# TITEL PAGE SAP FINAL ANALYSIS, V2.0 DATED 30-OCT-2020

**Study ALA-AK-CT015,**

*NCT04319159*

An open-label Phase I study to evaluate the pharmacokinetics of 5-aminolevulinic acid and protoporphyrin IX in human plasma under maximal use conditions after topical application of 3 tubes of BF-200 ALA 10% gel for photodynamic therapy (PDT) in subjects suffering from actinic keratosis

**Statistical Analysis Plan**
**for**
**final analysis**

**Version 2.0**

| | |
|---|---|
| Study: | An open-label Phase I study to evaluate the pharmacokinetics of 5-aminolevulinic acid and protoporphyrin IX in human plasma under maximal use conditions after topical application of 3 tubes of BF-200 ALA 10% gel for photodynamic therapy (PDT) in subjects suffering from actinic keratosis |
| Study-ID: | ALA-AK-CT015 |
| Sponsor / Contact: | Biofrontera Bioscience GmbH<br>Hemmelrather Weg 201<br>51377 Leverkusen, Germany |
| Evaluation: | [redacted] |
| [redacted] | [redacted] |
| Version: | 2.0 of 30Oct2020 |
| Previous versions: | 1.0 of 07Feb2020 |

**Revision history**

| Version | Author | Date | Reason for Revision |
|---|---|---|---|
| 1.0 | [redacted] | 07Feb2020 | 1st final version |
| 2.0 | [redacted] | 30Oct2020 | • Analysis set 'Enrolled Set 2' added to consider re-screened patients<br>• Description of recording of AEs and CMs within eCRF added<br>• Definition of TEAEs slightly adapted<br>• Distinction of concomitant medications and procedures added<br>• Minor inconsistencies in wording resolved<br>• Listing of protocol deviations (L5.1) added<br>• Footnotes added/adapted in Appendix A |

## 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:

| | |
|---|---|
| ADaM | Analysis Data Model |
| AE | Adverse event |
| AK | Actinic keratosis |
| ALA | 5-aminolevulinic acid |
| ATC | Anatomical therapeutic chemical classification |
| $AUC_{0-\infty}$ | Area under the curve (parameter for PK analysis) |
| BMI | Body mass index |
| $C_{max}$ | Maximal plasma concentration (parameter for PK analysis) |
| CDISC | Clinical Data Interchange Standards Consortium |
| CRF | Case report form |
| CS | Clinically significant |
| CV | Coefficient of variation |
| eCRF | Electronic case report form |
| ENR | Enrolled set |
| EoS | End of study |
| FSFV | First subject first visit |
| GmbH | Gesellschaft mit beschränkter Haftung, Ltd, limited liability company |
| ICH | International council of harmonization |
| IMP | Investigated medical device |
| MedDRA | Medical dictionary for regulatory activities |
| N | Number of subjects |
| NRS-11 | Numeric Rating Scale (11-point pain rating scale) |
| PDT | Photodynamic therapy |
| PK | Pharmacokinetic(s) |
| PP | Per-protocol |
| PpIX | Protoporphyrin IX |
| PT | Preferred term |
| SAE | Serious adverse event |
| SAF | Safety analysis set |
| SAP | Statistical analysis plan |
| SD | Standard deviation |
| SDTM | Study Data Tabulation Model |
| SOC | System organ class |
| $T_{1/2}$ | Terminal half-life after last administration (parameter for PK analysis) |
| TEAE | Treatment emergent adverse event |
| TLG | Tables, listings, graphs |
| $T_{max}$ | Time of maximum concentration (parameter for PK analysis) |
| Treatment area | Body area in which the treatment field is located |
| Treatment field | Area within the illumination area to which the medicinal product was applied |
| TS | Treated set |
| WHO-DD | World Health Organisation-Drug dictionary |
| $\lambda_Z$ | Terminal rate constant after last administration (parameter for PK analysis) |

## 1 General

This Statistical Analysis Plan (SAP) was defined by the Sponsor and the responsible Statistician. It is based upon the Study Protocol (version 2.0 of 17Jan2020) and contains detailed description of the statistical methods described therein.

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

The assessment and analysis of pharmacokinetics is covered in a separate SAP and is not part of this SAP.

### 1.1 Study Design

This is a non-randomized, open-label Phase I study to assess the pharmacokinetics (PK) of the parent drug 5-aminolevulinic acid (ALA) and its active metabolite protoporphyrin IX (PpIX) during photodynamic therapy applying 3 tubes of BF-200 ALA 10 % gel (Ameluz) in combination with the BF-RhodoLED® lamp in the systemic circulation of diseased individuals presenting with actinic keratosis (AK) on the face/scalp or in the periphery (neck/trunk/extremities) along with subjects' safety/tolerability during and after treatment. The study will be conducted in the US. All study visits and procedures will be conducted at one Phase I unit.

