OPIS	**Statistical Analysis Plan**
	Sponsor: ALMIRALL, S.A. **Protocol: M-14789-42**

M-14789-42

Open phase IV study to assess the impact of Tirbanibulin on the wellbeing of patients with actinic keratoses (TIRBASKIN)

Authors	PPD Statistician
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Date	13-Feb-2024

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Draft Version 0.1	19-Dec-2023	N.A.	N.A.
Draft Version 0.2	31-Jan-2024	- Sub-group analysis added for efficacy endpoints; - Clarification on LC-OCT data results analysis; - Analysis on LSR reported by patients/physicians added; - Section 6.5 Subgroup Analyses updated; - Minor changes.	Sponsor's request
Final Version 1.0	13-Feb-2024	- Almirall S.A. Medical Manager signature added; - First Final Release.	Sponsor's approval

Clinical Trial Protocol Title:

OPEN PHASE IV STUDY TO ASSESS THE IMPACT OF TIRBANIBULIN ON THE WELLBEING OF PATIENTS WITH ACTINIC KERATOSES (TIRBASKIN)

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TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AE	Adverse Event
AK	Actinic Keratoses
AESI	Adverse Events of Special Interest
BMI	Body Mass Index
EC	Exclusion Criteria
CI	Confidence Interval
CRF	Case Report Form
CRO	Clinician Reported Outcome
CSR	Clinical Study Report
EPQ	Expert Panel Questionnaire
FAS	Full Analysis Set
HRQoL	Health-related Quality of Life
IC	Inclusion Criteria
ICF	Informed Consent Form
IMP	Investigational Medicinal Product
MedDRA	Medical Dictionary for Regulatory Activities
LC-OCT	Line-field Confocal Optical Coherence Tomography
LSR	Local skin reactions
LTS	Local Tolerability Signs
QoL	Quality of Life
PRO	Patient-Reported Outcomes

SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
SE	Standard Error
SOC	System Organ Class
SoE	Schedule of Events
TSQM	Treatment Satisfaction Questionnaire for Medication
WHO	World Health Organization
WOCBP	Women Of Child-Bearing Potential

1. INTRODUCTION

The current Statistical Analysis Plan (SAP) for the Final Analyses refers to Clinical Trial Protocol Version 3.0 for Spain dated March 21st, 2023 and Clinical Trial Protocol Version 2.0 for Italy dated March 21st, 2023.

1.1. Changes from The Study Protocol

Clarification on LC-OCT analysis has been added.

2. STUDY OBJECTIVES AND ENDPOINTS

OBJECTIVES	VARIABLES	ENDPOINTS
Primary objective: To assess treatment satisfaction on Day 57 in patients with AK of the face or scalp following treatment with Tirbanibulin ointment 1% administered once daily for 5 consecutive days.	• TSQM-9 components (Convenience, Efficacy and Total Satisfaction) sub-scores. • Frequencies of each component's responses.	**Primary endpoint:** TSQM-9 questionnaire components sub-scores with 95% confidence interval.
Secondary objective(s): To evaluate patient-reported outcomes following treatment with Tirbanibulin ointment 1% administered once daily for 5 consecutive days.	• Skindex-16 components (symptoms, emotions and functioning) sub-scores and global score. • Frequencies of each component's responses.	**Secondary endpoint(s):** Change from baseline in Skindex-16 questionnaire score with 95% confidence interval.
	• Likert Scale score. • Frequencies of each response.	Likert Scale questionnaire score with 95% confidence interval.
	• TSQM-14 components (Convenience, Efficacy, Global Satisfaction and Total Satisfaction) sub-scores.	TSQM-14 questionnaire components sub-scores with 95% confidence interval.

OBJECTIVES	VARIABLES	ENDPOINTS
To evaluate physician-reported outcomes following treatment with Tirbanibulin ointment 1% administered once daily for 5 consecutive days.	• Frequencies of each component's responses. EPQ questionnaire (patient version)'s responses.	Proportion of EPQ questionnaire's responses with 95% confidence interval.
	EPQ questionnaire (physician version)'s responses.	Proportion of EPQ questionnaire's responses with 95% confidence interval.
To evaluate efficacy outcomes of patients following treatment with Tirbanibulin ointment 1% administered once daily for 5 consecutive days.	• Lesion clearance. • Number of lesions.	Proportion of patients with complete (100%) clearance of all lesions within the application area with 95% confidence interval.
	• Lesion clearance. • Number of lesions.	Proportion of patients with partial (>75%) clearance of all lesions within the application area with 95% confidence interval.
	• Total AK lesion count. • New AK lesion count. • Old AK lesion count.	Number of new and old AK lesions with 95% confidence interval.
	Olsen characterization.	Proportion of patients by Olsen characterization with 95% confidence interval.

OBJECTIVES	VARIABLES	ENDPOINTS
To evaluate safety outcomes of patients following treatment with Tirbanibulin ointment 1% administered once daily for 5 consecutive days.	LC-OCT data of clinical and subclinical lesions.	LC-OCT of clinical and subclinical lesions as Atypia scores.
	• Adverse events maximum severity grade. • Adverse events SOC. • Adverse events PT.	Proportion of patients reported at least one adverse event by incidence and severity.
	• LTS composite score. • LTS severity grade assessed by physicians. • Number of LTS reported reported by patients.	Proportion of patients reported at least one local tolerability sign (LTS).
To evaluate medication adherence of patients following treatment with Tirbanibulin ointment 1% administered once daily for 5 consecutive days.	Number of missing doses applied.	Proportion of patients applying Tirbanibulin doses.
Exploratory objective(s): NA	NA	**Exploratory endpoint(s):** NA

3. BACKGROUND AND RATIONALE

Actinic keratoses interchangeably referred to as solar keratoses are common skin lesions primarily caused by non-ionizing radiation, in particular ultraviolet light associated with chronic sun exposure. The clinical manifestations are red, irregular, scaly plaques, or papules on the sun exposed areas, usually found on the face, balding scalp, cleavage, ears, lips. If left untreated, the lesions are associated with the risk of malignant transformation in non-melanoma skin cancer, including squamous cell carcinoma (SCC).

The risk of AK progression rate to invasive cutaneous SCC is estimated to be 0.25% to 1% each year. However, the causative factor of nearly 60% of SCC develops from AK. The presence of AK lesions in photo-exposed areas, such as the face and scalp, is relatively frequent among the

population and constitutes one of the main reasons for consulting a dermatologist. In fact, AK prevalence is observed to increase with age for both sexes, reaching 60.4% of patients older than 80.

The mainline of AK treatment modalities includes cryosurgery, skin field therapy (topical agents like 5-fluorouracil, imiquimod, diclofenac) and photodynamic therapy. Relatively, these therapies demanded a long treatment period of approximately 4-16 weeks and were associated with numerous local skin reactions (LSR) like irritations, pain, ulcerations, erosions, pigmentations and scarring of the skin. These unmet factors increased the chance of low treatment compliance and undermine treatment success. In consequence, both current guidelines and expert consensus advocate choosing the treatment schedule based not only on factors associated with AK, but also on the characteristics, expectations, opinions and preferences of the patients themselves.

The novel microtubule inhibitor Tirbanibulin, its short treatment time (5 days) and minimal adverse reactions could address the unmet need of patients suffering from AK lesions, thereby increasing medication adherence and success.

In this study, the impact of Tirbanibulin on the wellbeing of patients will be evaluated in patients with a diagnosis of AK of the face or scalp following treatment with Tirbanibulin ointment 1% administered once daily for 5 consecutive days. The study will mainly focus on treatment satisfaction.

Refer to study protocol (section 5.1) for further details.

