AE or IMP application or uncertainty about worsening of AE, AEs will be assumed as TEAEs. AEs developing between signing the ICF and IMP application at Visit 2 will be considered as pre-treatment adverse events (PTAEs). TEAEs as defined above, will be tabulated by system organ class (SOC) and preferred term (PT) (MedDRA). The number of entries, as well as the number and rate of affected subjects will be reported for each treatment area and overall.
TEAEs will also be tabulated by relationship to IMP and/or medical device and by worst severity. TEAEs are considered being related to IMP or medical device, if causal relationship between IMP or medical device and the TEAE is at least possible or relationship assessment is missing. For AEs with changes in severity, the worst severity per AE and subject will be tabulated.

Frequency of adverse events

An overview table showing the number of entries, as well as the number and rate of affected subjects with AEs, SAEs, TEAEs, serious TEAEs, TEAEs leading to death, TEAEs related to IMP, TEAEs related to medical device, TEAEs leading to study discontinuation, and TEAEs related to IMP or medical device will be shown.

TEAEs leading to death, serious TEAEs, TEAEs related to IMP, and TEAEs related to medical device will be presented separately.

Subgroup analysis

Additionally, TEAEs will be tabulated by age groups (≥18 - ≤65 / ≥66 - ≤74 / ≥75), sex (male / female) and skin type subgroups (Type I-III, Type IV-VI).

A stratified analysis of related TEAEs will be done for subjects with major PDs as classified during DRM and subjects without major PDs.

Duration and severity of adverse events

Duration of TEAEs by severity will be presented for related PTs which occurred in at least 2 subjects. For all those TEAEs (related to IMP and/or medical device), the duration of the AEs will be tabulated by severity. For each of these TEAEs, the mean duration of each severity category, based on all subjects who experienced these TEAEs, will be presented in a stacked bar chart. The proportion of days in the different severity categories as a percentage of the total duration will be presented in a frequency table for each TEAE. For the frequency table and the stacked bar charts, the calculations for each severity category will be performed with reference to all subjects who experienced this TEAE, regardless of severity. If a severity category does not occur in a subject, the duration in this category is set to 0. Calculation of duration of severity will only be done for AEs with complete start and stop dates. AEs with incomplete start or stop dates or ongoing AEs will not be considered for this analysis.

If for a TEAE only one severity is recorded during the day, then the duration of the severity of this TEAE will be set to one day. If severity changes during the day, more than one change can be recorded. For the calculation of the duration of each severity category on this day, the day will be

divided by number of changes during the day (so that duration can be e.g. 0.5 day, 0.33 day). Note that on day 1 only one severity is recorded.

Listing will include the start day of TEAEs after PDT. Calculation of TEAE start after PDT is performed in days as follows: start date of TEAE- start date of PDT.

PTAEs will be listed separately and not included in any tables or other listings.

Adverse events of special interest

A medical review of all adverse events will be done during the data review meeting to identify adverse events of special interest (AESIs) as specified in Note to File no. 4 to the protocol version 3.0.

AESIs will be tabulated by SOC and PT stratified by SAE (yes/no).

Application site reactions

A frequency table showing number and proportion of subjects with application site skin reactions and application site discomfort will be displayed. Per subject the maximum intensity will be displayed if more than one application site skin reactions and application site discomfort occurred.

4.4.2 Assessment of New Lesions

Assessment of new lesions

The occurrence of any new lesions (AK, NMSC such as BCC, SCC or Bowens disease and melanoma, according to AE page) will be summarized and tabulated according to SOC and PT displaying the number of events as well as counts and percentages on a subject basis. Additionally, the occurrence of any new lesions will be tabulated stratified by location in relation to the treatment field (inside - / in close proximity (< 10 cm distance to treatment field)).

4.4.3 Overall Tolerability

Application site skin reactions during and post PDT

Details regarding application site skin reactions (erosion, erythema, exfoliation, oedema, fissure, induration, vesicles, ulceration, flaking, scabbing, discharge, other) will be listed.

Application site discomfort during and post PDT

Collected data about application site discomfort reported by the subjects for each category (Burning, Hyperaesthesia, Pain, Paraesthesia, Pruritus, Stinging, Warmth, other) will be listed.

Application site pain during PDT

Subjects will assess the maximum pain experienced during PDT using an 11-point Numeric Rating Scale (NRS-11) ranging from 0 (no pain at all) to 10 (worst possible pain). Basic statistics for application site pain will be given stratified by treatment area and overall.

Additionally, basic statistics for the NRS-11 score will be displayed stratified by concomitant medications given as pain relieving measures prior illumination (yes/no), concomitant medications given as pain relieving measures during illumination (yes/no) and physical measures taken during illumination (yes/no).