For each subject the following visits are planned:

- a screening visit within 14 days before treatment (Visit 1), at which eligibility of subjects for study participation will be assessed and 1 baseline PK blood sample will be taken
- a baseline/treatment visit, at which eligible subjects will receive PDT with application of 3 tubes of BF-200 ALA, and 14 PK blood samples will be collected, starting 0.5 h prior to BF-200 ALA application and then for up to 10 h afterwards (Visit 2)
- a phone call for safety assessment at 7 (± 3) days post PDT (Phone call)
- a further clinic visit, 28 (± 7) days after treatment, at which safety and tolerability assessments will be performed (Visit 3)

The primary endpoints of the study are:

- Baseline-adjusted plasma concentrations of ALA for obtaining baseline-adjusted plasma concentration-time curves
- Baseline-adjusted plasma concentrations of PpIX for obtaining baseline-adjusted plasma concentration-time curves

### 1.2 Objectives

Primary objectives

- Assessment of baseline-adjusted plasma concentration-time curves for ALA after a single PDT treatment applying 3 tubes of BF-200 ALA in conjunction with the BF-RhodoLED® under maximal use conditions in subjects with mild to severe actinic keratosis.
- Assessment of baseline-adjusted plasma concentration-time curves for PpIX after a single PDT treatment applying 3 tubes of BF-200 ALA in conjunction with the BF-RhodoLED® under maximal use conditions in subjects with mild to severe actinic keratosis.

Secondary objectives

- Evaluation of baseline adjusted pharmacokinetic parameters of ALA.
- Evaluation of baseline adjusted pharmacokinetic parameters of PpIX.
- Assessment of safety and tolerability of PDT with BF-200 ALA under maximal use conditions.

Tertiary objectives

- Evaluation of unadjusted plasma concentration-time curve for ALA and evaluation of unadjusted pharmacokinetic parameters of ALA.

- Evaluation of unadjusted plasma concentration-time curve for PpIX and evaluation of unadjusted pharmacokinetic parameters of PpIX.

### 1.3 Sample Size

Thirty-two (32) subjects will be included in this study, stratified into two groups of 16 subjects each. One of the groups will receive PDT applying 3 tubes of BF-200 ALA in the face/scalp, the other group will receive PDT applying 3 tubes of BF-200 ALA on the neck/trunk/extremities.

A previous PK Phase I study under maximal use conditions in AK subjects with 1 tube of BF-200 ALA (ALA-AK-CT006) had revealed homogeneous baseline-adjusted levels of ALA and PpIX in 12 subjects, with a CV of 53-73% in the key pharmacokinetic parameters after drug application. As statistical evaluation of PK and safety is intended to be performed as descriptive statistics only, no calculation of predictive power is required.

Based on the results of a previous PK study (ALA-AK-CT006), a group size of 12 subjects is considered to be sufficient for proper pharmacokinetic evaluation. In order to compensate for potential drop-outs of subjects as well as potentially higher variability of data due to combined investigation of two treatment areas, stratum size was adapted from 12 to 16 subjects. A drop-out rate of 25% (4 of 16 subjects) was assumed taking into account the complex nature of the study procedures comprising PDT treatment with three tubes and up to two illuminations (simultaneously and if needed), and frequent blood sampling on one single day. For this reason, the sample size of 32 subjects is considered to be adequate for pharmacokinetic evaluation in this PK study.

### 1.4 Terminology of Treatment Area, Visits and Timepoints

The notation displayed in table 1 and 2 will be used for table, listing and graph (TLG) presentation of treatment areas and visits, respectively.

Table 1: Treatment area

| **Treatment area/Stratum** | **Notation used for TLG presentation** |
|---|---|
| face/scalp | Face/scalp |
| periphery (neck/trunk/extremities) | Periphery |

Table 2: Visits and timepoints

| **Visit** | **Notation used for TLG presentation** |
|---|---|
| Screening visit (visit 1) | Visit 1 |
| Visit 2/Baseline (PDT) including rescheduled visit | Visit 2 |
| Visit 2 at timepoint -0.5 h in relation to BF-200 ALA application (Baseline for PK) | Visit 2 (-0.5 h) |
| Visit 2 at timepoint 0.0 h in relation to BF-200 ALA application (Baseline for PK) | Visit 2 (0 h) |
| Visit 2 at timepoint x* h in relation to BF-200 ALA application: | Visit 2 (x h) |
| Phone call | Phone call |
| Visit 3 End of study (EoS) | Visit 3 |
| Unscheduled visit y^ | Unscheduled visit y |

* x can be any of the following blood sampling timepoints: 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10

^ y = 1,2,3 ….

# 2 Endpoints

## 2.1 Primary Endpoints

The primary endpoints for the study are:

- Baseline-adjusted plasma concentrations of ALA for obtaining baseline-adjusted plasma concentration-time curves
- Baseline-adjusted plasma concentrations of PpIX for obtaining baseline-adjusted plasma concentration-time curves

The analysis of the primary endpoints is described in a separate pharmacokinetics SAP.