3.1. Overall Study Design and Plan Description

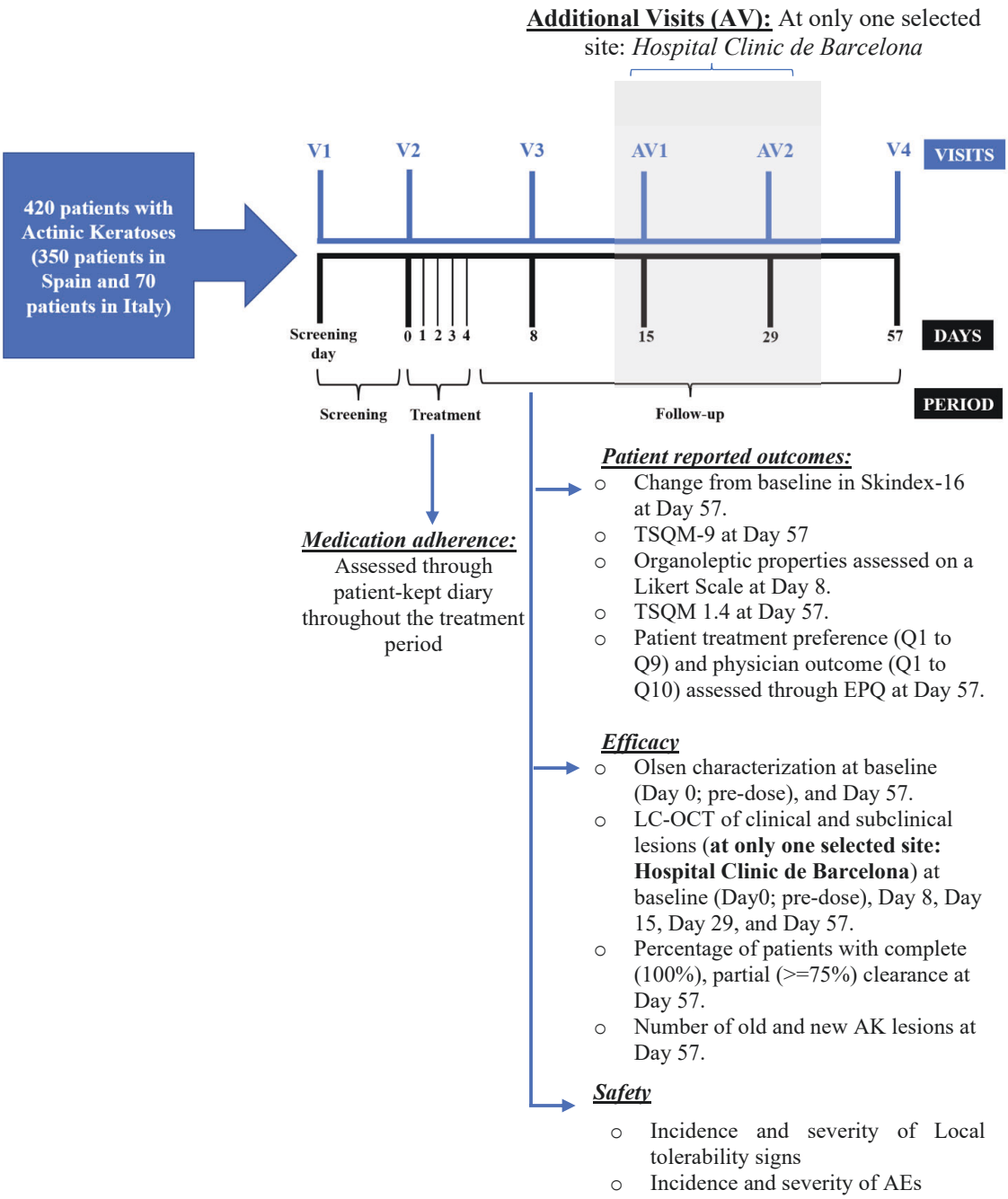
This is a multicentre single-cohort phase IV low-interventional clinical study that is conducted in 30 sites in Spain and 7 sites in Italy.
Sites have been selected on the basis of geographic region and institution size (in order to represent variations in current real-world patterns of care) and sites are hospitals where patients with AK are usually managed. The participating physicians are dermatologists.

Approximately 420 patients with AK lesions of the face or scalp will be enrolled (i.e. a minimum of 10 patients at each site): following informed consent and verification of eligibility criteria, patients will begin treatment with Tirbanibulin administered topically at a dose of 2.5 mg once daily for 5 consecutive days to a contiguous area. Tirbanibulin will be applied to evenly cover up to a 25 cm2 treatment field on the face or scalp.

Study visits will be held at screening and on Days 0 (baseline), Day 8, and Day 57. Only in 1 site (Hospital Clinic de Barcelona), study visits will be held at screening and on Day 0 (baseline), Day 8, Day 15, Day 29, and Day 57, but this site did not include patients in time for IA participation.

The total duration of the study will be approximately 9 months, including up to 6 months for recruitment, 1 month for screening and 2 months for treatment, and follow-up.

Figure 1 Schematic Trial Design



**Abbreviations: TSQM- Treatment Satisfaction Questionnaire for Medication; EPQ-Expert Panel Questionnaire; LC-OCT- Line-field Confocal Optical Coherence Tomography; AE-Adverse Event; AV1: Additional Visit 1; AV2: Additional Visit 2; Q: Question; Note: The duration of time window planned for each visit is +/- 3 days.*

3.2. Selection of Study Population

The study population will consist of male and female adult patients with 4-8 non-hyperkeratotic non-hypertrophic AK lesions of the face or scalp in an area of up to 25 cm2, patients have not been previously treated in the same area in the last 6 months. A minimum of 30% of patients previously treated in other small areas (up to 25cm2) in the last >1 to <6 months to avoid patient recall bias from previous treatments will be enrolled.

A complete list of all inclusion/exclusion criteria is provided in Sections 8.3 Inclusion criteria and 8.4 Exclusion criteria of the study protocol respectively.

3.3. Treatment

3.3.1. Treatment Administered

Treatments administered will be topical Tirbanibulin ointment 1%, which is the Investigational medicinal product (IMP) for this study.

Only participants enrolled in the trial will receive trial IMP which will be supplied in single use sachets, each of which contains 250 mg of the ointment. Each sachet will be for use as a single-dose application. Each eligible subject will be assigned a study kit containing 5 single-dose individual sachets filled with Tirbanibulin ointment 1%. Each sachet and study kit will be labelled in accordance with national regulations.

Patients should not discard the used and unused sachets and return all these sachets to the site at Day 8 (Visit 3), post dose, in order to check compliance.

Regardless of treatment administration, study treatment will be administered to all participants once daily for the following 5 consecutive days: Day 0, Day 1, Day 2, Day 3, Day 4. On Day 0, the first dose of the IMP will be administered. The first dose is recommended to be applied on-site, under the supervision of the study personnel. Following which, the subjects will self-administer the remaining individual sachets once daily at home. Preferably, the trial medication should be administered in the evening (potentially before bed) and at the same time. For approximately 12 hours after the administration, it is imperative that the application-site will remain untouched and dry. Subjects will be educated to wash their hands with soap and water after ointment application, to wash the treatment area gently with a mild, nonabrasive, non-medicated soap or shampoo. The treatment area should not be occluded with

bandages, and band aids. The treatment area should not be exposed to excessive sunlight or UV light.

In order to identify the treatment area for daily self-administration, the location and shape of the treatment area will be photographed and/or marked on an acetate transparency sheet (depending on clinical practice) for recording purposes on the subject's skin.

The complete procedure applied in order to Identify the treatment area using acetate transparency sheet or standardized photography at Screening is reported in Table 2 of Section 10.5.2 of Study protocol.

3.3.2. Method of Assigning Patients to Treatment Groups

This is a single-cohort study so all eligible participants will be assigned to study treatment Tirbanibulin ointment 1% at Baseline visit (Visit 2).

The Investigator will confirm that the participant has fulfilled all the inclusion/exclusion criteria in the source documents and the appropriate page on the eCRF. The Investigator will then assign the participant to treatment.

3.3.3. Prior and Concomitant Therapy

Since this is a single arm study, no control treatment or other treatments beyond IMP Tirbanibulin will be included in this study.

It will be mandatory to report on the appropriate electronic Case Report Form (eCRF) page information relative to concomitant prescription medications, over-the-counter medications, and supplements.

As regards to pre-trail medications, each patient's complete AK treatment history of face and scalp will be recorded on the eCRF as well as any treatment (prescription and non-prescription including vitamins and dietary supplements) and procedure taken during the 28 days before Day 0. Patients must not have received any IMP within the preceding 30 days from the first trial drug administration or any medication that could not be eliminated from the body (5 elimination half-lives of the IMP).