4.4.4 Safety Assessments

Vital signs

The analyses of variables for vital signs will focus on

A) the evaluation of the change from baseline (Visit 2, first measurement after the subject arrived at the site) to values on subsequent clinical visits and
B) The evaluation of the change on Visit 2 from baseline (Visit 2, first measurement after the subject arrived at the site) to values obtained prior and after illumination (until 60 min post illumination).

Basic statistics of vital signs by visit / timepoint and of changes from baseline to post baseline visits as well as from Visit 2 baseline to values obtained prior and after illumination will be presented.

Boxplots per treatment area for Heart rate [beats/min], Systolic blood pressure [mmHg], Diastolic blood pressure [mmHg] and Body temperature [°C] on Visit 2 will be displayed stratified by measurement timepoint (at arrival / 10 min before start …. / 10 min after end … / 60 (+/-10) min after end …).

Safety laboratory

Information about clinically significant findings will be displayed using frequency tables. Details regarding laboratory sampling will be listed. In addition, a listing of subjects with clinically significant values will be provided.

Physical examinations

Details of physical examination which involve head and neck, skin, lymph nodes, thorax including heart and lungs, abdomen, and musculoskeletal, peripheral vascular and nervous system status will be listed.

Neurological assessments

Neurological assessments comprise memory tests and neurological investigations.
Memory test results will be presented using frequency tables displaying whether all of the presented pictures were memorized correctly, and if not, the identified number of pictures, and which questions were answered correctly.

Information about neurological investigations will be presented using frequency tables showing results of assessment of clinical significance of pupils, coordination, gait and sensitivity. Information about clinically significant findings will be displayed using frequency tables.

4.5 Missing Values

No missing value imputation method will be applied.

4.6 Data Base Closure and Data Review

A data base closure will be performed prior to the analysis. All parameters will be checked, as specified in the data validation plan, and all queries resolved before data base closure and analysis.

SDTM datasets will be used to create ADaM datasets using ADaM Implementation Guide [redacted] and Analysis Data Model [redacted]. An ADaM specification document will be setup as a MS Excel spreadsheet, describing ADaM datasets to be created. A final define.xml [redacted] will be created when the ADaM datasets and the specification documents are final.

A data review will be conducted prior to data base lock based on all data to allocate the subjects to the analysis sets. Furthermore, the following items will be discussed:

- Any violation of inclusion/exclusion criteria
- Any dose changes/incompliance to study therapy
- Use of forbidden medication
- Premature discontinuation during the study
- Informed consent date not before application of IMP date or any other study related procedure

These evaluations and assessments will be done together and in agreement with the Sponsor, however, [redacted] will provide the Sponsor with the appropriate subject listings (as defined in appendix A). Data review can be done via a telephone conference. All protocol deviations detected during DRM will be listed.

4.7 Miscellaneous

For qualitative variables the frequencies (absolute and relative) will be calculated. For frequency tables, the number of non-missing values will be presented. Missing values (including user-defined missing values) will not be presented and not included in the calculation of percentages. Percentages will be presented to one decimal place. Details of format of tables, listings and graphs are given in Appendix A, but the real design will be determined by the technical possibilities within SAS and may not look identical to the description. However, all information as mentioned will be included. Only observed outcomes will be displayed in frequency tables. If for a category no data is observed (e.g., females will not be displayed if all subjects are male) this category will not be displayed.

Quantitative parameters will be described by declaring the mean value, standard deviation, minimum, first quartile, median, third quartile, and maximum. In the description of the tables this will be denoted by „basic statistics“. In general, minimum and maximum will be presented to the same level of precision as the raw data; means and medians, standard deviation, and quartiles will be presented to one further decimal place.

The listings are always sorted by treatment area and subject. If a different sorting order should be used for some listings this will be remarked separately. The variables for the special listings are explicitly given in the description of listings. All listings will be presented for the safety analysis set, if not stated differently.

Screening failures and enrolled but not treated subjects (withdrawal before treatment) will be considered in tables and listings describing analysis sets, eligibility and discontinuation and separate demographic listings. Listings including screening failures and enrolled but not treated subjects will be stratified by SAF.

The following title will be used for all generated tables, listings, and graphs:

Protocol: ALA-AK-CT018 [redacted] Page # of #
Final Analysis

The numbering NNN of the tables/listings/graphs will be stated in the detailed description (Appendix A).

Following footnote will be used for all generated tables, listings, and graphs:

Program: <Name of program> Run date/time: yyyy-mm-ddThh:mm

All tables, listings, and graphs will be generated in A4 paper format.

The statistical evaluation will be performed using SAS version 9.4 or higher.

Signatures

Statistician:
[redacted]
Date (ddmmmyyyy) Signature

Sponsor:
[redacted] Biofrontera Bioscience GmbH [redacted] Hemmelrather Weg 201 51377 Leverkusen Germany
Date (ddmmmyyyy) Signature