## 2.2 Secondary Endpoints

The following secondary efficacy parameters will be analyzed:

- Baseline-adjusted $AUC_{0-t}$, baseline-adjusted $AUC_{0-\infty}$, baseline-adjusted $C_{max}$, $T_{max}$, $T_{1/2}$, and $\lambda_Z$ for ALA
- Baseline-adjusted $AUC_{0-t}$, baseline-adjusted $AUC_{0-\infty}$, baseline-adjusted $C_{max}$, $T_{max}$, $T_{1/2}$, and $\lambda_Z$ for PpIX
- Frequency and severity of TEAEs / Overall tolerability
- Application site skin reactions in the treatment field(s) during and post PDT
- Application site discomfort during and after illumination reported by the subjects
- Application site pain during PDT on an 11-point Numeric Rating Scale (NRS-11)
- Vital signs
- Safety laboratory
- Physical examinations

All secondary endpoints will be analyzed overall and by stratum (face/scalp or periphery). The analysis of the pharmacokinetics parameters is described in a separate pharmacokinetics SAP.

## 2.3 Tertiary Endpoints

- Unadjusted plasma concentration-time curve for ALA
- Unadjusted plasma concentration-time curve for PpIX
- Pharmacokinetic parameters of ALA: unadjusted $AUC_{0-t}$, unadjusted $AUC_{0-\infty}$, unadjusted $C_{max}$, $T_{max}$, $T_{1/2}$, and $\lambda_Z$
- Pharmacokinetic parameters of PpIX: unadjusted $AUC_{0-t}$, unadjusted $AUC_{0-\infty}$, unadjusted $C_{max}$, $T_{max}$, $T_{1/2}$, and $\lambda_Z$

The analysis of the tertiary endpoints is described in a separate pharmacokinetics SAP.

# 3 Analysis Sets

## 3.1 Enrolled set (ENR)

All subjects enrolled in this study i.e. that provided informed consent to participate in the study.

## 3.2 Enrolled set 2 (ENR2)

All subjects enrolled in this study i.e. that provided informed consent to participate in the study will be included once. Due to COVID-19 implications 2 eligible subjects could not be treated within the visit window. Thus, these subjects were documented as drop-outs and re-screened later. Within enrolled set 2, these subjects will be included with their 2nd screening ID only.

## 3.3 Treated set (TS)

All subjects that received IMP treatment (including subjects whose PDT treatment was terminated for any reason without illumination). Subjects without any application of IMP will be excluded. If application of any IMP is not certain the subject will be included in the treated set.

## 3.4 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(s), application of BF-200 ALA, or illumination. If the conduct of any treatment procedures is not certain, the subject will be included in the SAF.

## 3.5 Pharmacokinetic Sets

The pharmacokinetic sets are defined for each of the two analytes separately. The pharmacokinetic set per analyte includes all subjects who have at least one evaluable pre-dose and post dose pharmacokinetic sample (i.e., samples taken after the application of BF-200 ALA) for the respective analyte. A post dose sample is regarded evaluable if the plasma concentration is at or above LLOQ. The two pharmacokinetic sets are defined as “PpIX Pharmacokinetic Set” and “ALA Pharmacokinetic Set”. These sets will be used for the statistical evaluation of the respective analyte.

The definition of the pharmacokinetic sets is described in detail within a separate pharmacokinetics SAP.

## 3.6 Assignment of Analysis Sets to Analysis

Pharmacokinetics variables including primary, secondary, and tertiary endpoints are analyzed with pharmacokinetic sets.

If not stated otherwise in the SAP appendix, the following assignment of analysis sets to analysis is used for non-pharmacokinetics variables: The enrolled set and enrolled set 2 are used for the disposition of subjects to analysis sets. The treated set is the analysis set for the summary of subject discontinuation. All other analyses (including all safety parameter) are based on the safety analysis set.

## 4 Statistical Evaluation

The last non-missing value until start of PDT procedure (preparation of treatment field, application of BF-200 ALA 10 % gel, illumination) will be used as baseline value. This means, assessments of Visit 1, of unscheduled visits, or of Visit 2 (pre-dose) could be used as baseline value.

No replacement of missing values will be done. Laboratory assessments of unscheduled visits will be listed but not included in tables. Thus, if laboratory assessments of a regular visit and of a subsequent unscheduled visit are available, the value of the regular visit will be displayed in tables. Apart from baseline, unscheduled visits will not be used to replace missing data of regular visits. In the unlikely event of missing laboratory assessments of a regular visit and available assessments of a subsequent unscheduled visit, it will be discussed on a case-to-case basis during the DRM whether the assessments of the unscheduled visit will be used for imputation.

The statistical evaluation of pharmacokinetic variables is described in a separate SAP.

All tables will be presented overall and by stratum (face/scalp, periphery). Listings will be sorted by stratum (face/scalp, periphery) and subject. In the unlikely event of actual treatment area differing from planned treatment area, analysis will be stratified by actual treatment area.

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 1.0).

### 4.1 Dispositions of Subjects and Analysis Sets

Disposition of subjects and analysis sets

The disposition of subjects and analysis sets, study visits and timepoints, inclusion and exclusion criteria, and the status at study termination will be tabulated.

Details regarding childbearing potential and pregnancy tests will be listed.

### 4.2 Demographics and Other Covariates

Demographic data

Demographic data (age, sex, race and ethnicity, skin type Fitzpatrick, body height [cm], body weight [kg], body mass index [BMI; calculated: (kg / $m^2$)]) will be tabulated.