Regardless to concomitant medications, during the follow-up period (period between Visit 3 and Visit 4, i.e. from Day 8 to Day 57), only medications/therapies for the treatment of Adverse Events (AEs) in the treatment area and those that may affect the assessment of AK lesion recurrence in the treatment area will be entered in eCRF page as well as any topical products for treatment of local skin reactions/local tolerability signs in the treatment area allowed by the Investigator from Day 8 up to Day 57.

Use of any treatment for AK lesions other than study drug on the treatment area will be prohibited during the study. Subjects will be reminded that AK lesions located outside the treatment area may be treated by lesion-directed treatment only, e.g., cryotherapy or biopsy. A detailed list of prohibited medications is provided in section 9.6.3 Prohibited Medications/Therapy of the study protocol. There are no restrictions during the study on smoking/tobacco use, diet, alcohol/caffeine, water or other beverages, or physical activity. Once the last Tirbanibulin dose will be applied and related trial measurements will be completed, patients should continue to take their usual medications, also allowed during the trial, and may resume other medications interrupted prior to trial enrolment as deemed appropriate by the Investigator; while it is not planned to treat patients with Tirbanibulin any further than scheduled in this trial.

3.4. Schedule of Time and Events

The flowchart of the follow-up visits and assessments scheduled for study centers is reported in Table 1 below.

Period	**Screening** [a]	**Treatment**					**Follow-up**			
			-	-	-	-		**Additional Visits *** (Applicable for one site: Hospital Clinic de Barcelona)		**Final visit**
Visit	**1**	**Baseline 2**					**3**	**AV1**	**AV2**	**4**
Days	**-28 to -1**	**0**	**1**	**2**	**3**	**4**	**8**	**15**	**29**	**57**
Visit time window (days)	**± 3**	**± 3**	**-**				**± 3**	**± 3**	**± 3**	**± 3**
Informed consent	X									
Inclusion & exclusion criteria	X									
Socio-demographic characteristics	X									
AK therapy history [b]	X									
Identification of treatment area [c]	X	X [d]								
Medical history	X									

Period	**Screening** [a]	**Treatment**					**Follow-up**			
			-	-	-	-		**Additional Visits *** (Applicable for one site: Hospital Clinic de Barcelona)		**Final visit**
Visit	**1**	**Baseline 2**					**3**	**AV1**	**AV2**	**4**
Days	**-28 to -1**	**0**	**1**	**2**	**3**	**4**	**8**	**15**	**29**	**57**
Prior and concomitant medications/therapies	X	X [d]	X	X	X	X	X	X	X	X
Physical Examination	X									X
Vital signs	X						X			X
Skindex-16		X [d]								X
Organoleptic properties (Likert scale)							X			
TSQM-9										X
TSQM 1.4										X
AK lesion count	X	X [d]								X
Standardized Photography		X [d]					X			X
Olsen grading		X [d]								X
LC-OCT of clinical and subclinical lesions [f]		X [d]					X	X	X	X
EPQ [g]										X
AEs/SAEs [e]		X [d]	X	X	X	X	X	X	X	X
Local Tolerability Signs		X [d]	X	X	X	X	X	X	X	X
Pregnancy test for WOCBP [h]	X	X [d]								
Tirbanibulin (Study drug) application		X	X	X	X	X				
Patient Diary		X	X	X	X	X				

Period	**Screening** [a]	**Treatment**					**Follow-up**			
			-	-	-	-		**Additional Visits *** (Applicable for one site: Hospital Clinic de Barcelona)		**Final visit**
Visit	**1**	**Baseline 2**					**3**	**AV1**	**AV2**	**4**
Days	**-28 to -1**	**0**	**1**	**2**	**3**	**4**	**8**	**15**	**29**	**57**
Return of Study drug sachets (used and unused) and patient diary							X			

Note: The duration of time window planned for this study is +/- 3 days

Abbreviations: TSQM=Treatment Satisfaction Questionnaire for Medication; LC-OCT = Line-field Confocal Optical Coherence Tomography; EPQ=Expert Panel Questionnaire; AK=Actinic Keratoses; AE=Adverse Event; SAE=Serious Adverse Event; WOCBP=Women of Childbearing Potential;

* Additional visits 1 and 2 (AV1 and AV2) will be performed only in one clinical site: Hospital Clinic de Barcelona

a. The duration for screening is 1 month.
b. This includes history of treatments namely topical medications freezing, laser, scraping, etc.
c. The location and shape of the treatment area will be marked on an acetate transparency sheet or captured through standardized photography that will be kept for recording purposes. The treatment area will be also outlined with dots and dashes with an indelible marker on the patient' skin, to help him/her with daily self-administration of study ointment.
d. These are baseline procedures/assessments and will be performed before application of study medication.
e. AEs and local tolerability signs will be reported separately. At each study visit, AEs will be recorded **before** assessment of local tolerability signs in the treatment area. From Day 0 to Day 4, AEs and local tolerability signs will be recorded using patient diary. During rest of the study period, AEs and local tolerability signs will recorded and evaluated by the investigator at each visit or through telephonic follow-up in case of urgency.
f. LC-OCT assessments are applicable only for one site: Hospital Clinic de Barcelona. LC-OCT assessment will be done for 2 lesions, which includes 1 clinical lesion and 1 subclinical lesion. eCRF will collect only the information about LC-OCT performed or not at each scheduled visit.
g. EPQ comprises of 2 versions: patient version and clinician version. Patient version of the EPQ should be completed by the patient and clinician version by the clinicians.
h. Initially, **urine** pregnancy test will be performed. If pregnancy is tested positive, **serum** pregnancy test will be performed for confirmation.

The total duration of each patient's participation in the trial is estimated to be 3 months, including 1 month for screening and 2 months for treatment, and follow-up.

- **Screening period** (period between Informed Consent Form (ICF) signature day, that could occur up to 28 days before to Day 0, and Day 0): before patient's signing the ICF, Investigators will describe in detail to the patient all activities and requirements,

in order to ensure their understanding and compliance with sample collection and clinical examinations.

After that, Investigators will evaluate eligibility of patients for entry in the trial by comparing past and current medical status, as documented in the patient's medical records, the inclusion/exclusion criteria of the trial and Investigators will complete eCRF specifying whether the subject will be eligible to participate in the study and reasons for screen failure, in case he/she will not.

- **Treatment period** (from Day 0 to Day 4): visits and assessments will be performed in accordance with the schedule detailed in Table 1. They cannot be repeated or skipped but may be postponed (if not started yet) within the time window of ± 3 days.

  Trial visits will be scheduled in the morning. At the scheduled visit (Day 0), it is recommended IMP dosing occur while being witnessed by the research personnel at the clinic in order to ensure the proper IMP application, as well as the correct timing of activities to occur before and after dosing.

  At Baseline (Day 0, pre-dose), the Investigator must obtain a standardized photograph, using their own cameras, of each subject's treatment area. These photographs will be attached with the eCRF. In advance, Investigators will provide to each patients a diary to record usage pattern of the Tirbanibulin ointment applications during the Treatment period.
- **Follow-up period** (Visit 3 and Visit 4, while 2 additional visits (AV1 and AV2) will be performed only in one clinical site: Hospital Clinic de Barcelona): at the scheduled visits both patient and physician will report outcome assessments, while efficacy and safety assessments will be recorded by the research personnel.

  At Day 8 and Day 57, the Investigator must obtain a standardized photograph, using their own cameras, of each subject's treatment area ensuring that the same lighting, background, subject positioning relative to the camera and camera settings will be used for each photograph (including photograph taken at Baseline). These photographs will be attached with the eCRF in order to document the appearance of the subjects' treatment area and to assist with the identification and confirmation of the location of the treatment area throughout the study (further information about standardized photographs in Section 10.4.4 of study protocol).

  At Day 8, patients should return diary in order to Investigator or other authorized trial personnel will transcribe all information recorded inside the diary in the corresponding eCRF page.

  At each scheduled visits (also included visits in Treatment period) a booklet will be dispensed to the patients for completing questionnaires named Skindex-16 (at baseline and Day 57), Organoleptic properties on Likert Scale (at Day 8), TSQM-1.4 and TSQM 9 (at Day 57), and patient version of the EPQ (at Day 57). Similarly, a booklet that consists of only 1 questionnaire (namely clinician version of the EPQ) will be completed by the investigators at Day 57.