Treatment area and assessment of AK lesions

The treatment area (face/scalp or periphery) of subjects, the location of treatment fields (forehead, nose …), the total area of treatment field [$cm^2$] per subject, the number of treatment fields per subject, the total lesion size of lesions ≥ 4mm [$cm^2$] and the number of AK lesions (stratified by diameter < 4mm / ≥ 4mm) will be tabulated per subject. The size of each AK lesion will be determined by multiplying the largest diameter and the perpendicular diameter. In addition, for lesions ≥ 4mm (target lesions) the diameter (calculated) and severity will be tabulated per lesion.

Medical history and concomitant diseases

The proportion of subjects with relevant medical history or concomitant diseases, with first diagnosis of AK, and with past AK lesions in same region or proximity to treatment field will be tabulated. Medical History and concomitant diseases will not be coded.

Concomitant medication

Concomitant medications (CMs) will be coded by WHO-DD of September 2019. Any medication taken by the subject during the course of the study including over-the-counter medicinal products (dietary supplements, herbal medications, etc.) will be recorded in the eCRF. A CM will be documented once in the eCRF if it is used for several adverse events (AEs) in the same way considering dosing, time frame etc. If a CM is used in different ways for several AEs then it is documented multiple times. For example, if Aspirin is given 1 day for AE1 and AE2, Aspirin is documented once. If Aspirin is given 1 day for AE1 and 2 days for AE2, then Aspirin is documented twice.

Concomitant medications will be tabulated by Anatomic Group (Anatomical therapeutic chemical classification [ATC] level 1), ATC level 4, and preferred name.
Concomitant procedures will be coded by Medical Dictionary for Regulatory Activities (MedDRA) and tabulated by Preferred Term (PT) and System Organ Class (SOC).

### 4.3 Study Drug Administration

PDT details (duration of incubation, duration of illumination, number of interruptions) will be displayed using basic statistics. Furthermore, it will be tabulated if preparation of lesions, application of dressing and illumination in all illumination areas was done according to protocol and if all three tubes of IMP were completely used.

### 4.4 Analysis of Endpoints

#### 4.4.1 Analysis of Primary Endpoints

The analysis of pharmacokinetics is described in a separate SAP.

#### 4.4.2 Analysis of Secondary Endpoints

Frequency and severity of TEAEs / Overall tolerability

AEs will be coded by using the Medical dictionary for regulatory activities (MedDRA). The latest MedDRA version at the day of data base closure will be used. If an AE occurs in multiple treatment fields, it is reported separately for each treatment field within the eCRF. Thus, some AEs are counted repeatedly for the analysis.

Analysis will focus on TEAEs. TEAEs are defined as all AEs or SAEs that occur after lesion preparation at Visit 2, which is at maximum one hour before IMP application (i.e. AE onset date/time ≥ date/time of IMP application (Visit 2) – 1h).. If unclear due to incomplete start date/time of AE or IMP application, AEs will be assumed as TEAEs.

TEAEs 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 intensity.

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

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.

AEs which occurred before lesion preparation, which is at maximum one hour before IMP application (i.e. AE onset date/time < date/time of IMP application (Visit 2) - 1h) will be listed separately but are not included in any other tables or listings.

Application site skin reactions in the treatment field(s) during and post PDT

The frequency of application site skin reactions for each category (erythema, edema, induration, vesicles, erosion, ulceration, scaling/flaking, scabbing/crusting, discharge/exudate, other) will be presented by intensity and treatment area.

Application site discomfort during and after illumination reported by the subjects

The frequency of application site discomfort for each category (burning, itching, pain, stinging, warmth, other) will be presented by intensity and treatment area.

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.

Vital signs

Blood pressure (systolic and diastolic) and heart rate will be analyzed descriptively. Absolute change from baseline to follow-up visits will be descriptively tabulated.

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

The physical examination will involve head and neck, skin, lymph nodes, thorax including heart and lungs, abdomen, and musculoskeletal, peripheral vascular and nervous system status. Information about clinically significant findings will be displayed using frequency tables. General physical examination will be listed. All clinically significant physical findings will be listed separately.

### 4.4.3 Analysis of Tertiary Endpoints

The analysis of pharmacokinetics is described in a separate SAP.

## 4.5 Missing Values

No missing value imputation method will be applied. In case an AE cannot clearly assigned as TEAE yes/no due to partial or completely missing AE/IMP application dates, it will be considered as TEAE.

## 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 set-up as a MS Excel spreadsheet, describing ADaM dataset to be created. A final define.xml 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
- Informed consent date not before application of IMP date or any other study related procedure
- Discrepancies between actual and planned treatment area
- Use of forbidden medication
- Premature discontinuation during the study
- Sample processing errors that may lead to inaccurate bioanalytical results

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 will 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) are calculated. If no further remark is given in the description of the tables, following format will be used for all tables with qualitative variables:

[redacted]

For this standard format the description of the tables in Appendix A determines only the X- and Y-variables. If another format of table is described in the details to the tables, the real design will be determined by the technical possibilities within SAS and may not look identical to the provided example. However, all information as displayed will be included.

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.

For frequency tables, missing values (including user-defined missing values) will not be included in the calculation of percentages. Percentages will be presented to one 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.