3.5. Sample Size and Power Estimation

A sample size of 420 patients allows to estimate the mean of a specific component of TSQM-9 at Day 57 with a precision (half width) of the 95% confidence interval (CI) ranging from ±1.6 to ±2.1 if we assume a standard deviation between 15 and 20.

4. DEFINITIONS AND GENERAL METHODOLOGY

4.1. General Methodology

All statistical tables, listings and analyses will be produced using SAS® release 9.4 or later (SAS Institute, Inc, Cary, NC, USA).

The data from all sites will be pooled and summarized.

Continuous data will be summarized with mean, standard deviation (SD), median, first (Q1) and third (Q3) quartiles, minimum and maximum. Categorical data will be presented by absolute and relative frequencies (n and %) or contingency tables. The percentage calculation will be based on total values (i.e., count will be shown also in the missing category, unless otherwise specified). Bilateral 95% confidence limits will be presented as appropriate. Unless stated otherwise, a two-sided alpha level 0.05 will be considered.

4.2. Definitions

Study Phases

The following phases are identifiable:

- **Screening period**: Day -28 to Day -1
  Period of up to 4 weeks before Baseline used to assess patient eligibility. The screening period starts with the signing of the informed consent. No re-screening of patients is allowed.
- **Treatment period**: Day 0 (Baseline) to Day 4
  This period started with the first treatment administration (Day 0) and ended at the last treatment administration planned at Day 4.
  At the Baseline visit (Day 0), after confirmation of eligibility, patients will be included and treated with Tirbanibulin administered topically at a dose of 2.5 mg once daily for 5 consecutive days to a contiguous up to a 25 cm^2 area field on the face or scalp.
- **Follow-up period**: Day 5 to Day 57
  In this period 2 visits are scheduled at Day 8 (Visit 3) and at Day 57 (Visit 4, Final Visit), but only for 1 site (i.e. Hospital Clinic de Barcelona), study visits will be held at Screening and on Day 0 (baseline), Day 8, Day 15, Day 29 and Day 57.

- **End of Study**
  Day 57 visit (Visit 4) will constitute the End of Study Visit.

Definition of Screening Failures

Participants who sign an informed consent form and subsequently found to be ineligible (i.e., due to non-meeting of inclusion/exclusion criteria) will be considered screen failures. The reason for screen failure (i.e., protocol deviation and non-protocol deviation) will be properly summarized.

Definition of Baseline

Baseline date (Day 0) is defined as the date of the first Tirbanibulin administration, i.e., Visit 2 date.

First/Last Administration of Study Treatment

The date of first treatment administration is the first "Date of study medication application" in TIRBANIBULIN (STUDY DRUG) APPLICATION eCRF page (and it should coincide with Baseline date).

The date of last treatment administration is the "Last known date subject took study treatment" in END OF TREATMENT DISPOSITION eCRF page.

End of Trial

The "end of trial" is defined as the date when all patients complete the Day 57 visit or prematurely discontinue from the study, i.e., "Date of Discontinuation /Study completion" date in END OF STUDY DISPOSITION eCRF page.

Completed Patient

A participant is considered to have completed the study if he or she has completed all phases of the study including the last visit and the last scheduled procedure shown in the Table 1 reported in Section 2.4 of this document. Only patients who perform the last scheduled follow-up contact, i.e., who performed Visit 4 will be considered trial completers.

4.3. Coding of Therapies and Medical Terms

Medications/therapies reported in PRIOR AND CONCOMITANT MEDICATIONS/THERAPIES eCRF pages will be coded using version B3_Q1_2023 of the World Health Organization (WHO) dictionary. Medical terms reported in the MEDICAL HISTORY, SURGICAL AND MEDICAL PROCEDURES or ADVERSE EVENTS eCRF pages are coded using version ENG 26.0 of the Medical Dictionary for Regulatory Activities (MedDRA).

Versions of the dictionaries can be upgraded during the study according to Sponsor's request.

4.4. Handling of Drop-Outs or Missing Data

4.4.1. Missing or Partial Dates

No imputation of missing/partial dates will be performed.

4.4.2. Handling of Missing Data/Imputation/Censoring Rules

Patients will be included in each analysis based on available data. No replacement of missing values will be performed. In case of missing values, they will be displayed as "Missing" in the descriptive analysis.

4.4.3. Handling of Drop-Out Patients

Screening failure patients and drop-out patients will not be replaced in this study.

4.5. Multiple Comparison/Multiplicity

Not applicable.

4.6. Multicentre Studies/Center Pooling

The study will be conducted at about 33 sites in Spain and 7 sites in Italy. The data from all centers will be pooled and summarized.

5. ANALYSIS POPULATIONS

Starting from Screened patients (i.e., all patients who signed the informed consent form), the following analysis populations will be defined for statistical analysis:

- **Enrolled population**: all patients who signed the informed consent form and completed the screening disposition.
- **Full analysis set (FAS) population:** all patients who signed the informed consent form and applied at least one dose of Tirbanibulin.
- **Evaluable patient population:** FAS patients who completed the 57 days of observation and have the TSQM-9 assessment.
- **Safety patient population:** Full analysis set patients.
- **LC-OCT Population**: FAS patients who performed at least one valid LC-OCT assessment (i.e., FAS patients enrolled at site 101).

6. STATISTICAL METHODOLOGY

For some specific endpoints, sub-group analysis will be performed by factors reported in Section 6.5.

6.1. Study Patients

An illustration of Screening Disposition will be provided on Screened patients (i.e., who signed the informed consent form), providing the number of Enrolled, the number of patients who completed the screening defined as eligible patients at screening (i.e., patients for whom "Status = Completed" in the SCREENING DISPOSITION eCRF page) and the number who fail the screening (i.e., patients not completed, patients who reported "Status = "Screen Failure" or "Status = "Other") together with the reason for screen failure that will be tabulated according to the inclusion/exclusion criteria violated. Screening disposition summary will be provided by country (i.e., Italy and Spain) and overall.

Furthermore, the proportion of Screened patients who met/did not meet each IC/EC criterion at Screening will be provided. Since IC n.8 will be evaluated also at Baseline Visit, the female patients' pregnancy status will be reported also at Baseline Visit.

A description of patient disposition will be provided on the Enrolled patients by country and overall providing both treatment and study patient status. Treatment status will be described based on END OF TREATMENT DISPOSITION eCRF page specifying the number of patients who completed the treatment, and the number of patients who discontinued the study treatment (i.e., patients with Status not equal to "Completed" in the END OF TREATMENT DISPOSITION eCRF page) will be summarized together with reason for treatment discontinuation. Similarly, study disposition will be described based on END OF STUDY DISPOSITION eCRF page specifying the number of patients who completed the study and the number of patients who discontinued the study with the reason for discontinuation.

The numerousness of the analysis populations will also be described by country and overall and the reasons for excluding a patient from an analysis population will be provided on Enrolled patients.

Protocol deviations will be summarized for the Enrolled patients. Non-protocol deviations (i.e., criteria leading to the exclusion from an analysis population, even if they do not themselves constitute a deviation from the study protocol) will be also summarized on Enrolled patients. The Non-protocol deviation considered for this study is listed in Table 5.1-1.

Table 5.1-1

Deviation code	Description	Exclusion from analysis population
NOPD01	Patient without at least one application of study drug.	FAS, Evaluable population, SAF
NOPD02	Patient who discontinued the study before Visit 4 (Day 57).	Evaluable population
NOPD03	Patient without at least one valid LC-OCT assessment.	LC-OCT population

NOPD=Non-protocol deviation; FAS=Full Analysis Set; SAF=Safety Set.

TIRBANIBULIN (STUDY DRUG) APPLICATION eCRF page will be used to check if a patient performed at least one application of study drug while END OF STUDY DISPOSITION eCRF page will be used to identify early discontinued patients (i.e., patients with status different from "Completed" as end of study status will be considered as discontinued patients).

Patients without valid LC-OCT assessment are patients not enrolled in Hospital Clinic de Barcelona, since patients enrolled in Hospital Clinic de Barcelona sign a specific Informed Consent including also LC-OCT assessment.

All protocol and non-protocol deviations observed will be also listed.