For screening failures, demographic data, date of informed consent as well as date and reason for withdrawal will be entered into the clinical database and will not be passed through the data cleaning process. Enrolled but not treated subjects (e.g. withdrawal before treatment) will be considered in tables and listings describing analysis sets, eligibility, and visits, as well as listings for subject demographics and discontinuation. Listings including enrolled but not treated subjects will be stratified by SAF.

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

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[redacted]

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:

Date: <Actual date(ddmmmyyyy)> Program: <Name of program>

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.

## 5 Changes from Protocol

In the following any changes on statistical aspects as described in the protocol are given:

- The protocol version 2.0 of 17Jan2020 states in section 14.3, that confidence intervals will be presented for maximal pain score. As all planned analyses are of exploratory nature and without any formal statistical hypotheses, no confidence intervals will be conducted.
- In addition to the analysis sets specified in protocol version 2.0 of 17Jan2020, the analysis set ‘Enrolled 2’ is defined.

## 6 Signatures

| **Statistician:** |
|---|
| [redacted] |
| Date (ddmmmyyyy) Signature |

| **Sponsor:** |
|---|
| [redacted]<br>Biofrontera Bioscience GmbH<br>Head Clinical Trial Project Management<br>Hemmelrather Weg 201<br>51377 Leverkusen<br>Germany |
| Date (ddmmmyyyy) Signature |

| **Sponsor:** |
|---|
| [redacted]<br>Biofrontera Bioscience GmbH<br>Project Manager Clinical Trials<br>Hemmelrather Weg 201<br>51377 Leverkusen<br>Germany |
| Date (ddmmmyyyy) Signature |

# TITEL PAGE SAP PK, V2.0 DATED 21-SEP-2020

**Study ALA-AK-CT015,**

*NCT04319159*

An open-label Phase I study to evaluate the pharmacokinetics of 5-aminolevulinic acid and protoporphyrin IX in human plasma under maximal use conditions after topical application of 3 tubes of BF-200 ALA 10% gel for photodynamic therapy (PDT) in subjects suffering from actinic keratosis

# Statistical Analysis Plan for Pharmacokinetics

## Study title: An open-label Phase I study to evaluate the pharmacokinetics of 5-aminolevulinic acid and protoporphyrin IX in human plasma under maximal use conditions after topical application of 3 tubes of BF-200 ALA 10% gel for photodynamic therapy (PDT) in subjects suffering from actinic keratosis

## Protocol Number: ALA-AK-CT015

[redacted]

Version: Final 2.0
Date: 21-SEP-2020

PREPARED BY: _____ _____
[redacted]
Date
Senior Biostatistician
[redacted]

REVIEWED BY: _____ _____
[redacted]
Date
Team Lead Programming
[redacted]

APPROVED: _____ _____
[redacted]
Date
Director of Exploratory Development
Biofrontera Bioscience GmbH

# INDEX

## 1. INTRODUCTION

The objective of this statistical analysis plan (SAP) is to specify calculation of pharmacokinetic parameters and the statistical analysis of the pharmacokinetic data in more detail than stated in the protocol for this Phase I clinical trial. The SAP does not change the analysis described in the protocol, but it should be precise enough to serve as a guideline for statistical programming and creation of tables for pharmacokinetics.

Safety and demographics evaluations will be described in another analysis plan provided by [redacted]

This SAP was developed with reference to the Protocol Version 2.0, 17-JAN-2020. Supplementary text gives a full specification of analyses and presentation. Deviations from the planned methods should also be summarized in Section 14. If additional analyses are required to supplement the planned analyses described in this SAP, they may be performed and will be identified in the CSR.

## 2. RESPONSIBILITIES

The following persons are responsible for the statistical analysis (including SAS®-programming), the biometrical interpretation and the internal quality control:

Responsible biometrician / project biometrician:
Name: [redacted]
Title: Senior Biostatistician

Responsible project SAS®-programmer / data analyst:
Name: [redacted]
Title: Senior Data Analyst / SAS Programmer

## 3. STUDY OBJECTIVES AND ENDPOINTS

**Primary objectives**

- Assessment of baseline-adjusted plasma concentration-time curve for ALA after a single PDT treatment applying 3 tubes of BF-200 ALA under maximal use conditions in subjects with actinic keratosis.
- Assessment of baseline-adjusted plasma concentration-time curve for PpIX after a single PDT treatment applying 3 tubes of BF-200 ALA under maximal use conditions in subjects with actinic keratosis.

**Secondary objectives**

- Evaluation of pharmacokinetic parameters of ALA: baseline-adjusted $AUC_{0-t}$, baseline-adjusted $AUC_{0-\infty}$, baseline-adjusted $C_{max}$, $T_{max}$, $T_{1/2}$, and $\lambda_Z$ if data permit
- Evaluation of pharmacokinetic parameters of PpIX: baseline-adjusted $AUC_{0-t}$, baseline-adjusted $AUC_{0-\infty}$, baseline-adjusted $C_{max}$, $T_{max}$, $T_{1/2}$, and $\lambda_Z$ if data permit
- Assessment of safety and tolerability of PDT with BF-200 ALA under maximal use conditions (not part of this SAP)

**Tertiary objectives**

- Assessment of unadjusted plasma concentration-time curve for ALA and evaluation of pharmacokinetic parameters of ALA: unadjusted $AUC_{0-t}$, unadjusted $AUC_{0-\infty}$, unadjusted $C_{max}$, $T_{max}$, $T_{1/2}$, and $\lambda_Z$ if data permit
- Assessment of unadjusted plasma concentration-time curve for PpIX and evaluation of pharmacokinetic parameters of PpIX: unadjusted $AUC_{0-t}$, unadjusted $AUC_{0-\infty}$, unadjusted $C_{max}$, $T_{max}$, $T_{1/2}$, and $\lambda_Z$ if data permit

Data will be analyzed overall and according to strata (treatment area: face/scalp or periphery (neck/trunk/extremities)).

## 4. DESIGN OF THE STUDY

This is a non-randomized, open-label Phase I study that will be conducted in the US. All study visits and procedures will be conducted at one Phase I unit.

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**) and 1 baseline PK blood sample will be taken
- a baseline/treatment visit, at which eligible subjects will receive PDT with application of 3 tubes of BF-200 ALA, and 14 PK blood samples (with 2 additional baseline PK blood samples) will be collected, starting 0.5 h prior to BF-200 ALA application and then for up to 10 h afterwards (**Visit 2**)
- a phone call for safety assessment at 7 (± 3) days post PDT
- a further clinic visit, 28 (± 7) days after treatment, at which safety and tolerability assessments will be performed (**Visit 3**)

## 5. SAMPLE SIZE AND POWER ESTIMATION

Thirty-two (32) subjects will be included in this study, stratified into two groups of 16 subjects each. One of the groups will receive PDT applying 3 tubes of BF-200 ALA on the face/scalp, the other group will receive PDT applying 3 tubes of BF-200 ALA on the neck/trunk/extremities.

A previous PK Phase I study under maximal use conditions in AK subjects with 1 tube of BF-200 ALA (ALA-AK-CT006) had revealed homogeneous baseline-adjusted levels of ALA and PpIX in 12 subjects, with a CV of 53-73% in the key pharmacokinetic parameters after drug application. As statistical evaluation of PK and safety is intended to be performed as descriptive statistics only, no calculation of predictive power is required.

Based on the results of a previous PK study, a group size of 12 subjects is considered to be sufficient for proper pharmacokinetic evaluation which is in agreement with FDA guidance (1). In order to compensate for potential drop-outs of subjects as well as potentially higher variability of data due to combined investigation of two treatment areas, stratum size was adapted from 12 to 16 subjects. For this reason, the sample size of 32 subjects is considered to be adequate for pharmacokinetic evaluation in this PK study.

## 6. ANALYSIS SETS

For purposes of analysis of PK, the following population is defined:

**Pharmacokinetic sets**

The pharmacokinetic sets are defined for each of the two analytes separately. The pharmacokinetic set per analyte includes all subjects who have at least one evaluable pre-dose and post dose pharmacokinetic sample (i.e., samples taken after the application of BF-200 ALA) for the respective analyte. A post dose sample is regarded evaluable if the plasma concentration is at or above LLOQ.

The two pharmacokinetic sets are defined as “PpIX Pharmacokinetic Set” and “ALA Pharmacokinetic Set”. These sets will be used for the statistical evaluation of the respective analyte. Data listings will include all subjects that have concentration data.

Plasma concentration data of ALA and PpIX are considered separately, e.g. a subject can be in the pharmacokinetic set for evaluation of PpIX but not for evaluation of ALA.

Subjects who develop bleeding during preparation of the treatment area are not to be excluded from the pharmacokinetic set.

The allocation of subjects to analysis sets will be fixed in a Data Review Meeting (DRM) prior to database lock to those who decide on the allocation of subjects to analysis sets. The pharmacokinetic set may exclude subjects with major/important protocol deviations with respect to factors that likely affect the comparability of pharmacokinetic results. All protocol deviations and the classification of major/important will be evaluated.

Major/important deviations are a subset of deviations that might affect the completeness, accuracy and/or reliability of the PK data . Major/important protocol deviations affecting PK may include:

- Deviations from inclusion and exclusion criteria
- Use of prohibited medicines
- Subjects that receive incorrect treatment or dose
- Sample processing errors that may lead to inaccurate bioanalytical results

## 7. PRESENTATION OF DATA

Tables, listings, and graphs will be produced in accordance with the principles outlined by the International Council of Harmonisation (ICH) E3 guideline.

Analysis of pharmacokinetics will be based on the pharmacokinetic analysis sets, if not stated otherwise.

If not stated otherwise, descriptive statistics for qualitative data include counts and percentages. The percentages will be calculated taking into account the number of subjects in the population under consideration. Descriptive statistics for quantitative data include: total number of subjects in the population under consideration, minimum, median, maximum, arithmetic mean, standard deviation, coefficient of variation (CV; if appropriate). For plasma concentrations and PK parameters related to concentrations, geometric mean, geometric standard deviation and geometric CV will also be presented.

Listings will be prepared including data that were not used for descriptive analysis for whatever reason. Data not used for descriptive analysis will be annotated accordingly.

In this Phase I PK study, missing observations will be assumed to be missing completely at random. Data will therefore be used to the largest possible extent without imputation of missing results.