6.2. Background and Demographic Characteristics

Demographic and other patient's characteristics will be obtained by the Investigator or qualified designee at the Screening/Baseline visit and they will be summarized for FAS population and the Evaluable population. In particular:

- **Demographic information**: descriptive summary of age (years) and number and percentages of patients by sex and race. For female patients will be provided the proportions of female reproductive status (i.e. Premenarchal, Childbearing potential, Menopause, Sterile). Each patient's year of birth will be listed also. The same description will also be provided by country and for patients valid for LC-OCT Population.
- **Fitzpatrick skin type scale**: it classifies skin into six separate categories based on colour and response to sunlight: Type I is the lightest in colour and most sensitive to sun exposure; type VI is the darkest and most sun-resistant.
  The number and percentage of patients who answered "Was the Fitzpatrick Skin Type assessment performed? = Yes" on FITZPATRICK SKIN TYPE eCRF page and the

proportion of patients classified by the Fitzpatrick skin type scale (Type I, Type II, Type III, Type IV, Type V, Type VI) will be provided.

- **Medical history**: patient history should include information on clinically significant personal history including identification of current and past smoking history, current alcohol use, past history of infections, AK clinical history, history of non-melanoma skin cancer (NMSC) malignancies, and major cardiovascular conditions.
  Medical history will be summarized by System Organ Class and Preferred Term according to the MedDRA dictionary, presenting the number and percentage of patients with at least one medical history finding (i.e., reported in the "History/Condition category" of MEDICAL HISTORY eCRF page) considering separately relevant medical history, i.e. conditions reported as "Ongoing = No", and current medical condition, i.e. conditions reported as "Ongoing = Yes".
- Similarly, **AK therapy history** (history of treatments namely topical medications cryotherapy, photodynamic therapy, laser, scraping, etc.) will be described reporting the number and percentage of patients with at least one AK therapy history finding (i.e., reported in the "Therapy category" of AK THERAPY HISTORY eCRF page) considering separately relevant AK therapy history, i.e. conditions reported as "Ongoing = No", and current AK therapy condition, i.e. conditions reported as "Ongoing = Yes". This summary will be provided by country and overall.
- **Count of AK lesions**: AK lesion should be counted only if it is completely inside the treatment area. Descriptive statistics on the number of AK lesions counted at Screening and Baseline visits will be provided by country and overall.
- **Identification of treatment area**: Tirbanibulin will be applied to evenly cover up to a 25 cm^2 treatment field on the face or scalp. At each timepoint (i.e., Screening and Baseline visits), descriptive statistics on AK lesion area (cm^2) and the proportions of treatment area's location will be provided. Moreover, the proportion of patients previously treated for AK on the current treatment area of the face or scalp in the last 6 months will be computed. The other treatment area's characteristic (i.e., patient previously treated in other small areas (up to 25cm^2) in the last >1 to <6 months) will be listed at each timepoint.
- **Standardized photographs**: Care must be taken by the research personnel to ensure that the same lighting, background, subject positioning relative to their own camera and their camera settings are used for each photograph. The number and percentages of patients with standardized photographs of treatment area performed before application of study medication will be only listed for all timepoints.
- **Prior medications/therapies** are defined as therapies starting prior to the study and ending prior to the first Tirbanibulin administration.
  Since no imputation will be performed in case of partial/missing medication's start or end date, the presence of at least one of the following conditions will be used to identify prior medication:

- If medication end date is complete, medication status is not ongoing or missing and medication end date < Tirbanibulin start date;
- If day and month of medication end date are missing, medication status is not ongoing or missing and medication end date year < Tirbanibulin start date year;
- If only day of medication end date is missing, medication status is not ongoing or missing and medication end date year < Tirbanibulin start date year;
- If only day of medication end date is missing, medication status is not ongoing or missing, medication end date year = Tirbanibulin start date year and medication end date month < Tirbanibulin start date month;
  Otherwise, medication will be identified as Concomitant medication.

Prior medications will be described by ATC code (2nd level class) and Preferred Term, presenting the number and percentage of patients taking at least one prior medication. The same summary will be performed for surgical and medical procedures.

6.3. Efficacy Evaluation

6.3.1. Primary efficacy analysis

The primary efficacy analysis will be evaluated on the Evaluable population. All data collected in the database will be taken into account.

Definition of primary endpoint

The primary objective is to assess treatment satisfaction on Day 57 in patients with AK of the face or scalp following treatment with Tirbanibulin ointment 1% administered once daily for 5 consecutive days. Primary objective will be evaluated through Treatment Satisfaction Questionnaire for Medication Version 9 (TSQM-9) score.

TSQM-9 is a 9-item clinically validated psychometric instrument developed from the TSQM 1.4 (an extended version of TSQM-9 composed by 14 items more accurately descripted in Section 5.3.2.3 of this document): in fact, the 5 items associated with side effects related to the medication (question 4 to 8 of Version 1.4) are excluded in TSQM-9. It comprises 3 domains: efficacy (questions 1-3), convenience (questions 4-6), and global satisfaction (questions 7-9) of the drug application.

Similarly to TSQM 1.4, the TSQM-9 global total scores [1] vary from 0 to 100 with higher score indicating higher treatment satisfaction. In order to calculate the three domain scales, the responses to items will be summed and transformed. Specifically, the scores will be computed by adding the items loading on each domain. The lowest possible score (for each item the lowest possible score is 1, therefore for all the three subscale the lowest possible score is 3) will be subtracted from the composite score

and divided by the greatest possible score. This will provide a transformed score between 0 and 1 that is then multiplied by 100. For a more schematic explanation, see the table below:

Score calculation formula	
Effectiveness	
If no missing item	$\frac{(\sum item1, item2, item3) - 3}{18} * 100$
If one missing item	$\frac{(\sum the\ 2\ completed\ items) - 2}{12} * 100$
Convenience	
If no missing item	$\frac{(\sum item4, item5, item6) - 3}{18} * 100$
If one missing item	$\frac{(\sum the\ 2\ completed\ items) - 2}{12} * 100$
Global satisfaction	
If no missing item	$\frac{(\sum item7, item8, item9) - 3}{14} * 100$
If item7 or item8 missing	$\frac{(\sum the\ 2\ completed\ items) - 2}{10} * 100$
If item9 missing	$\frac{(\sum the\ 2\ completed\ items) - 2}{8} * 100$

If more than one item is missing from a domain of the TSQM-9 for a particular patient, this domain should be considered invalid for that respondent.

Analysis methodology

All the analysis on TSQM-9 questionnaire will be provided by country, by AK lesion localization (i.e., face and scalp) and overall.

Considering only the answers to the questions 1 to 3 and 9 to 14 (i.e. excluding the 5 questions related to side effects in TSQM 1.4 eCRF page) provided by the patients at Day 57, a first summary of patients who completed the questionnaire (i.e., patients with no more of one missing answer for each component) will be provided, while the total sub-scores computed as explained above, will be described.

In advance, 95% CI for mean value of Convenience, Efficacy and Total Satisfaction items' scores at day 57 will be estimated using Student's t distribution, regardless response's distribution.

Moreover, the proportion of patients for each answer at questions of TSQM-9 questionnaire will be provided.

Data handling rules

No missing imputation will be performed.

Sensitivity of primary analysis

Not applicable.

6.3.2. Secondary efficacy analysis

The secondary efficacy analysis will be evaluated on the Evaluable population if not differently specified. All data collected in the database will be taken into account.

The secondary objective is to evaluate patient-reported outcomes, physician-reported outcomes, efficacy, and safety following treatment with Tirbanibulin ointment 1% administered once daily for 5 consecutive days.

6.3.2.1. Skindex-16 questionnaire

Definition

Skindex-16 [2] may be used for patients to rate skin conditions that have occurred within the previous week. It is a short 16-item patient-completed assessment that are classified into three domains: symptoms (four items, 1-4), emotions, (seven items, 5-11) and functioning (five items, 12-16). All items are scored on a seven-point numerical analogue scales (0=never bothered to 6=always bothered). Each item is then transformed to a linear scale from 0 (never bothered) to 100 (always bothered). The higher the score, the more severe is the impairment.

One of the secondary objectives of this study is to assess change from baseline in Skindex-16 of Evaluable patients at Day 57.