In general, the scheduled measurement (rather than the control, if any) will be used in the statistical calculations for presentation in the tables of descriptive statistics (unless the scheduled measurement was considered unreliable, e.g. due to technical reasons, and needed to be replaced by an unscheduled repeat measurement). Although only one value per time point is selected for analysis, all data are presented in the data listings. Unscheduled readings will be annotated accordingly in the listings.

Data listings will include all subjects, i.e. evaluable and not evaluable. Subjects will be identified by the unique subject number.

## 8. SOFTWARE

Software to perform statistical analyses will be SAS® version 9.4 or higher. The non-compartmental pharmacokinetic analysis of the data will be accomplished by using Phoenix WinNonlin® version 7.0 or higher (linear trapezoidal linear/log interpolation). If a pharmacokinetic parameter is not automatically calculated within WinNonlin®, then WinNonlin® derived parameters will be used to determine these subsequent PK parameters and will be calculated within SAS® version 9.4 or higher.

## 9. DEMOGRAPHIC DATA AND BASELINE CHARACTERISTICS

The analysis of demographics will not be performed by [redacted] and is therefore not part of this statistical analysis plan.

## 10. PHARMACOKINETIC PARAMETERS

The pharmacokinetic parameters for ALA and PpIX which will be derived from plasma using non-compartmental methods are listed in the table below. Values < LLOQ and negative values after baseline-adjustment will be set to zero for calculation of PK parameters. The mean of the three pre-dose samples (Visit 1, -0.5 h and 0 h predose at Visit 2) will be used for baseline adjustment.

| Parameter | Description |
|---|---|
| $C_{max}$ | Observed maximum baseline-adjusted or unadjusted plasma concentration |
| $AUC_{0-t}$ | Area under the baseline-adjusted as well as unadjusted plasma concentration-time curve from time zero to the last sampling time point at which the concentration was at or above lower limit of quantification (LLOQ); t(last) is defined as the last value > 0 after baseline adjustment.<br><br>$AUC_{0-t}$ will be calculated according to the linear trapezoidal formula |
| $AUC_{0-\infty}$ | Area under the baseline-adjusted as well as unadjusted plasma concentration-time data extrapolated to infinity<br><br>($AUC_{0-\infty} = AUC_{0-t} + C_t/\lambda_z$) |
| $\%AUC_{t-\infty}$ | Proportion of extrapolated part ($\%AUC_{t-\infty}$)<br><br>$(1- [AUC_{0-t}/AUC_{0-\infty}]) \bullet 100$ |
| $t_{max}$ | time to reach $C_{max}$ |
| $t_{1/2,\lambda z}$ | apparent terminal half-life, calculated by $\ln 2/\lambda_z$ |
| $\lambda_z$ | Terminal rate constant, assuming first order elimination kinetics:<br><br>$C(t) = \beta * \exp(-\lambda_z t)$.<br><br>$\lambda_z$ denotes the terminal rate constant estimated by linear regression analysis from a range of concentrations in the terminal phase estimated for each treatment and subject by log-linear regression from the linear portion of the logarithmic transformed concentration-time plot. The algorithm will start with the last 3 points with quantifiable concentrations and increases the number of involved points by 1 until the time point after $C_{max}$ restricted on time points after removing of the BF-200 ALA gel (all PK samples after the gel is wiped off [after 3h±10 min).<br>For each regression an adjusted $R^2$ will be computed:<br>Adjusted $R^2 = 1 - \frac{(1-R^2)\cdot(n-1)}{n-2}$,<br>where n denotes the number of data points ($n \geq 3$).<br>The regression with the largest adjusted $R^2 > 0.8$ will be selected for the estimation of $\lambda_z$ using WinNonlin option “BestFit”. |

The pre-dose sample always will be considered as if it had been taken simultaneously with the application of BF-200 ALA. If there should have been any deviations in post-dose sampling, the actual sampling times relative to preceding drug administration will be used unless stated otherwise. Missing data will not be replaced or imputed in any way.

$AUC_{0-t}$ and $AUC_{0-\infty}$ will be regarded as unreliable if more than three consecutive results are missing or if the concentrations were quantifiable for fewer than 5 time points.

$C_{max}$ and $t_{max}$ will be regarded as unreliable if the maximum was observed preceding or following a sample with missing data. In case of multiple peaks, $C_{max}$ and $t_{max}$ refer to the highest measured concentration even if there should be earlier peaks. In case of two or more samples with the same concentration (as supplied by the analyst), $t_{max}$ refers to the earlier of these.

Concentrations for PK analyses will be used as supplied by the analytical laboratory.

The calculation of $\lambda_z$ will be considered unreliable in case of $r^2<0.8$. The starting time ($T_k$) for calculation of $\lambda_z$ and the number of data points in the regression ($N_z$) as well as the coefficient of determination $r^2$ will also be tabulated.

$\lambda_z$, $t_{1/2}$, $AUC_{0-\infty}$ will only be determined in subjects in which the log-linear terminal phase can clearly be defined. The value of $AUC_{0-\infty}$ will be considered unreliable but will be reported if the terminal area beyond the last quantified sample is greater than 20% of the total $AUC_{0-\infty}$.

If any pharmacokinetic parameter should be classified as unreliable, all calculations that use this parameter will be considered missing.

Unreliable parameters will be listed and flagged accordingly and set to missing for calculation of descriptive statistics and statistical analysis.

### 10.1. Tabulation of Individual Data

Values < LLOQ and negative values after baseline-adjustment will be set to zero and disregarded on logarithmic scale.

The results of unadjusted and baseline-adjusted plasma concentrations for ALA and PpIX measured will be listed for each subject with PK concentration data and tables will be prepared overall as well as by strata showing descriptive statistics for concentrations at each sampling time. Samples with an unquantifiable low concentration will be identified (i.e. <LLOQ).

Results of subjects excluded from the pharmacokinetic analysis sets will be listed together with the data of the other subjects and will be annotated accordingly. Subjects excluded from a pharmacokinetic analysis set will not be used for descriptive statistics of the respective analyte. Similar listings and tables (overall and by strata) will be prepared for pharmacokinetic parameters.

For each subject in the respective Pharmacokinetic Set, unadjusted and baseline-adjusted concentration-time curves will be plotted (showing each subject in one graph) overall as well as by strata on a linear as well as on a log-linear scale. Similar plots will be generated for the unadjusted and baseline-adjusted mean concentration-time curves (incl. figures showing the mean course without standard deviation on a linear as well as on a log-linear scale and a figure showing the mean course with standard deviation on a linear scale) overall as well as by strata.

For unadjusted concentrations, the mean overall baseline concentration will be presented as a line in the individual and geometric mean concentration graph

## 11. STATISTICAL METHODOLOGY FOR PHARMACOKINETIC ENDPOINTS

### 11.1. Primary Hypothesis to be tested

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

### 11.2. Analysis Pharmacokinetics

Pharmacokinetic analyses will be performed on the PpIX Pharmacokinetic Set for the evaluation of PpIX and on the ALA Pharmacokinetic Set for the evaluation of ALA.

Summary tables by treatment with the descriptive statistics mentioned above will be provided.

## 12. SAFETY PARAMETERS

### 12.1. Analysis for Safety

The analysis of safety will not be performed by [redacted] and is therefore not part of this statistical analysis plan.

### 12.2. Drug Administration and Blood Sampling

A listing of date and time of each drug administration and each blood sampling including time deviations will be provided sorted by subject.

## 13. STATISTICAL METHODOLOGY FOR SAFETY ENDPOINTS

Not applicable.

## 14. CHANGES FROM THE PROTOCOL

Not applicable.

## 15. INTERIM ANALYSES

Interim analyses are not anticipated.

## 16. GENERAL FORMAT OF TABLES, FIGURES AND SUBJECT DATA

There are no Sponsor-specific guidelines or SOPs that have to be observed for the analysis or report generation.

The Sponsor does not require any specific formats (e.g. footer, header, margins, fonts) to be observed. The TLFs provided for the report will be formatted with font courier new with at least 7points.

Treatment labels to be used in listings and tables will be:

BF-200 ALA: BF-200 ALA containing 7.8 % ALA in a lecithin-based nanoemulsion gel with preservatives (sodium benzoate) and xanthan gum in purified water.

The following lists (Section 17) provide an overview of a possible Table of Content (TOC).

## 17. Appendix

### 17.1. List of End-of-Text-Tables

Pharmacokinetic Data

- Summary Data on Plasma Concentrations (Unadjusted and Baseline-Adjusted)
  - Summary Data on Plasma Concentrations of ALA (ALA Pharmacokinetic Set)
  - Summary Data on Plasma Concentrations of PpIX (PpIX Pharmacokinetic Set)
- Summary Data on Pharmacokinetic Parameters (Unadjusted and Baseline-Adjusted)
  - Summary Data on Pharmacokinetic Parameters of ALA (ALA Pharmacokinetic Set)
  - Summary Data on Pharmacokinetic Parameters of PpIX (PpIX Pharmacokinetic Set)

### 17.2. List of End-of-Text-Figures

- Mean Plasma Concentration-Time Profiles of ALA (Unadjusted and Baseline-Adjusted, ALA Pharmacokinetic Set)
- Mean Plasma Concentration-Time Profiles of PpIX (Unadjusted and Baseline-Adjusted, PpIX Pharmacokinetic Set)

### 17.3. List of Individual Data

Drug Concentration Data (Unadjusted and Baseline-Adjusted)

- Drug Concentration Data of ALA (Lists and Figures)
- Drug Concentration Data of PpIX (Lists and Figures)

Pharmacokinetic Parameters (Unadjusted and Baseline-Adjusted)

- Pharmacokinetic Parameters of ALA
- Pharmacokinetic Parameters of PpIX

Drug Administration, Pharmacokinetic Blood Sampling Times and Time Deviations

## 18. Reference

1. Guidance for Industry: Bioavailability and Bioequivalence Studies for Orally Administered Drug Products - General Considerations. 1st ed.; 2003 [cited 2019 Aug 28]. Available from: https://www.fda.gov/files/drugs/published/Guidance-for-Industry-Bioavailability-and-Bioequivalence-Studies-for-Orally-Administered-Drug-Products---General-Considerations.PDF.