The score will be computed as follows:

1. All responses will be transformed to a linear scale of 100 multiplying each raw score by 16.6667, in this way it's possible to obtain a scale from 0 (never bothered) to 100 (always bothered).

2. The score will be equal to the mean of a patient's non-missing responses to the items transformed in the linear scale.

If an item has multiple responses, then it will be considered as missing.
If a patient has more than 25% of missing responses in a component (symptoms, emotions and functioning) then the component score will be considered missing for that patient.

The global score will be computed as the mean of the 3 non-missing scale scores.

Analysis methodology

All the analysis on Skindex-16 questionnaire will be provided by AK lesion localization and overall.

The number and percentages of patients who completed the questionnaire (i.e., patients with no more of 25% of missing answers for each component) at each timepoint (i.e. at Baseline Visit and at Final Visit) will be summarized.

Proportions of each response provided by patients will be reported.

Descriptive analysis on change from Baseline of the global score and each of the 3 components' scores (i.e., symptoms, emotions and functioning) at Day 57 will be provided including 95% CI estimated by Student's t distribution, regardless response's distribution.

As regard to the global score computed as above and the 3 components' scores, descriptive statistics for at each timepoint will be provided.

Lastly, two listings of Skindex-16's responses including patients who were treated in face and scalp separately, will be provided in Section 16.

Data handling rules

No missing imputation will be performed.

Sensitivity of secondary analysis

Not applicable.

6.3.2.2. Likert Scale for organoleptic properties of Tirbanibulin

Definition

Likert scale is an instrument used to measure the individual's degree of agreement and disagreement with a variety of statements about some attitude, options, or their

feelings. In this study, the product's organoleptic properties are evaluated with Likert scale. The questionnaire is built with questions related to the product's characteristics namely appearance, color, convenience, texture, smell, and the feelings experienced during drug application. The Likert scale offers 7 possible answers, from "totally agree" to "totally in disagreement".

In this secondary objective the main purpose is the description of organoleptic properties (i.e appearance, color, convenience, texture, smell, and the feelings experienced during drug application) of Tirbanibulin through the Likert Scale at Day 8.

Analysis methodology

The number and percentages of patients who completed the questionnaire at Day 8 (i.e. Visit 3) will be summarized.

Scores of each question obtained by patients will be summarized with descriptive statistics and 95% CI (calculated for the multinomial proportion) of each question' response will be provided. In case the lower limit of CI is negative due to small frequency of the category, 0 will be imputed as lower limit.

Data handling rules

No missing imputation will be performed.

Sensitivity of secondary analysis

Not applicable.

6.3.2.3. TSQM 1.4 questionnaire

Definition

TSQM 1.4 is one of the two versions of TSQM questionnaires, the largest one. It is a 14-item robust instrument that psychometrically evaluates the treatment satisfaction of the administered medication. The instrument is designed with 4 scales consisting of 14 questions. These 14 questions were derived from an original set of 55 questions extracted from exhaustive literature review and treatment groups through multistep iterative process. The 4 scales focussed on efficacy (questions 1 to 3), side effects (questions 4 to 8), convenience (questions 9 to 11) and global satisfaction (questions 12 to 14). The global total score ranges from 0 – 100, where lower scores imply less satisfaction whilst higher scores imply higher satisfaction.

To reduce patients' burden in answering similar questions (on comparing the 2 versions of TSQM, Q1 to Q3 in TSQM 1.4 is equivalent to Q1 to Q3 in TSQM-9, and Q9 to Q14 in TSQM 1.4 is equivalent to Q4 to Q9 in TSQM-9), only the most extensive version of TSQM i.e., TSQM-1.4 will be administered to the patients in PRO Booklet.

One of the secondary objectives of interest in this study is to evaluate TSQM 1.4 responses obtained at day 57, assessing with in particular accuracy the items' results, i.e. Tirbanibulin Convenience, Efficacy, Side Effects and Total satisfaction.

Similarly to TSQM-9, global score will be computed as follows:

Score calculation formula	
Effectiveness	
If no missing item	$\frac{(\sum item1, item2, item3) - 3}{18} * 100$
If one missing item	$\frac{(\sum the\ 2\ completed\ items) - 2}{12} * 100$
Side effects	
If item4 is answered = "No"	Score =100
Else	
If no missing item	$\frac{(\sum item5, item6, item7, item8) - 4}{16} * 100$
If one missing item	$\frac{(\sum the\ 3\ completed\ items) - 3}{12} * 100$
Convenience	
If no missing item	$\frac{(\sum item9, item10, item11) - 3}{18} * 100$
If one missing item	$\frac{(\sum the\ 2\ completed\ items) - 2}{12} * 100$
Global satisfaction	
If no missing item	$\frac{(\sum item12, item13, item14) - 3}{14} * 100$
If item12 or item13 missing	$\frac{(\sum the\ 2\ completed\ items) - 2}{10} * 100$

If item14 missing	$\frac{(\sum the\ 2\ completed\ items) - 2}{8} * 100$

If more than one item is missing from a domain of the TSQM-1.4 for a particular patient, this domain should be considered invalid for that respondent.

Analysis methodology

All the analysis on TSQM-14 questionnaire will be provided by country, by AK lesion localization and overall.

The number and percentages of patients who completed the questionnaire (i.e., patients with no more of one missing answer for each component) at Day 57 (i.e. Visit 4) will be summarized.

As regard the total score for Convenience, Efficacy, Side Effects and Total satisfaction of TSQM 1.4 computed as above, it will be summarized at Day 57 with descriptive statistics and 95% CI for mean value of each item will be provided using Student's t distribution, regardless response's distribution.

Moreover, the proportion of patients for each answer at questions of TSQM 1.4 questionnaire will be provided.

Data handling rules

No missing imputation will be performed.

Sensitivity of secondary analysis

Not applicable.

6.3.2.4. EPQ questionnaires

Definition

EPQ was developed to accommodate an adequate validity as a real-world evidence tool to solicit patient's treatment preference and physician treatment outcomes. EPQ consists of 10 specific items to identify both patient and physical treatment preference. EPQ comprises of 2 versions, namely clinician and patient version. Clinician's version comprises 10 questions and patient's version includes 9 questions. The questions used in both the versions are similar, but they have been reworded to address both clinician and patient. Clinician version of the EPQ will be administered

to the clinician through physician reported outcome booklet. Patient version of the EPQ will be administered to the patients through patient reported outcome booklet.

Patient treatment preference at Day 57 will be assessed through question 1 (Q1) to question 9 (Q9) of the EPQ (whose outcome are reported in EPQ (PATIENT VERSION) eCRF pages) as well as physician treatment preference at Day 57 through question 1 (Q1) to question 10 (Q10) of EPQ (whose outcome are reported in EPQ (CLINICIAN VERSION) eCRF pages).

Analysis methodology

All the analysis on EPQ questionnaires will be provided by country, by AK previous naïve status (i.e., naïve patients are patients never previously treated for AK lesions; not naïve patients are patients already treated for AK lesions) and overall.

The number and percentages of patients and physicians who completed the respective questionnaire at Day 57 (i.e., Visit 4) will be summarized. The proportion of patients for each response provided by patients and clinicians will be computed with the corresponding 95% CI for the multinomial proportion. In case the lower limit of CI is negative due to small frequency of the category, 0 will be imputed as lower limit.

Data handling rules

No missing imputation will be performed.

Sensitivity of secondary analysis

Not applicable.

6.3.2.5. AK lesions characteristics

Definition

For each patient the following assessments (reported in AK LESION COUNT and in AK LESION COUNT AND OLSEN GRADING eCRF pages) will be provided:

- Presence of AK lesions (counted only if they are inside the treatment area) at Screening and Baseline and count of new and old AK lesions ad Day 57
- Reduction of number of lesions at Day 57 will be calculated considering only old lesions as:

$$\frac{N.lesions\ at\ Baseline - N.\ old\ lesions\ at\ Day\ 57}{N.lesions\ at\ Baseline} * 100\%$$

N. old lesions at day 57 is reported in AK LESION COUNT AND OLSEN GRADING eCRF page.

- Reduction of number of lesions at Day 57 will be calculated considering considering both old and new lesions, as:

$$\frac{N.lesions\ at\ Baseline - N.\ lesions\ at\ Day\ 57}{N.lesions\ at\ Baseline} * 100\%$$

Where, N. lesions at day 57 = N. new lesions + N. old lesions reported in AK LESION COUNT AND OLSEN GRADING eCRF page.

- Occurrence of complete clearance of all AK lesions within the application area, is defined as a reduction from baseline in the number of lesions = 100% at day 57
- Occurrence of partial clearance is defined as a reduction ≥ 75% in the number of lesions within the application area at Day 57.

Analysis methodology

All the analysis reported in this section will be provided by country, AK lesion localization and overall.

The number and percentages of patients who performed the AK lesions count at each timepoint (i.e., Screening Visit, Baseline Visit and Final Visit) will be reported.

The number of the AK lesions at Screening, Baseline and Final Visit (new, old and total AK lesions) will be provided.

The change from Baseline Visit of the AK lesions count will be described at Final Visit reporting also the corresponding 95% CI estimated by Student's t distribution, regardless response's distribution.

The number and percentages of patients with a complete clearance will be provided with the corresponding 95% CI calculated with the Binomial exact method.

Similarly, the same analysis will be performed for patients with partial clearance. In order to verify the association between Olsen Grade (defined in Section 5.3.2.6) and clearance, a cross-table between their categories will be produced.

Data handling rules

No missing imputation will be performed.

Sensitivity of secondary analysis

Not applicable.

6.3.2.6. Olsen characterization of AK lesions

Definition

The lesions in the identified treatment area will be classified based on Olsen characterization. It consists in the following grades:

- *Olsen Grade I:* Early AK appear as single or few, differently sized, from 0.1-0.3-5.0 cm, in size, rough, blurred, less visible than palpable (palpable on the roughness of the surface), red, rough spots or very flat, non-edged plaques which reach into the reddish colour and are easier to feel than to see;
- *Olsen grade II:* describes advanced AK as clearly visible and palpable, flat, and irregularly raised, with sharp or blurred boundaries, red, rough keratinized surface. If the surface is more strongly keratinized, the AK can also be white, yellow, or light brown. After scratching effects (frequently), a black or blue-black shade may appear (older bleeding);
- *Olsen grade III*: denotes "late" AK that have existed for a longer period of time and are firmly anchored on the lower surface, with an irregular, humpy surface, also wart-like and of different colours (white, brown, black). When the horn deposits are removed, an erosive subsurface is formed.

Olsen characterization (whose outcome will be reported in “AK LESION COUNT AND OLSEN GRADING” eCRF pages) will be evaluated for each patient at Baseline Visit and at Final Visit.
Therefore, in this analysis, the following groups will be considered:

- Olsen I: patients with all lesions graded as Olsen I;
- Olsen II: patients with all lesions graded as Olsen II;
- Olsen III: patients with all lesions graded as Olsen III;
- Olsen I/II: patients with some lesions graded as Olsen I and some lesions graded as Olsen II;

- Olsen I/III: patients with some lesions graded as Olsen I and some lesions graded as Olsen III;
- Olsen II/III: patients with some lesions graded as Olsen II and some lesions graded as Olsen III;
- Olsen I/II/III: patients with some lesions graded as Olsen I, some lesions graded as Olsen II and some lesions graded as Olsen III;
- Complete clearance (Only for Final Visit): patients who performed Olsen Grade evaluation (i.e., patient who answered "Was OLSEN Grading performed? = Yes" in AK LESION COUNT AND OLSEN GRADING eCRF Page) and reported no lesions or patients who did not perform Olsen Grade evaluation because he/she specified no lesions observed (specified in AK LESION COUNT AND OLSEN GRADING eCRF Page);
- Missing: patients with AK lesions who did not perform Olsen Grade evaluation for at least one of them.

Analysis methodology

The number and percentages of patients who performed the AK lesions Olsen Grading at each timepoint (i.e., Baseline Visit and Final Visit) will be reported.

The proportions of patients with lesions classified as described above at Baseline and at Final Visit will be provided with the corresponding 95% CI calculated for the multinomial proportion. In case the lower limit of CI will be negative due to small frequency of the category, 0 will be imputed as lower limit.

Furthermore, a shift table Baseline vs Final Visit with frequency distribution will be produced.

Data handling rules

No missing imputation will be performed.

Sensitivity of secondary analysis

Not applicable.

6.3.2.7. LC-OCT of clinical and subclinical lesions

Definition

Line-field Confocal Optical Coherence Tomography (LC-OCT) is a high-resolution imaging tool that provides real-time, and non-invasive diagnosis. Comparatively, higher diagnostic accuracy of AK was achieved using LC-OCT than conventional techniques. The histopathology of the skin is evaluated based on the estimated atypia

score at cellular level of the LC-OCT images. Atypia score is calculated using certain quantitative and qualitative metrics.

In this study, only one Spanish site will perform LC-OCT: Hospital Clinic de Barcelona.

Each patient will be assessed for 2 lesions (1 clinical lesion and 1 subclinical lesion) at each visit. Information regarding whether assessment is performed or not in the respective patient will be collected in the eCRF with Yes/No responses. Data pertaining to individual cellular metrics of the atypia score and the atypia score will be collected only in the source documents externally and not entered in the eCRF.

Analysis methodology

LC-OCT data of clinical and subclinical lesions at only one selected site, i.e., Hospital Clinic de Barcelona (Site # 101) will be collected at baseline (Day 0; pre-dose), Day 8, Day 15, Day 29, and Day 57 and atypia scores will be calculated on those 3D images externally, i.e., by the site.

Results of this analysis will be presented separately and included in the Clinical Study Report (CSR).

A table summarizing data collected in Line-field Confocal Optical Coherence Tomography (LC-OCT) eCRF page, in terms of number of patients who performed LC-OCT assessment at each timepoint, will be provided.
Patient data listings for LC-OCT population will be provided, reporting the assessment's performance and the related assessment date, for each visit.

Data handling rules

No missing imputation will be performed.

Sensitivity of secondary analysis

Not applicable.

6.3.3. Exploratory efficacy analysis

Not applicable.

6.3.4. Measurement of Treatment Compliance

Medication adherence will be recorded using a self-administered Patient Diary from Day 0 to Day 4. Questions reported in Patient Diary will be related to usage pattern

(dosage, frequency, and site) of study medication application, adverse reactions, and reasons for any missed or delayed doses.

Medication adherence assessed with patient-kept diary will be summarized with frequency distribution from Day 0 to Day 4, reporting the number of patients who applied Tirbanibulin and the number of recorded missing applications (i.e., no missing doses, 1 missing dose, 2 missing doses, 3 missing doses, 4 missing doses or 5 missing doses) at each considered day and overall.

In advance, patients' compliance will be evaluated computing the number and percentage of patients who received all Tirbanibulin doses and the number and percentage of patients who didn't received all ones.

6.4. Safety Evaluation

The safety analysis will be performed on the Safety population.

Adverse events and local tolerability signs during the treatment period will be collected through patient diary and evaluated at each visit or through telephonic follow-up as final safety evaluation.

6.4.1. Extent of Exposure

Exposure to the study treatment will be based on the number of Tirbanibulin applications administered from Day 0 to Day 4 which will be summarised as well as number of missed treatment's applications.

Moreover the proportion of patients who applied all Tirbanibulin doses, and the proportion of patients by number of missed doses will be provided.

6.4.2. Concomitant medications

Concomitant medications are defined as therapies ending or ongoing after the first Tirbanibulin application.

Since no imputation will be performed in case of partial/missing medication's start or end date, the presence of at least one of the following conditions will be used to identify concomitant medication:

- If medication status is ongoing;
- If medication end date is missing and medication status is not ongoing or missing;
- If medication end date is complete, medication status is not ongoing or missing and medication end date ≥ Tirbanibulin start date;

- If day and month of medication end date are missing, medication status is not ongoing or missing and medication end date year ≥ Tirbanibulin start date year;
- If only day of medication end date is missing, medication status is not ongoing or missing and medication end date year > Tirbanibulin start date year;
- If only day of medication end date is missing, medication status is not ongoing or missing, medication end date year = Tirbanibulin start date year and medication end date month ≥ Tirbanibulin start date month.

Concomitant medications/therapies and surgical/medical procedures will be described by ATC code (2nd level class) and Preferred Term, presenting the number and percentage of patients taking at least one concomitant medication/procedures.

Moreover, surgical and medical procedures administrated to each patient will be described by System Organ Class and Preferred Term, presenting the number and percentage of patients undergoing at least one surgical or medical procedure.

6.4.3. Adverse Events

Adverse Events (AEs) are defined as any untoward medical occurrence associated with the use of an intervention in humans after providing written informed consent for participation in the study until the end of study visit.

Tables reporting a summary of AEs will be produced including:

- the total number of events and absolute and relative frequencies of patients with AEs;
- the total number of events and absolute and relative frequencies of patients with Serious AEs (SAEs);
- the total number of events and absolute and relative frequencies of patients with AEs related to treatment (i.e., for patients who reported “Relationship to Study Treatment=Related or Possibly Related” in ADVERSE EVENTS eCRF page);
- the total number of events and absolute and relative frequencies of patients with fatal AEs;
- the total number of events and absolute and relative frequencies of patients with AEs leading to treatment discontinuation.

AEs summaries will be presented by SOC, PT and maximum severity using the MedDRA dictionary. All related AEs with study treatment, SAEs, AEs with an outcome of death, AEs leading to treatment discontinuation will be summarized by MedDRA SOC and PT.

If a patient reported more than one AE coded to the same PT, in the analysis the patient will be counted only once in the incidence calculation for that PT. Similarly, if a patient has more than one AE in the same SOC, the patient will be counted only once in the total number of patients with an AE for that SOC.

SAE with fatal and non-fatal outcome will be listed by patient.

All the AEs, SAEs and Adverse Events of Special Interest (AESIs - identified as Basal Cell Carcinoma (BCC), Squamous Cell Carcinoma (SCC) and melanoma and distinguished through the PTs included in Standardised MedDRA Query (SMQ): "Skin neoplasms, malignant and unspecified") will be also listed as well as all reported deaths.

6.4.4. Laboratory parameters

Not applicable.

6.4.5. Vital signs/Physical examination

Vital Signs

Measurement of vital signs will include body temperature (°C), systolic and diastolic blood pressure (mmHg), heart rate (beats/min), respiratory rate (beats/min).

Measurements of blood pressure will be carried out after at least 5 minutes resting in the supine position and always on the same arm. In case of any suspicion of unreliable measurement, blood pressure will be measured again and the value obtained the second time will be considered as definitive.

Vital signs will be summarized at each timepoint as well as absolute changes from baseline.
In advance, the proportion on patients by overall evaluation (i.e., Normal, Abnormal and not clinically significant and Abnormal and clinically significant) will be provided at each timepoint.
In order to display the number of patients with potentially clinically significant vital signs at screening vs post-screening timepoint, shift tables will be produced.

Physical examination

A complete physical examination will be performed at screening and Visit 4 and should include assessments of the cardiovascular, respiratory, gastrointestinal, and neurological systems. Height and weight will also be measured and recorded to

allow calculation of body mass index (BMI). For this, patients should be in light indoor clothes without shoes.

Summary descriptions of weight, height and BMI will be provided as well as the proportions of patients according to the reported results and the clinically relevance of the following assessments: General appearance, Eyes, ears, nose and throat, Cardiovascular, Respiratory, Gastrointestinal, Urogenital, Neurological, Musculoskeletal, Lymph nodes, Dermatology and in case Other assessments at each timepoint.
In order to display the number of patients with potentially clinically significant results (i.e., Normal, Abnormal and not clinically significant and Abnormal and clinically significant) at screening vs Final visit, shift table will be produced.

6.4.6. Other safety parameters

Local tolerability signs and Local skin reaction.

Local tolerability signs (LTSs) and local skin reactions (LSRs) will be assessed at Day 57, based on the Investigator's review of the following signs in the treatment field: Erythema/redness, Flaking/scaling, Crusting/scabs, Swelling, Vesiculation/pustulation and Erosion/ulceration.

The signs will be evaluated at each scheduled site-visit both individually and as a composite score (total of the 6 individual scores).
These signs will be assessed using a 4-point grading scale, composed as follows:

- 0=absent;
- 1=mild (slightly, barely perceptible);
- 2=moderate (distinct presence);
- 3=severe (marked, intense).

Only signs (LTSs and LSRs together) evaluated at Baseline, Visit 3 (Day 8), Visit 4 (Day 57) by the Investigators (i.e., collected in LOCAL SKIN REACTION or LOCAL TOLERABILITY SIGNS eCRF pages) will be summarized by country and overall reporting the following information:

- Number of patients at each visit;
- Frequency of patients who provided information on signs at each visit (calculated on patients who performed the visit);
- Frequency of patients who did not provide information on signs at each visit (calculated on patients who performed the visit);
- Frequency of patients with at least one sign of at least Mild severity (calculated on patient who performed the visit and on patients with information on sign at visit);

- For each of the six signs (erythema, flaking/scaling, crusting, swelling, vesiculation/pustulation, and erosion/ulceration at the application-site) the number and percentage (on patients with information on sign at visit) of patients corresponding to each evaluated sign;
- A composite score will be calculated and summarized as the sum of the scores for all six signs (range 0 to 18, with higher scores indicating more severe reactions) at each visit.

Signs reported by patients (i.e., collected in LOCAL SKIN REACTIONS/LOCAL TOLERABILITY SIGNS REPORTED BY PATIENT eCRF page) will be summarized by country and overall. The proportion of patients with at least one sign as well as the number of signs reported by each patient will be described.

Moreover, all local tolerability signs and local skin reactions will be listed in Section 16.

Pregnancy

Considering female patients, at screening and baseline visit a urine pregnancy test should be performed for all women of childbearing potential.
Moreover, women who reported positive urine pregnancy test will perform an additional serum pregnancy test for confirmation.

Pregnancy test results will be listed only.

Other

Information about kit assignment, Instructions for self-administration, patient diary and return of patient diary will be listed.

6.5. Subgroup Analyses

Subgroup	Definition	Analysis Type
Olsen grade at Baseline	Olsen grade I, Olsen grade II, Olsen grade III, Olsen grade I/II, Olsen grade I/III, Olsen grade II/III, Missing.	E

Olsen grade at Visit 4	Olsen grade I, Olsen grade II, Olsen grade III, Olsen grade I/II, Olsen grade I/III, Olsen grade II/III, Complete clearance, Missing.	E
Country	Italy, Spain	E
AK lesion localization	Face, Scalp	E
Previous AK treatment naïve status	Naïve, not naïve	E

E/S: efficacy or safety analysis

The definitions above reported will be derived from the following rules:

- Olsen Grade: see section 6.3.2.6.
- Country: country will be identified through the first number of site-id.
  - Spain: if first number of site-id is 1;
  - Italy: if first number of site-id is 2.
- AK lesion localization: reported in IDENTIFICATION OF TREATMENT AREA eCRF page:
  - Face;
  - Scalp.
- Previous AK treatment naïve status:
  - Naïve patients: patients who answered “No” to “Has the subject received any previous AK Therapy?” question in AK THERAPY HISTORY eCRF page;
  - Not naïve patients: patients who answered “Yes” to “Has the subject received any previous AK Therapy?” question in AK THERAPY HISTORY eCRF page.

6.6. Interim Analysis and Data Monitoring

An Interim Analysis was performed to provide preliminary evaluation of the impact of Tirbanibulin on the well-being of AK patients, to provide a descriptive analysis of the patients' characteristics and the study endpoints.

The IA was performed when 50% of patients (i.e., 210 of 420 patients expected to enrol for this study) have completed the study, i.e. when they have reached Day 57 (Final Visit) or early discontinued the study.

7. REFERENCES

[1] Treatment Satisfaction Questionnaire for Medication (TSQM) User Manual, Version 2.4, 21 Apr 2022.

[2] Skindex-16, Scaling and Scoring. Version 2.0: February 2012. MAPI Research Trust.

8. APPENDIX

Not applicable.

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Envelope Summary Events	**Status**	**Timestamps**
Envelope Sent	Hashed/Encrypted	13-Feb-2024 | 11:07
Certified Delivered	Security Checked	13-Feb-2024 | 12:30
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